UNIVERSAL ANTENATAL SCREENING FOR GROUP B *STREPTOCOCCUS* COLONISATION IN THE UK

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS

BPSU	British Paediatric Surveillance Unit
CC	Clonal complex
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
CI	Confidence intervals
CSF	Cerebrospinal fluid
E. coli	*Escherichia coli*
EOGBS	Early-onset neonatal group B *Streptococcus* disease
FCS	Functional Conditional Specification
FMI	Fraction of missing information
G	Gram
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GBS	Group B *Streptococcus*
IAP	Intrapartum antibiotic prophylaxis
INT	International
IQR	Interquartile range (25^{th} to 75^{th} percentile)
LOG	Logarithmic notation
LOGBS	Late-onset neonatal group B *Streptococcus* disease
MBRRACE-UK	Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK
MeSH	Medical Subject Heading
MICE	Multiple Imputation by Chained Equations
ML	Milliliter
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NPV	Negative predictive value
NSC	National Screening Committee
ONS	Office of National Statistics
OR	Odds ratio
QUIPS	Quality in Prognosis Studies
P	Probability
PCR	Polymerase chain reaction
PRISMA-P	Preferred Reporting Items for Systematic Review and Meta-analysis
PHE	Public Health England
PMM	Predictive mean matching
PROSPERO	International Prospective Register of Systematic Reviews
PPP	Purchasing power parity
PPV	Positive predictive value
QALY	Quality-adjusted life year
QUIPS	Quality in Prognosis Studies
RCOG	Royal College of Obstetricians & Gynaecologists
RCT	Randomised controlled trials
RoB	Risk of Bias

RoBANS	Risk of Bias Assessment Tool for Nonrandomised Studies
RD	Risk difference
RR	Relative risk/Risk ratio
S. *aureus*	*Staphylococcus aureus*
S. *pneumoniae*	*Streptococcus pneumoniae*
SD	Standard deviation
ST	Sequence type
UN	United Nations
UNDP	UN Development Programme
UNICEF	UN Children's Fund
UK	United Kingdom
US	United States of America
USPSTF	US Preventive Services Task Force
VIF	Variance Inflation Factor
WHO	World Health Organization

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DECLARATION

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree. The work presented (including data generated and data analysis) was carried out by the author except in the cases outlined below:

List of data provided or data analysed by collaborators: Data for chapters 11 to 13 were collected from institutions across the globe who completed a questionnaire providing data (see Appendix 21). The original meta-analysis in Chapter 6 was performed by my supervisor, Olalekan Uthman, for a UK National Screening Committee evidence review that I led. I re-ran the meta-analysis for this chapter and conducted all of the sensitivity analyses.

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Le Doare K, O'Driscoll M, Turner K, Seedat F, Kampmann B. Intrapartum antibiotic chemoprophylaxis policies for the prevention of Group B Streptococcal Disease worldwide: systematic review. Clinical Infectious Disease, in press.

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Seedat F, Cooper J, Cameron L, Stranges S, Kandala NB, Burton H, Taylor-Phillips S (2015). International comparisons of screening policy-making: A systematic review. Department of Health.

Seedat F, Stranges S, Kandala NB, Taylor-Phillips S (2014). Written evidence submitted to the National Health Screening inquiry, House of Commons Science and Technology Committee.

Seedat F, Brown CS, Stinton C, Patterson J, Geppert J, Uthman OA *et al.* Bacterial load and bacterial molecular markers associated with neonatal Group B Streptococcus: A systematic review. The Pediatric Infectious Disease Journal, submitted.

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Abstracts

Seedat F, Geppert J, Stinton C, Patterson J, Freeman K, Johnson S *et al*. Universal antenatal culture-based screening for maternal Group B Streptococcus (GBS) carriage to prevent early-onset GBS disease: A systematic review for the UK National Screening Committee (NSC). Journal of Epidemiology and Community Health, 71(Supplement 1): OP34.

Seedat F, Brown CS, Stinton C, Patterson J, Geppert J, Uthman OA *et al.* Bacterial load and bacterial molecular markers associated with neonatal Group B Streptococcus. 27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Conference Proceedings Handbook. Vienna, ECCMID; April 2017.

Conference presentations and workshops

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International policy-making processes for health screening. Presented at the UK National Screening Committee Meeting, Belfast, Northern Ireland, June 2014.

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Group B *Streptococcus* screening: a UK NSC evidence review. Presented at the expert workshop, Department of Health, London, England, September 2016.

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Group B *Streptococcus* screening: a UK NSC evidence review. Presented at the 61st Annual Scientific Meeting for the Society for Social Medicine, Manchester, England, September 2017.

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DEDICATION

TO MY HUSBAND, FAHIM

For your constant love, support and humour
throughout this roller coaster ride.

ABSTRACT

Background: Group B Streptococcus (GBS) is the leading cause of neonatal sepsis and meningitis. Currently, the UK recommends against universal antenatal screening to prevent early-onset GBS disease (EOGBS, <7 days). Key gaps around GBS natural history, harms from screening and a lack of high-quality data to prove screening effectiveness make it difficult to ensure the benefits of GBS screening outweigh the harms. There is also a wider gap on policy-making processes for screening. The overall aim of this thesis is to address these gaps and examine whether the UK should introduce universal GBS screening as a result.

Methods: In addition to a literature review, I used two approaches: systematic review/meta-analysis and ecological trend analysis. The systematic reviews synthesised evidence on the screening policy-making processes, mechanisms of EOGBS and adverse events from intrapartum antibiotic prophylaxis (IAP) to prevent EOGBS. In the absence of RCTs, I combined ecological data on the benefits and harms of GBS screening, then analysed their trends across time compared with other prevention strategies in regression analyses adjusting for context differences.

Results: Evidence from 17 countries showed that most GBS screening recommendations were not developed by screening organisations and it is not known whether screening principles and the likely unseen harms of GBS screening were considered. Seventeen studies revealed that we do not fully understand the natural history of why some mothers, but not others, transmit GBS to their neonates, or which neonates will develop EOGBS. There was consistent evidence that heavy bacterial load was associated with transmission and progression to EOGBS. Neonates colonised with serotype III were also twice as likely to develop EOGBS compared with serotype Ia and II. However, the evidence was old and at high risk of bias. The selective culture test at 35 to 37 weeks gestation is not an accurate predictor of EOGBS and at least 99% of screen-positive and treated mothers (and their neonates) would be over-treated. Seventeen observational studies and 13 RCTs showed a wide range of potential harms from IAP, including cerebral palsy, functional impairment and antibiotic resistance. However, there was little high-quality and applicable evidence to quantify the frequency of adverse events.

The three ecological trend analyses combining data from 59 geographical areas showed that EOGBS incidence decreased by approximately 0.02 per 1,000 livebirths per year in areas that most recently reported GBS screening, whereas it increased by approximately 0.01 to 0.02 per 1,000 livebirths in areas most recently reporting risk-based prevention. Areas that recently did not have GBS prevention displayed conflicting EOGBS trends. By contrast, there was no evidence that screening impacted annual early-onset sepsis trends compared with other, or no prevention strategies; however, this study did not have a sufficient sample size. The was no harmful impact of GBS screening on LOGBS trends compared with other, or no prevention. There was also no evidence that screening increased early-onset *E. coli* incidence and the percentage of GBS cases resistant to clindamycin and erythromycin, compared with risk-based or no prevention; again, these analyses did not have a sufficient sample size. The findings of these studies must be treated with caution as some results may be due to low statistical power and others were unstable across analyses. The findings also contain numerous limitations as covariates were poorly collected in most countries. Therefore, the evidence on the benefits and harms of universal GBS screening remains inconclusive.

Conclusion: GBS infection is an important health condition and its persistence, poor screening tests and the IAP harms stress the need for a better understanding of the natural history of GBS and more effective prevention. Evidence on the harms and benefits of GBS screening is limited, therefore, screening should not be introduced in the UK. Ecological trend analysis was not an adequate method to inform GBS screening decisions, however, it may be useful for screening decisions on other conditions.

PART I. INTRODUCTION

1. INTRODUCTION

In this chapter, I will introduce the main issues underpinning this PhD thesis and describe the structure of my thesis. In Chapter 2, I will provide a detailed review of the background literature surrounding the thesis and in Chapter 3, I will introduce the thesis aims and objectives.

1.1 Health problem

Streptococcus agalactiae, or Group B *Streptococcus* (GBS), is a gram-positive bacterium that was first recognised as a serious child health concern in the 1960s and 1970s.[1] Since then, GBS has remained the leading cause of neonatal sepsis and meningitis in the United Kingdom (UK) and other developed countries.[2, 3] Early-onset GBS disease (EOGBS) in the first six days of life accounts for around 60% to 70% of neonatal GBS disease and originates from GBS passing from mothers to their neonates during labour.[3] It is not known why 36.4% of mothers colonised with GBS pass it on to their neonates or why 1% to 3% develop EOGBS.[4]

The incidence of EOGBS in the UK is approximately 0.57 per 1,000 livebirths,[5] which equates to approximately 443 neonates every year. EOGBS progresses rapidly, presenting with sepsis in 63% of cases, meningitis in 13% and pneumonia in 24%.[5] Ten percent of EOGBS cases will die as a result,[6] though the most recent surveillance reported a case fatality rate of 5.2% in the UK.[5] Neonates who survive with EOGBS can suffer from long-term neurodevelopmental abnormalities in around 8.7% or 15.8% of cases.[7 8] EOGBS meningitis, in particular, can lead to long-term neurological impairment in up to 50% of cases.[9] Although EOGBS is relatively rare, it is an important health condition as it can lead to long-term and fatal consequences.

1.1 GBS prevention treatment

The current recommendation to prevent EOGBS is to offer antibiotic prophylaxis during labour to mothers at risk of having a neonate with GBS.[1, 3] With vaccinations currently in development and licensure awaiting, intrapartum antibiotic prophylaxis (IAP) treatment is the only prevention option. A Cochrane review of all randomised controlled trials (RCTs) assessing the effectiveness of IAP found that it reduced the incidence of EOGBS by 83% compared with no treatment.[10] However, the evidence was at high risk of bias making the estimate uncertain.[10] Nevertheless, the current IAP recommendation is intravenous penicillin (or ampicillin in the US and Italy) given as soon as possible after the onset of labour and then

every four hours until delivery.[11-14] For mothers allergic to penicillin, second-line treatment recommended in the UK was intravenous clindamycin, [13] however, this has changed in the new guidelines published in September 2017 to cephalosporin.[15] If a woman has a history of severe allergy to beta-lactams vancomycin is recommended. Similarly, in the United States of America (US) since 2010, the first alternative is intravenous cefazolin followed by clindamycin.[14]

1.2 GBS prevention strategies

There are different prevention strategies to identify women at risk of having a baby with EOGBS in order to offer IAP. The UK adopts risk-based prevention, whereby women who present with known EOGBS risk factors in labour, such as maternal GBS colonisation, bacteriuria, a previous baby with GBS disease or intrapartum fever are offered IAP.[12, 13] There are currently no high-quality studies on the effectiveness of risk-based prevention. Under risk-based prevention in the UK, EOGBS incidence has not decreased, but instead, has increased from 0.48 to 0.57 per 1,000 livebirths.[5] The risk-based prevention strategy has also been criticised as above 30% of EOGBS cases without risk factors are excluded from prevention.[5]

To increase the identification of pregnant women colonised by GBS an alternative strategy is universal antenatal screening. This mainly involves culturing rectal and/or vaginal swabs from all pregnant women and offering IAP to those with positive screening results. Testing is administered at 35 to 37 weeks gestation as culture takes 24 to 48 hours to process and the results would not be available in time to treat in labour. Observational evidence, at high risk of bias, reports that the odds of EOGBS is 55 to 75% lower during periods of screening compared with historical periods of risk-based or no prevention.[16, 17] There has been no RCT assessing the effectiveness of GBS screening on the morbidity and mortality of EOGBS making it increasingly difficult to calculate the impact of screening. Concerns have also been raised about the potential harms from widespread IAP under screening, including increases in antibiotic resistance,[18] neonatal infections caused by gram-negative bacteria (as a result of selection pressure) and changes in the neonatal microbiota leading to long-term health problems.[4, 19, 20] Widespread IAP may also cause a maternal predisposition to *Clostridium difficile* infection[21] maternal anaphylaxis[14] and the increased medicalisation of labour.[4, 13]

1.3 UK GBS screening recommendation

In the UK, screening programmes are national, and the UK National Screening Committee (NSC) is tasked with providing triennial evidence-based recommendations about whether or not to introduce nominated screening programmes. To reach a decision, the UK NSC assesses the evidence against internationally recommended screening criteria.[22] In 2012, the UK NSC reviewed the evidence for universal GBS screening, concluding that, it is difficult to ensure that the benefits of screening would outweigh the harms due to evidence gaps around key screening criteria.[23] In particular, there was no evidence about why some neonates develop EOGBS, and others do not (natural history), nor the frequency and severity of screening associated IAP harms. Furthermore, a lack of RCT evidence inhibited information on the effectiveness of screening.

1.4 Chapter summary

EOGBS is an important health condition, and with the increasing rate of EOGBS in the UK, an effective prevention strategy is required to reduce the morbidity and mortality that neonates suffer as a result. Universal GBS screening is a potential strategy to prevent EOGBS, however, there is a need to address the current gaps in the evidence base in order to assess whether it should be introduced in the UK.

2. BACKGROUND

In this chapter, I will examine the literature on EOGBS and the strategies to prevent it, particularly universal screening. This will involve the key issues of GBS epidemiology and natural history, IAP treatment and its effectiveness, GBS prevention strategies, screening as a concept, universal GBS screening and the evidence on the benefits and harms from universal screening.

2.1 Data sources

Much of this chapter is informed by the 2016 UK NSC evidence review on the policy of universal GBS screening,[24] which I successfully led with the help of my supervisors and a multi-disciplinary team of experts. The team included public health and screening experts, infectious diseases consultants, clinical microbiologists, gynaecologists and systematic reviewers and meta-analysts. While the overarching objectives of the review were set by the NSC, I led the design of the specific research questions and the methodology to address them. I also performed the searches, study selection, data extraction and report writing. My supervisors and other team members contributed their technical expertise by reviewing and advising me throughout. Some of the team members were also involved with study selection, data extraction and report writing.

For the 2016 NSC review, I conducted comprehensive literature searches in well-known and recommended electronic databases: Medline, Medline In-process, Embase and the Cochrane Library, from January 2012 to 21st April 2016. I applied a comprehensive search using the following MeSH and text terms that I combined with the Boolean operator OR: *Streptococcus agalactiae*/, group b adj *streptococc** and *Streptococc** *agalactiae*. I also searched Public Health England for published reports, unpublished data from the British Paediatric Surveillance Unit (BPSU) and unpublished data from Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries (MBRRACE-UK). As the NSC review was only from 2012 to 2016, for the purposes of this chapter, I have supplemented the literature review with evidence published before 2012 or after 2016 if it was of higher quality.

2.2 The health problem

2.2.1 Natural history

GBS is a gram-positive commensal bacterium that principally colonises the gastrointestinal and genitourinary tract in approximately 30% of healthy adults.[25, 26] GBS colonisation can be persistent, transient or intermittent.[27] As a natural reservoir for GBS is the genitourinary tract, GBS can colonise the vagina during pregnancy and labour. Globally, GBS colonisation in pregnant women varies and, in developed countries, GBS has been identified from vaginal and/or rectal swabs in between 10% and 30% of women tested.[28, 29] A recent meta-analysis of 78 studies including 73,791 pregnant women across 37 countries estimated a global prevalence of 17.9% (95% confidence intervals [CI] 16.2 to 19.7).[30] The highest incidence was reported in Africa (22.4%, 95% CI 18.1 to 26.7), followed by the Americas (19.7%, 95% CI 16.7 to 22.7), Europe (19%, 95% CI 16.1 to 22), and southeast Asia (11.1%, 95% CI 6.8 to 15.3). In the UK, maternal GBS colonisation is estimated at 21%,[31] although prevalences of 14%,[32, 4] 19%[33] and as high as 29%[34] have been reported. Differences in prevalences could be attributable to socio-demographic factors, such as the ethnic make-up of the participants, the geographical location, as well as the methodology used. For example, culturing swabs from the vagina and rectum with a selective culture medium, compared with a vagina-only swab and/or a standard culture medium, can increase the detection of GBS.[35] However, in the recent meta-analysis, differences in the timing of specimen collection in pregnancy or selective culture methods did not explain heterogeneity.[30]

When a pregnant woman is vaginally colonised during labour, a meta-analysis estimated that there is a 36.4% (95% CI 28.1 to 45.0) risk that GBS can be transmitted to her neonate, either through the neonate passing the colonised birth canal or GBS ascending *in utero*.[4] Rates around 50% have also been reported.[3, 36] The majority of the colonised neonates will be asymptomatic. However, 3.0% (95% CI 1.6 to 4.7) of colonised neonates will suffer from GBS in the first six days of life,[4] although rates as low as 1% have also been reported.[3] The natural history of GBS is summarised in Figure 1 on a hypothetical cohort of 718,126 term pregnant women. The estimates for each point in the natural history pathway are based on the best available data, however, they do contain uncertainties. For example, the estimate for the risk of EOGBS in colonised neonates is calculated from a small number of cases pooled from non-UK studies published between 1979 and 1998, therefore, the estimate may not be applicable to the UK today.[4] The risk of EOGBS in colonised term neonates may also be lower than 1.6% as the burden of EOGBS is lower in term compared to preterm neonates. Indeed, the total number of term EOGBS cases in Figure 1 (916/718,126, 1.28 per 1,000 term

livebirths) is much higher than the enhanced surveillance estimates in section 2.3.1. Therefore, these estimates should be used cautiously for a sense of scale but not as exact estimates.

The natural history of GBS in the gastrointestinal and genitourinary tract is poorly understood. In particular, the reasons why 36% of colonised mothers transmit GBS to their neonates, while the other 64% do not, or the reasons why, in 1% to 3% of GBS colonised neonates, the commensal becomes a pathogen causing invasive GBS disease, are largely not known. As for many other pathogens, the polysaccharide capsule appears to be important. There are 10 GBS capsular serotypes – Ia, Ib, II-IX.[25, 26, 37] Types Ia, Ib, II, III and V are more commonly responsible for GBS disease,[38-43] with 80.3% of neonatal GBS disease within six days attributed to serotypes III and Ia.[39] Isolates of GBS that are not typeable on capsular serotyping are also found to be less associated with invasive disease, supporting the association of the capsule with virulence. Interestingly, a study in South Africa exploring the temporal changes in invasive GBS serotypes found a 9.4% increase in the incidence of serotype Ia and a 7.4% decrease in serotype III invasive disease.[44] Although there were some changes in serotype distribution, both serotypes Ia and III were still the most dominant across time. In the UK, serotype III is the most commonly reported serotype in neonatal GBS disease (60%) followed by Ia (17.2%).[5] The number of serotype III cases increased from 2000/01 to 2014/15, mirroring an increase found in disease incidence.[5]

Congruent with this is the evidence, summarised in a recent systematic review, that low levels of GBS serotype-specific capsular antibody levels are associated with increased risk of GBS disease in neonates. However, the reviewers found substantial heterogeneity in assay methods and a lack of standardised reference ranges for functional antibody levels.[45] Recently, a study in Gambia also found that no infant was colonised with GBS above a serotype-dependent antibody threshold, and that a higher antibody concentration was also associated with clearance of GBS between birth and 60 to 89 days.[46]

A number of other virulence factors such as resistance to antimicrobial peptides, factors for immune evasion and pore-forming toxins have been proposed in laboratory and clinico-epidemiological studies as important in the pathogenesis of GBS disease.[47-49] The majority of these have little or no clinical or experimental evidence to confirm their role. A clonal complex, known as CC-17 has been described as hypervirulent in a number of geographical locations, with enhanced risk of disease in the neonate and higher rates of meningitis.[41, 40, 50, 51]A CC-17 specific surface protein of GBS, which promotes attachment to intestinal and meningeal cells, has been described as an important determinant of hypervirulence.[52] It has also been demonstrated that sequence types do not always follow serotypes.[51]

2.2.2 Clinical presentation and prognosis

An invasive GBS diagnosis is most commonly confirmed by culture of the organism from blood, cerebrospinal fluid or another sterile site. Invasive GBS disease in the neonate is separated into early-onset GBS (EOGBS) and late-onset GBS (LOGBS). EOGBS occurs during the first six days of life (although definitions can vary across countries), with the majority of cases occurring within the first day of life.[25, 26] The most recent estimate in the UK reported that approximately 67% of EOGBS cases present within the first 24 hours.[5] EOGBS cases progress rapidly with the majority presenting with sepsis, pneumonia and meningitis; in in rare cases, bone and joint involvement occur.[25, 26] In the UK, the relative proportion of cases with each type of morbidity has been remained stable between 2000/01 and 2014/15; 63% present with sepsis, 23% to 26% with pneumonia and 11% to 13% with meningitis.[5] The clinical symptoms of EOGBS are not very specific to this pathogen but neonates could be irritable, lethargic, feed poorly or present with respiratory disease.[53]

Conversely, LOGBS presents between seven and 89 days of life and is associated with localised infections, particularly meningitis.[25, 26] In the UK, the percentage of LOGBS cases presenting with meningitis decreased from 43% to 28.9%.[5] While maternal colonisation is the direct cause for EOGBS, LOGBS is predominantly caused by perinatal, nosocomial and community sources.[3] The remaining review will focus more heavily on EOGBS as it is amenable to maternal prevention strategies.

Further to the initial morbidity, EOGBS can result in death and disability. Without treatment, case fatality rates from EOGBS in the 1970s were reported at 20 to 50%. This has substantially declined to 10% or lower as a result of antibiotic treatment.[25, 26, 1] Therefore, neonates with symptoms of EOGBS should be tested for culture confirmation and treated with intravenous antibiotics as soon as possible.

For GBS survivors, there is the risk of chronic neurodevelopmental sequelae, particularly from meningitis. Eastwood *et al.* (2014) found that 8.7% of surviving EOGBS cases had abnormal neurodevelopment although it was uncertain whether the sequelae were related to GBS or prematurity.[7] In Japan, a study found that 15.8% of surviving EOGBS cases and 33% in EOGBS meningitis cases had neurological sequelae, with no difference in preterm and term neonates.[8] In another Japanese study, sequelae were observed in 11.3% of EOGBS cases, with around 30% suffering neurological sequelae from EOGBS meningitis. The neurological sequelae included one or more of the following: brain atrophy, cerebral infarction, cerebral palsy, mental retardation/developmental delay, diabetes insipidus, encephalomalacia, hearing

impairment, hydrocephalus, seizure disorder and visual impairment.[54]An earlier study in the UK showed that, after GBS meningitis, 50% had neurodevelopment impairment at five years of age. Of the 98 neurological meningitis cases, 13% had severe disability, 17% had moderate disability and 18% had mild disability.[9] A study in the US in 2012 found that GBS meningitis resulted in 25% of cases having mild to moderate impairment while 19% had severe impairment.[55] Relatedly, a recent published meta-analysis reported a pooled relative risk (RR) of 4.99 (95% CI 3.17 to 7.86) for low IQ (less than 70) as well as developmental delay in survivors of bacterial meningitis, compared with controls.[56]

2.3 GBS Epidemiology

2.3.1 Incidence and mortality

Despite prevention, treatment and management efforts, GBS remains the most important cause of neonatal sepsis and meningitis. Globally, a meta-analysis of data from 2000 onwards, estimated that the incidence of neonatal GBS is 0.53 per 1,000 livebirths (95% CI 0.44 to 0.62), although this is likely an underestimate.[6] The GBS burden varies geographically, with the highest incidence per 1,000 livebirths found in Africa (1.21), followed by the Americas (0.67), Europe (0.57), Eastern Mediterranean (0.35), Western Pacific (0.15) and very low estimates in Southeast Asia (0.016).[6] EOGBS incidence is estimated at 0.43 per 1,000 livebirths (95% CI 0.37 to 0.49), double the incidence of LOGBS (0.24).[6] EOGBS incidence is the highest for Africa and lowest for Southeast Asia (see Table 1).[6]

Table 1. Incidence of early onset group B *Streptococcus* per 1000 livebirths by region[6]

Region	Incidence of early onset group B *Streptococcus*
Africa	0.53 (0.15 to 0.92)
Americas	0.50 (0.43 to 0.57)
Europe	0.45 (0.34 to 0.56)
Southeast Asia	0.11 (0.012 to 0.220)

The burden of GBS differs by country. In the US, multi-state, population-based active surveillance reported that the incidence of EOGBS per 1,000 livebirths was 1.7 per 1,000 livebirths in the early 1990s but is now around 0.23 per 1,000 livebirths in 2015.[14, 57] It is important to note that these estimates are of culture-proven EOGBS and although this is the primary and standard outcome of interest, the more recent figures during periods of antibiotic prevention are likely to underestimate the true burden of the EOGBS due to antibiotics given

to women. The presence of the antibiotics in the blood of neonates could prevent the isolation of GBS from neonatal blood in the laboratory, despite the organism being present. LOGBS rates from the 1990s until 2015 has remained between 0.3 and 0.4 per 1,000 livebirths.[14] In the 1990s, it was estimated that approximately 7600 cases of GBS cases occurred in the US, with 310 deaths.[3] In Alberta, Canada, EOGBS increased from 0.15 per 1,000 livebirths in 2003 to 0.34 per 1,000 livebirths in 2013.[58] In Europe before 2000, the EOGBS incidence per 1,000 livebirths was 3.35 in Czech Republic,[59] 0.2 to 0.3 in Denmark,[60] 0.6 to 0.7 in Finland,[61, 62] 0.69 to 4.5 in France,[63, 64] 0.11 to 0.54 in the Netherlands,[65, 66] 0.46 in Norway,[67] 2.4 in Spain,[68] and 5.4 in Vienna.[69, 1] Since then, EOGBS incidence rates have been 1.96 per 1,000 livebirths in Czech Republic,[59] 0.19 in the Netherlands,[66] 0.46 in Norway,[70] 0.33 in Spain,[68] and 0.47 in Germany[71] In Australia and New Zealand, the incidence of EOGBS was 1.43 per 1,000 livebirths in 1993, which reduced to 0.25 in 2001.[72] The lower incidence rates reported more recently are attributed to prevention and treatment strategies.

In Africa, a recent meta-analysis estimated a high EOGBS incidence of 1.3 per 1,000 livebirths (95% CI 0.81 to 1.90) despite methodological limitations that would lead to an underestimation.[73] A small recent study of 500 women in Nigeria reported an incidence of 2.00 per 1,000 livebirths, while, in Kenya, a study of 7,967 women reported a rate of 0.76 per 1,000 livebirths in the hospital, but a reduced rate of 0.13 using a population denominator.[41] As indicated, the incidence of EOGBS is relatively lower in Asian countries, for example, a recent study in Japan, reported a rate of 0.09 per 1,000 livebirths.[54] In Malaysia, the EOGBS incidence per 1,000 livebirths was reported at 0.26, in India 0.15 to 0.17 and in Bangladesh 0.10.[74] In Thailand, EOGBS incidence was 0.27 per 1,000 livebirths in 1996 but 0.10 in 2001.[75] Across Latin America, in Brazil, EOGBS incidence was reported at 0.39 to 1.0 per 1,000 livebirths and recently 0.90 per 1,000 livebirths.[76] In Mexico, it was reported at 0.60 per 1,000 livebirths.[74]

In the UK, single centre studies in England reported neonatal GBS incidence rates of 0.5 to 1.4 per 1,000 livebirths through the 1990s.[77-79] In 2000/01, enhanced surveillance across the UK and the Republic of Ireland reported a neonatal GBS incidence of 0.72 per 1,000 livebirths (568 cases), EOGBS incidence of 0.48 per 1,000 livebirths (377 cases) and LOGBS incidence of 0.24 per 1,000 livebirths (191 cases).[80] The enhanced surveillance was reproduced for 2014/15 and the authors found an increase in the GBS burden. The neonatal GBS incidence across all five countries in the British Isles was 0.94 per 1,000 livebirths (856 cases), EOGBS incidence was 0.57 per 1,000 livebirths (518 cases) and LOGBS incidence was 0.37 per 1,000 livebirths (339 cases).[5] EOGBS incidence in the latest survey varied slightly across the different countries with the lowest incidence in the Republic of Ireland (0.45 per 1,000

livebirths) and the highest in Northern Ireland (0.64 per 1,000 livebirths). The incidence of both EOGBS and LOGBS has increased from the 2000/01 survey with the biggest increase observed in Scotland (from 0.21 to 0.49 per 1,000 livebirths). While these are the best available data in the UK, the enhanced surveillance studies were only at two points in time (14 years apart), thus, it is not clear how the incidences might fluctuate year by year. The authors of the surveys have also mentioned that the observed increase in the EOGBS incidence might be, in part, due to technical improvements in the increased awareness of neonatal GBS, increased case ascertainment and bacterial culturing practices. Nevertheless, voluntary annual surveillance collected by Public Health England between 2000 and 2010 has also shown that EOGBS incidence increased from 0.28 to 0.41 per 1000 livebirths and LOGBS increased from 0.11 to 0.29 per 1,000 livebirths.[81] Again, the limitations of focussing the surveillance to culture-proven EOGBS may underestimate reported incidences.

The global mortality rate of neonatal GBS disease has been estimated at 9.6% (95% CI 7.5 to 11.8%), EOGBS at 12.1% and LOGBS at 6.8%.[6] Case fatality of neonatal GBS was three times higher in low-income countries at 12.6% compared with high income countries at 4.6%.[6] Case fatality rates of EOGBS have been reported at around 4.7% in the US,[82], 8% in Finland,[61] 4.3% in Germany,[71] 6.6% in Portugal[83] and 4.5% in Japan.[54] In the UK, the enhanced surveillance study found a case fatality rate of 5.2%; a statistically significant decrease from 10.6% in 2000/01 (p=0.01).[5] Data from MBRRACE-UK reported that, in 2014, there were 17 GBS-related neonatal deaths within 7 days of life, 13 of whom had GBS as a primary cause of death and four who had GBS as a co-factor of death among 777,764 livebirths. This equates to a rate of 2.2 per 100,000 livebirths and 1.72% (17/991) of all early neonatal deaths.[84]

Related to GBS mortality is the burden of GBS related stillbirths, which has been relatively less researched. A systematic review searching the literature up to 2015, concluded that the incidence of GBS related stillbirths varied substantially between 0.04 to 0.9 per 1,000 births between studies.[85] The proportion of stillbirths associated with GBS varied between zero to 12.1% across studies. In the UK, data from MBRRACE for 2014 identified 31 GBS related stillbirths among 780,979 total births corresponding to a rate of 0.04 per 1,000 total births and 0.96% of all stillbirths.[84] GBS was reported as the primary cause of death in 24 cases and as a co-factor of death in seven cases.

2.3.2 Risk factors

There are a number of maternal, obstetric and neonatal risk factors that increase the risk of EOGBS disease. Some of these factors are well-defined and provide an important

understanding into the development of EOGBS. Other factors are not clear due to contradictory results in the literature and the interrelated relationships between them, making them less helpful to direct clinical management.[3] It has been well-established and internationally reported that babies born preterm and colonised with GBS are at an increased risk of developing EOGBS as their immune systems are immature.[35, 3, 6, 86, 87] Daniels *et al.* (2009) report that the pooled incidence of EOGBS disease from five studies showed that 40% of cases were preterm, which gave a 5.5 times higher risk for preterm compared with term neonates.[35] In a case-control study, the odds of having GBS for preterm neonates was 10.4 (95% CI 3.9 to 27.6).[88] Indeed, the recent enhanced surveillance in England and Wales reported that EOGBS incidence was inversely associated with gestational age at birth decreasing from 4.42 per 1,000 livebirths before 28 weeks to 0.41 per 1,000 livebirths after 37 weeks of gestation (see Table 2).[5] Overall, 21.9% of EOGBS were preterm (<37 weeks). Prematurity was also an independent risk factor for EOGBS mortality. Case fatality decreased from 47.1% in neonates born before 28 weeks of gestation to 2.8% in neonates born after 27 weeks (see Table 2).[5] Low birthweight, related to prematurity, is also inversely related to EOGBS incidence.[87] For example, EOGBS incidence in the enhanced surveillance was 2.24 per 1,000 livebirths in neonates weighing less than 1500g, 1.17 in neonates 1500 to 2499g and 0.43 in neonates born 2500g and above.[5]

Table 2. EOGBS incidence and mortality by gestational age at birth in England and Wales[5]

Gestational weeks	EOGBS cases	EOGBS incidence per 1,000 livebirths (95% CI)	Number of EOGBS deaths	Case fatality rate
<28	14	4.42 (2.42 to 7.40)	8/17	47.1%
28-36	68	1.27 (0.99 to 1.61)	7/77	9.1%
≥37	283	0.41 (0.36 to 0.46)	9/321	2.8%
All	343	0.46 (0.41 to 0.51)	24/415	5.8%

EOGBS early-onset GBS, GBS group B *Streptococcus*

Vaginal GBS colonisation has been identified as an important risk factor for EOGBS. In one study, GBS colonised women had a 29 times higher likelihood of EOGBS compared with women who were not colonised.[89] In a case-control study where birthweight and birth time were controlled for, Heath *et al.* (2009) reported that compared with uncolonised mothers, vaginal colonisation increased the risk of GBS by OR 8.47 (95% CI 3.73 to 19.22) (74% EOGBS).[88] In the study, it was not clear at what point GBS colonisation was assessed and this is important as GBS colonisation can be transient and intermittent. Nevertheless, GBS colonisation remained a statistically significant risk factor in the multivariable analysis (odds ratio [OR] 6.88, 95% CI 2.77 to 17.1). Heavier GBS colonisation, in particular, has been more strongly associated with EOGBS than light colonisation.[90, 91]

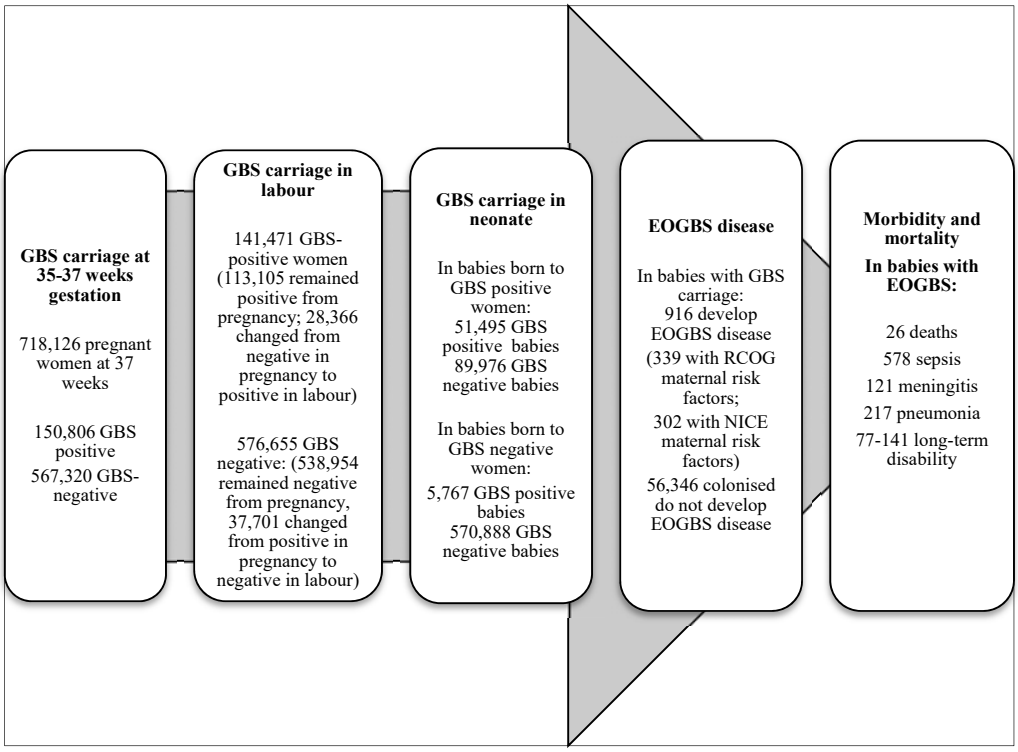
Another important risk-factor for EOGBS is the premature or prolonged rupture of membranes. It is argued that the prolonged rupture of membranes increases the risk of GBS colonisation and disease by increasing the opportunity and likelihood of GBS infection ascending in utero.[35, 92] However, it is also argued that GBS may be causing the prolonged rupture of membranes.[35, 92] Recently, Surve *et al.* (2016) demonstrated in a rat model that, GBS produces membrane vesicles, which lead to chorioamnionitis and damage to the membrane resulting in preterm birth or fetal death.[93] The rupture of membranes for more than 18 hours prior to delivery has been associated with a 25.8 (95% CI 10.2 to 64.8) times higher risk of EOGBS in one study.[94] However, in the case-control study by Heath *et al.* (2009), prolonged rupture of membranes (≥ 18 hours) had an OR of 2.69 (95% CI 1.67 to 4.34) for GBS disease in the unadjusted analysis, which lost statistical significance in the multivariable analysis (OR 1.82, 95% CI 0.99 to 3.35).[88] The latest enhanced survey found that 31.7% of EOGBS cases had prolonged rupture of membranes (≥ 18 hours, term and preterm), 9.6% had preterm prolonged rupture of membranes (≥ 18 hours) and 11.4% had preterm, pre-labour rupture of membranes.[5] The current understanding of prolonged rupture of membranes is limited, especially the differences in the association of preterm, term and preterm pre-labour rupture of membranes with GBS.[95]

Neonates born to mothers suffering from intrapartum fever or pyrexia are also at an increased risk. In their case-control study, Heath *et al.* found that intrapartum fever was associated with GBS even in multivariable analyses (OR 2.16 for every °C increase in maximum temperature, 95% CI 1.32 to 3.53).[88] In the recent survey, 19.3% of EOGBS cases had intrapartum fever. In the US, a case-control study, matched for gestational age, also found that intrapartum fever increased the odds of EOGBS (OR 4.1, 95% CI 1.2 to 13.4).[96]

In addition to these key risk factors, Heath *et al.* (2009) found that women having their first baby, GBS bacteriuria during pregnancy, an epidural during labour, having one or more vaginal examinations during labour, emergency intervention during labour, infection after delivery, foetal tachycardia, foetal distress, foetal blood sampling during labour or maternal post-delivery antibiotics also increased the risk of EOGBS in univariable analyses.[88] In the multivariable analysis, besides maternal GBS colonisation and intrapartum fever, maternal infection after delivery also remained statistically significant (OR 4.17, 95% CI 1.12 to 15.4). Other studies have similarly found that black ethnicity, teenage maternal age, and history of miscarriage increased the risk of EOGBS.[97, 98] However, when birthweight is controlled for, these factors do not remain statistically significant, probably as a result of the association between low birthweight and these factors.[88, 96]

Despite these associations, there have been varied results on how prevalent the risk factors are or how many EOGBS cases have risk factors. An observational study found that among GBS cases, 67% had at least one risk factor and 44% had two or more risk factors.[99] Another study, in Rome, found that only 17.4% of GBS cases had risk factors,[100] and a UK HTA study found no association between the presence of a risk factor and neonatal colonisation.[35] In the US, risk factors were found in 49% of EOGBS cases.[96] In the most recent enhanced UK survey it was found that, depending on which risk factors were considered, there were 59% to 65% of EOGBS cases whose mothers had no risk factors and 52% to 63% of EOGBS deaths had no risk factors.[5]

One systematic review has explored country-level determinants of neonatal GBS disease.[6] The authors found that studies having less than 20% of infants with GBS who had low birthweight decreased the odds of GBS infection compared with 20 to 39% (OR 0.51 9% CI 0.32 to 0.81). Countries with low gross national income had higher odds of GBS compared with high gross national income, however, this was not statistically significant. Intrapartum antibiotic prophylaxis use, skilled attendance at delivery, site of delivery and specimen type were not related to neonatal GBS disease. In the analysis on EOGBS alone, no use of prophylaxis had 2.20 times higher odds of EOGBS compared with those that did (95% CI 1.59 to 3.40). The incidence of EOGBS was 0.23 per 1,000 livebirths in studies with any intrapartum antibiotic prophylaxis compared with 0.75 per 1000 livebirths in studies with no intrapartum antibiotic prophylaxis.



GBS Group B *Streptococcus*, EOGBS early-onset GBS, NICE National Institute for Health and Care Excellence, NPV Negative predictive value, PPV Positive predictive value, RCOG Royal College of Obstetricians and Gynaecologists

a. Term pregnant women available for screening at 35-37 weeks: cohort based on 776,352 livebirths in the UK in 2014 (Office for National Statistics [ONS], 2015).[101] Of all livebirths, 7.5% delivered < 37 weeks (applied from England and Wales ONS, 2015)[102] and removed from cohort. Assumes no stillbirth, multiple births or miscarriages in third trimester.
b. 21% GBS maternal carriage in the UK.[31]
c. Carriage in labour: Estimates were approximately 60.6%, 78.6% & 67.4% for PPV, and 96.7%, 93.5% & 89.5% for NPV.[103-106] An average estimate of PPV of 75% and NPV of 95% was used.
d. GBS colonisation in neonates: 36.4% GBS transmission from women positive for GBS intrapartum;[4] 1% from uncolonised women.[35]
e. EOGBS disease based on transition rate from neonatal colonisation to EOGBS disease: 1.6% colonised neonates taken as the most appropriate.[4] Three percent gives an estimate of 1,718 cases (2.4/1000), which is almost five times the number of cases seen in the UK in all pregnancies, therefore the lower confidence interval from the meta-analysis is used instead.
f. Maternal risk factors: 37% had at least one RCOG risk factor; 33% had at least one risk factor based on NICE guidelines.[5]
g. Mortality in this review: 2.8% taken as most appropriate.[5]
h. Short-term morbidity in this review: Meningitis: 13.2%; Sepsis 63.1%; Pneumonia 23.7%[5]
i. Long-term disability in this review: 8.7-15.8% of surviving EOGBS cases.[7, 8]

Figure 1. Natural history of GBS on a hypothetical cohort of 718,126 term pregnant women

2.4 Detection of maternal GBS colonisation

The most frequently used and recommended method for detecting maternal GBS colonisation is bacterial culturing as it is considered the definitive approach.[28, 107] Bacterial culturing involves taking swabs from pregnant women and plating them on blood agar plates, where GBS grows forming white colonies surrounded by β-haemolysis areas if a woman is colonised with GBS, followed by tests to confirm the identity of GBS.[35] If GBS colonies grow, the woman would be diagnosed as positive for GBS colonisation and if they are absent, the woman would be diagnosed as negative.

The site of swab, timing of swab and use of selective enriched media can vary the sensitivity of the detection of GBS colonisation.[86] The currently recommended test in the UK is a selective enriched culture of rectovaginal swabs.[107] The recommendation for swabbing sites are rectal and vaginal as studies have found that they are more sensitive than other sites or one of these sites alone, increasing detection by 40%.[28] Using a selective enrichment broth before plating compared with standard plating can also increase the isolation of GBS by inhibiting the growth of competing organisms. Although its necessity has been questioned,[35] there is evidence showing that selective enrichment can increase detection by 50%.[108] The most widely used enrichment media is Lim broth with Todd-Hewitt base, nalidixic acid and colistin before plating on blood agar.[35] Finally, as culture tests take 24 to 48 hours to process, culture testing cannot be offered at the point of treatment in labour as results would not be available in time to treat. Thirty-five to 37 weeks has been selected as the time to test for GBS using culture, in order to balance the changes in colonisation status with sufficient time to obtain results. Therefore, culture testing only detects babies born at term or nearly at term, missing the majority of preterm pregnancies, who have a higher incidence, morbidity and mortality of EOGBS.

Studies investigating the test accuracy of selective enriched GBS culture focus on the positive predictive value (PPV) of culture at 35 to 37 weeks to detect maternal GBS carriage at birth; however, the outcome of interest in a prevention programme would be EOGBS. Using the number of EOGBS cases from the enhanced survey and the number of term pregnancies from the Office for National Statistics,[5, 101, 102] in the 2016 GBS NSC review, I calculated the positive predictive value for antenatal culture detecting EOGBS. If enriched culture testing at 35 to 37 weeks was a perfect test with 100% uptake, the PPV of the test would be 0.2% (350/150,806) for detecting EOGBS in the neonate.[24] The incidence of EOGBS in this study was during a period of intrapartum antibiotic prophylaxis prevention and this could underestimate the PPV. The PPV of selective enriched culture testing at 35 to 37 weeks

detecting EOGBS can also be calculated by combining studies which estimate each point in the natural history pathway from Figure 1. In 150,806 women colonised in the third trimester (21% of 718,126), 659 neonates would go on to develop EOGBS without treatment. This would increase the PPV to around 0.4% (659/150,806). This figure is based on the best available data for each point in the natural history pathway, however, as noted above, it does contain uncertainties. Furthermore, this rate does not account for the proportion of multiple births, nor does it remove elective caesarean sections or women with maternal risk factors who would not benefit from the screening test. With these PPVs, around 99.6% or 99.8% of the women positive for GBS colonisation at 35 to 37 weeks would be over-detected.

There are no agreed test accuracy values for antenatal selective enriched culture detecting maternal GBS colonisation at birth, and a range of values have been reported. A systematic review found that the ability of a selective rectovaginal culture at 35 to 37 weeks to predict maternal GBS colonisation at birth using rectovaginal culture as the reference standard was reported in two prospective studies.[104] The reported PPVs were 67.4%[103] and 78.6%,[106] and the negative predictive values (NPVs) were 96.7%[103] and 93.5%.[106] Recent but small studies show similar PPVs of 71.7%,[109] 72.8%,[110] 77.0%[111] and 89.1%[112] and NPVs of 91.2%[112] and 94.9%.[109] To summarise the varying ranges, approximately 20 to 30% of pregnant women who test positive for GBS at 35 to 37 weeks test negative during labour. The largest study since the systematic review looking at the accuracy of culture in routine clinical practice in the US reported a lower PPV of 60.6% and an NPV of 89.5%.[105] It is difficult to ascertain the reason for the change in colonisation status between 35 to 37 weeks and birth as it could be a result of false test results at 35 to 37 weeks or because of natural fluctuations in GBS colonisation during late pregnancy.

Due to the restrictions in culture methods, rapid tests have been developed that can be administered during labour and can include women in preterm labour. Of all the available rapid tests, real-time polymerase chain reaction (PCR) testing is the most promising.[35] In real-time PCR, after taking the rectovaginal swab (enriched or standard), the DNA is extracted by targeting specific areas of the bacterial chromosome by primers. The DNA then undergoes logarithmic iterative amplification to identify whether there is any evidence of GBS DNA.[35] In older real-time PCR tests, first the swab has to be prepared by extracting the DNA from it and adding the primer and polymerase. To ensure biases and false results are avoided, positive and negative controls for each kit are also prepared.[113, 35] All three samples are then placed into the real-time PCR machine where the target DNA for GBS is amplified and the presence of GBS is analysed from fluorescent signals.[113, 35] More recently, the GeneXpert GBS PCR (Cepheid) has automated the process of DNA extraction, amplification and detection. The

rectovaginal swab is inserted into a single-use cartridge that contains the PCR reagents and controls which goes into the machine to process and analyse.[114-116] The results for any of the real-time PCR tests present as negative, positive or inhibitory (inconclusive and patient needs to be re-tested). The average time to complete a real-time PCR test is 40 to 50 minutes.[116, 117]

An early systematic review of real-time PCR studies from 1996 to 2005 found a median sensitivity of 96% and specificity of 98% across two studies using intrapartum culture as reference standards.[117] Since then, compared with intrapartum culture, reports of real-time PCR sensitivity have ranged between 62.5% and 100%, specificity between 84.6% and 100%, PPVs between 65% and 100% and NPVs between 92.3% and 100%.[118] A literature review in 2015 specifically on GeneXpert GBS compared with intrapartum culture, found eight studies reporting good sensitivity of the test, which was superior to antenatal culture.[116] Sensitivity ranged between 83.3% and 98.5%, specificity between 64.5% and 99.6%, PPVs between from 90.9% and 97.8% and NPVs between 95.2% and 99.7%. Since the reviews, studies in France, Brazil and Demark have similarly found that real-time PCR had better test accuracy than antenatal culture when intrapartum culture was used as the reference standard.[119-121] The largest of the three studies, on 902 women in Denmark, reported a sensitivity of real-time PCR of 83%, specificity of 97%, PPV of 78% and NPV of 98%.[120] Again, the outcome of interest in a GBS prevention programme is EOGBS although real-time PCR studies use intrapartum culture as the reference standard.

There are some practical limitations of real-time PCR that restricts their use. Firstly, real-time PCR is much more expensive than enriched selective culture. A cost-effectiveness analysis reported that real-time PCR was less cost-effective compared with culture,[122] although this was an older version of real-time PCR and studies are needed on the latest available technology. In addition, the test needs to be simple for it to be administered and interpreted by midwives or nurses and would require training and additional support. Quality assurance of point of care tests remains an important issue. One of the studies investigating PCR was operated by midwives and found a sensitivity of 85%, specificity of 96.5%, PPV of 85.7% and NPV of 96.3% compared with intrapartum culture, with no difference between the performance of antenatal culture at 35 to 37 weeks and real-time PCR.[123] In addition to these feasibility concerns, real-time PCR cannot determine antibiotic sensitivity which directs the choice of antibiotic to use for treating women who are allergic to penicillin.[124] Culture testing is currently the only method to determine antibiotic susceptibility and, as indicated earlier, culture testing cannot be performed with sufficient time to direct antibiotic selection if GBS colonisation was only identified by real-time PCR in labour. It is estimated that approximately 9% of women, identified as culture-positive for GBS, are allergic to penicillin.[125] Without

testing antibiotic sensitivity, these women and their neonates could be put at risk making culture methods a compulsory pathway for them. Therefore, even though guidelines have incorporated rapid testing into the maternal GBS colonisation testing guidelines,[118, 14] this is in addition to antenatal culture testing, which remains the main recommendation and is most frequently utilised. Real-time PCR is not currently recommended in the UK.[116, 15]

2.5 Intrapartum antibiotic prophylaxis for EOGBS prevention

With no vaccination currently available, the widely recommended prevention for EOGBS is intrapartum antibiotic prophylaxis (IAP).[3, 1] GBS vaccination is the most promising prevention strategy for GBS disease as it would not only prevent EOGBS, but also LOGBS, and possibly miscarriage, stillbirths and preterm delivery related to GBS and have greater feasibility in low-resource settings.[27] Advances have been made in the development of a GBS vaccine, however, they are still being trialled and regulatory licence for a vaccination is eagerly awaited.[27, 3, 126]

IAP treatment involves administering IAP to eligible mothers at risk of vertical GBS transmission in order to prevent GBS vertical transmission and EOGBS disease. Giving IAP to at-risk mothers was first demonstrated to be effective in reducing EOGBS in 1986.[127] The current recommendation for IAP is intravenous penicillin (or ampicillin in the US) given as soon as possible after the onset of labour and then, every four hours until delivery. Second-line treatment for mothers allergic to penicillin varied across countries.[13, 14] In the UK, until September 2017, intravenous clindamycin was recommended.[13] However, in the latest guideline published in September 2017, the recommendations have been modified due to the evidence of increasing clindamycin resistance.[15] If a woman has a history of allergy to beta-lactams that is not severe, i.e. does not have a history of anaphylaxis, angioedema, respiratory distress or urticaria, a cephalosporin is recommended. If a woman has a history of severe allergy to beta-lactams, vancomycin is recommended instead. Similarly, in the US since 2010, intravenous Cefazolin is the first alternative, followed by clindamycin if there is a history of anaphylaxis, respiratory distress, urticaria or angioedema after penicillin or cephalosporin.[14]

A Cochrane review of three randomised controlled trials (RCTs) assessing the effect of IAP found that IAP substantially reduced the incidence of EOGBS (risk ratio [RR] 0.17, 95% CI 0.04 to 0.74) and the incidence of probable EOGBS (RR 0.17, 95% CI: 0.03 to 0.91) by around 83%, compared with no treatment.[10] IAP did not reduce the incidence of all-cause mortality, mortality from GBS infection or mortality from other bacteria. Due to the high risk of bias

identified in the three small RCTs more than 20 years ago, the authors concluded that there was no valid information to inform clinical practice. While this is the best available evidence, findings from two observational studies have suggested that the timing and duration of IAP had an impact on its effectiveness, with rates of EOGBS and clinical sepsis higher in mothers who received IAP for less than 4 hours compared with those who received it for four or more hours.[128, 129] Furthermore, patients who received substandard IAP of clindamycin due to reported penicillin-allergy also showed a reduced effectiveness of IAP. [128] As the studies were observational, the results may be due to bias from detection bias and confounding variables. Better quality evidence is required to address the effectiveness of IAP, although RCTs may not be feasible when IAP has become the recommended treatment.

2.6 EOGBS prevention strategies

Different strategies are used across countries to identify eligible women at risk of having a baby with EOGBS in order to treat them with IAP. One strategy is risk-based prevention, whereby women who present with risk factors such as those identified in Section 2.3.2, are offered IAP in labour.[35, 1] As GBS maternal carriage is a pre-requisite for EOGBS disease, an alternative strategy is universal antenatal screening. Screening principally involves using the selective enriched culture test of recto-vaginal swabs from all pregnant women at 35 to 37 weeks and offering IAP to those with positive results.[35, 1] If feasible, national guidelines have suggested the use of rapid testing.[14, 118] It is important to note, that in most cases, screening is applied in addition to risk-based prevention. Therefore, women with antepartum or intrapartum risk factors would be offered IAP irrespective of screening results.[14] As mentioned above, screening at 35 to 37 weeks would miss the majority of preterm births who have a higher burden of GBS disease. For these women or women with missing results,[14] IAP is either offered automatically or based on other risk-factors.[35]

A third strategy is a combination of the risk-based prevention and universal screening, whereby women are tested in pregnancy and only if they then present with a risk factor in labour are they offered IAP. If there is no risk factor present, IAP is not offered.[35] In Australia, there is one national guideline stating either a risk-based or screening prevention strategy be implemented across hospitals and a review indicated that across the country different strategies are implemented.[130]

2.6.1 Risk-based GBS prevention strategy

In the UK, risk-based prevention was first introduced in 2001 by the GBS Working Group of the Public Health Laboratory Service.[131] The Royal College of Obstetricians and Gynaecologists (RCOG) endorsed the recommendations in 2003, 2012 and most recently in September 2017.[13, 15] In 2012, the National Institute for Health and Clinical Excellence (NICE) developed their guidelines for intrapartum antibiotics also following a risk-based strategy.[12] Women presenting with intrapartum fever, incidental GBS colonisation. GBS bacteriuria, a previous baby with invasive GBS disease or chorioamnionitis are offered IAP.[12, 13] Previously, IAP was not recommended for pre-labour rupture of membranes in term or preterm mothers or for preterm labour,[12, 13] although NICE guidelines did suggest considering IAP for preterm pre-labour and prolonged rupture of membranes but not for term rupture of membranes or preterm birth alone.[12] In the most recent RCOG guidelines in 2017, IAP is now recommended for women in confirmed preterm labour.[15] In addition, if GBS was detected in a previous pregnancy, enriched selective culture testing is recommended in late pregnancy with IAP offered if the result is positive.[15]

The Netherlands,[66] New Zealand,[132, 133] Denmark,[134] Norway[135] and Sweden[136] all recommend risk-based prevention. However, guidelines in New Zealand, Sweden and Denmark state that women with preterm labour with evidence of imminent labour and women with prolonged rupture of membranes should also be offered IAP.[133, 134, 137] In the Netherlands, if women present with preterm labour or prolonged rupture of membranes, a culture is taken and IAP is offered if the results are positive.[138]

There are currently no high-quality studies on the effectiveness of risk-based prevention. Population surveillance shows that in the UK EOGBS incidence appears to have increased under risk-based prevention from 0.48 in 2000/01 to 0.57 per 1,000 livebirths in 2014/15, although mortality has decreased from 10% to 5%.[5] A criticism of the risk-based prevention strategy is that approximately 30 to 40% of cases without risk factors are excluded from prevention. The enhanced survey found that the 2012 national GBS prevention guidelines provided by the RCOG and NICE, which are based on the presence of at least one clinical risk factor, only identified 35.4% to 41.3% of UK and Irish EOGBS cases.[5] Only 44% of those women with RCOG risk factors were then treated with IAP; 50% received IAP for less than two hours and only 25% received IAP for at least four hours. Among EOGBS deaths, 37% had at least one RCOG risk factor for IAP and 48% had at least one NICE risk factor. Of the EOGBS deaths, only one woman had received IAP in labour. The percentage of EOGBS cases born at term with no RCOG or NICE risk factors was 63% and 67% respectively. Similarly,

60% to 70% of 10 EOGBS deaths in neonates born after 35 weeks had no NICE or RCOG risk factors respectively, while 56% to 67% of nine EOGBS deaths in neonates born after 37 weeks of gestation had no risk factors.[5] These are the EOGBS morbidities and mortalities that the current risk-based strategy would not be able to prevent. Similarly, in the Netherlands EOGBS has increased from 0.10 per 1,000 livebirths in 1987 to 0.19 per 1,000 livebirths in 2011, though it is not clear whether this could be an increase in reporting[66] and another study reported a decrease from 0.54 in 1997/98 to 0.36 per 1,000 livebirths in 1999 to 2001.[65]

By contrast, in New Zealand, the incidence of EOGBS has more than halved under risk-based prevention from 0.5 per 1,000 livebirths in 1998/99[139] to 0.23 per 1,000 livebirths in 2009 to 2011.[140] In Denmark, there was a decrease of EOGBS incidence from 2.27 per 1,000 livebirths in 2002 to 1.30 per 1,000 livebirths in 2010.[141] Similarly, in Sweden the incidence of EOGBS decreased from 0.40 per 1,000 livebirths in 2006 to 2008 to 0.30 per 1,00 livebirths in 2009 to 2011,[136] while in Norway it has remained steady.[70] It is possible that the difference in trends between the UK and the Netherlands and the remaining countries are a result of treating preterm births with EOGBS. Preterm births have an increasing burden of GBS and it can be hypothesised that treating all could have a substantial impact. Now that the RCOG guidelines recommend treating all preterm births, the impact this has on EOGBS incidence in the UK may shed some light on the differences.

While the trends of EOGBS are informative about the effects of risk-based prevention on EOGBS incidence, they are observational in nature, which means it is possible that they are a result of factors within or beyond the prevention programme. For example, there could be differences in GBS awareness, adherence to guidelines or the management of neonates across time that could contribute to the trends. The low adherence to the risk-based prevention policy in the UK makes it difficult to identify its impact, particularly as the reasons for the low proportion of women with indication receiving IAP, are not known. As there were no contemporaneous control groups, it is not known how the EOGBS rates would have fluctuated over time without risk-based prevention.

2.6.2 Universal GBS screening

Screening as a concept

An alternative strategy to increase the detection of GBS in pregnant women is universal antenatal screening. Before discussing the adoption and effectiveness of universal GBS screening, it is important to understand health screening as a concept and prevention

programme. Health screening is a public health service in which members of a defined population who do not perceive that they are at risk of a disease are offered a test to identify individuals who can be diagnosed in a timely way and treated primarily to reduce the risk of mortality and severe morbidity from the disease, via early diagnosis.[142-144] While screening is favoured as a valuable tool in disease prevention, since the 1960s there has been a recognition that all screening programmes cause harms. The potential benefits of screening are better prognosis and less radical treatment for some individuals, reassurance for others and more cost-effective care. The potential harms from screening include hazards of the screening and diagnostic tests, side effects from the treatment, anxiety, time and financial burden to the patient, false reassurance and the opportunity cost of spending resources on a screening programme instead of other services.[145] For individuals with false positive results, the anxiety and complications from testing would be unnecessary as they were not actually at risk of the disease. Similarly, screening can also cause over-diagnosis and overtreatment, whereby individuals are true positives for latent conditions, however, these latent conditions would never become symptomatic. Again, the testing and treatment could be invasive, cause severe adverse physical and psychological harm and would be unnecessary.[146, 142, 144] Many of these harms would also be concealed once a screening programme is implemented.

To ensure that benefits of screening outweigh the harms to an asymptomatic population, it is essential to undertake a robust evaluation of screening programmes prior to implementation.[144] To do so systematically, Wilson and Jungner developed 10 criteria to assess screening programmes for any disease.[147] The criteria were: important condition, with a recognisable latent phase, whose natural history is known, available and acceptable treatment, available facilities, a suitable and acceptable test, agreed policy on whom to treat as patients and screening to be cost-effective and continual. Policy-makers and academics in different countries have accepted these criteria and modified them to incorporate evidence-based healthcare, quality assurance, consumer choice, the advent of genetic screening and accountability.[148] For example, the NSC had developed these principles into a 22-item list of criteria,[22] which they have now modified into a 20-item list.[149] It is not currently known which criteria policy-makers use within other countries or how they are evaluated.

Equally, there has been limited discussion about the systems or structures responsible for screening within countries. For criteria to be valuable, countries need to have screening systems that encourage the implementation of evidence-based screening decisions. There has been limited discourse on the optimum system to ensure screening care reflects evidence-based decisions. It has been proposed that there should be one national organisation to make national screening recommendations and decisions and to implement national screening

programmes.[142, 143, 150] However, the financing and structure of general health systems can affect the structure for screening.[148, 142, 150] Countries that have decentralised screening policies usually have general health systems that are decentralised, insurance-based, autonomous and less regulated. On the other hand, countries that have national agencies for the decision-making and implementation of screening are more likely to have tax-based general health systems and greater regulation.[151] In the UK, the NSC is nationally responsible for making screening recommendations and for implementing the recommended programmes. Within other countries, we do not know which organisations assess screening programmes for their introduction, decide whether or not to introduce screening programmes or implement screening programmes as a result.

Screening for GBS

Many countries internationally recommend and adopt universal GBS screening. The first national guidelines were recommended by the Centers for Disease Control and Prevention (CDC) in the US in 1996.[152] In this guideline, the CDC recommended that a risk-based strategy or universal screening be adopted. The CDC revised its guidelines in 2002 recommending universal screening only,[86] which was then reinforced in 2010.[14] After the CDC guidelines were published, many countries developed universal GBS screening guidelines.[130, 1] In the Americas, Canada and Argentina introduced screening guidelines.[153-155] Across Europe: Belgium,[156, 157] Czech Republic,[158, 59] France,[159] Germany,[160] Italy,[161] Poland,[162] Spain[163] and Switzerland[164] also reported screening guidelines, while in Asia: Hong Kong[165] and Japan[166] have reported screening guidelines.

In the UK, the NSC developed the screening policy for GBS by assessing the scientific evidence against the international screening criteria. They recommended against introducing universal GBS screening (see Section 2.6.5). In the other countries, it is not clear what the responsibility is of the organisations which made the policy for GBS screening within each country nor the processes used to make the policy. In the US, GBS screening guidelines were made by the CDC which is a public health institution whose goal is to protect health and safety by controlling and preventing disease. They developed guidelines in conjunction with experts from relevant disciplines and provided a report outlining an epidemiologic basis for prevention protocols, summarised results of clinical trials demonstrating IAP efficacy and examined the limitations of different prevention strategies. In 2002, the CDC stated that the recommendation was based on available evidence and expert opinion where evidence was lacking. Similarly, in 2010, clinical and public health representatives revaluated the 2002 guidelines on the basis of available evidence and expert opinion when evidence was

insufficient. We do not know whether there are organisations responsible for screening in the US or other countries that may have made GBS screening guidelines.

Clinical Effectiveness

As with risk-based prevention, there is only observational evidence investigating the effectiveness of universal GBS screening on morbidity and mortality. Without RCT evidence it is difficult to calculate the impact of screening due to confounding variables. In investigations of screening this is even more problematic due to the bias of the healthy screenee effect, where individuals who opt for screening are healthier and more likely to heed medical advice compared with those who do not opt for screening, as well as the problem of over-diagnosis and over-treatment, whereby individuals who might have been positive at 35 to 37 weeks would have never been at risk of having a neonate with EOGBS.[144] Efforts to undertake an RCT have been made, and whilst it would be possible, it would require a very large sample size. At the beginning of this research project, a previous RCT proposal had not been executed due to the large sample size requirements. The authors calculated that for an RCT in the UK a sample size of 540,000 pregnant women would be required,[167] which is approximately 70% of the annual UK birth population. RCTs in other developed countries (or the possibility of an international multicentre study) may also be difficult as the public in countries with universal screening might consider it unethical not to receive screening and would do so independently, contaminating the trial.

Nevertheless, there are increasing reports from several countries that have implemented universal screening. These observational studies compare EOGBS incidence during periods of universal screening to historical controls (no screening and/or risk-based strategy) that precede the universal screening periods. There is a high risk of bias from this kind of approach as data are collected retrospectively, participants in the study and the control period are not contemporaneous and confounding factors are not usually adequately considered. Consequently, the results could be due to other factors in the population that may have changed between the two periods of comparison, for example, the number of women with risk factors or the serotype distribution. Another limitation is that many studies report the rate of all EOGBS and not in term births alone, the population eligible for screening. Many of these preterm births would not have been screened and their reduction could be a result of routine IAP administration. Combining all of the results together blurs the effect of screening.

Since the US introduced IAP guidelines, EOGBS has decreased from 1.7 per 1000 livebirths during the 1990s to 0.23 in 2015.[57, 168] However, the largest decreases occurred during the

period in which both strategies were recommended, making it difficult to attribute all the decrease to universal screening. As an indication of guideline implementation, in 1997, 28% of hospitals across eight states adopted universal screening, 20% adopted risk-based strategies, 4% adopted both strategies, and 42% did not have a policy.[169] Recently, Schrag *et al.* (2016) reported a reduction in EOGBS sepsis incidence from 0.27 in 2005 to 0.22 in 2014 during screening recommendations (p=0.02).[87]

A systematic review in 2011 including eight observational studies from the US (four studies), Austria, Australia, Italy and Switzerland compared the rate of neonatal GBS sepsis before and after the introduction of universal screening.[16] The incidence of neonatal GBS sepsis was less common during universal screening compared with risk-based (OR 0.25, 95% CI 0.16 to 0.37) or no prevention (OR 0.43, 95% CI 0.25 to 0.73). However, this review has been heavily criticised. In addition to the limitations identified above, discrepancies have been detected in the data extracted from the included reviews, rendering the meta-analysis potentially unreliable.[23] It has also been noted that the review did not thoroughly consider clinical heterogeneity and differences between studies, such as the timing and method of screening, which can influence the validity of the meta-analysis.[23] Another systematic review in 2013 pooled nine studies from Turkey, Australia and the US (seven studies) comparing risk-based prevention to screening from 2000 to 2013. They also found that the odds of EOGBS under risk-based prevention were higher than under screening (OR 0.45, 95% CI 0.37 to 0.53). This review did analyse EOGBS in term births only (three studies, 27,630 livebirths) finding that the odds were still higher under risk-based prevention compared with universal screening (OR 0.45, 95% CI 0.36 to 0.57). While the heterogeneity as assessed by χ^2 (chi-squared) tests was adequate for meta-analysis, the influence of the heterogeneity was not further assessed in sensitivity analyses or meta-regression. Furthermore, most of the studies in the meta-analyses were observational cohort studies with no adjustment for confounding factors, therefore, the risks of potential healthy screenee bias and confounding variables still apply. Finally, although combining the data from the studies and finding consistent results across them strengthens the evidence, there were only two countries outside of America. As most of the data were from the same country, there is a risk that population differences within the US could be contributing to the result, questioning their generalisability.[17]

Since the systematic reviews, recent studies have consistently shown a decrease in EOGBS incidence during the era of universal screening compared with the era without any screening. However, the results are inconsistent when comparing screening with the era of a risk-based strategy. In the US, Bauserman *et al.* (2013) found lower odds of developing EOGBS (denominator admissions to neonatal intensive care units [NICUs]) during universal screening

compared with the risk-based or screening prevention period in an adjusted analysis (OR 0.69 95% CI 0.59-0.80). Factors adjusted for were gestational age, sex, race, inborn status, 5-minute Apgar, ventilator support on first postnatal day, prenatal steroid exposure, prenatal antibiotic exposure and mode of delivery. By contrast, Ecker *et al.* (2013) found that EOGBS incidence decreased after risk-based prevention (from 2.06 to 0.96 per 1,000 livebirths) but did not reduce further after the introduction of universal screening (1.11 per 1,000 livebirths).[170] In the UK, a study in one maternity unit found that EOGBS incidence fell from 0.99 per 1,000 livebirths in the risk-based period to 0.33 per 1,000 livebirths during the screening period; however, this did not reach statistical significance (p=0.08).[34] The authors acknowledged that several maternal characteristics were different between the two periods. Mothers during the screening period were older, a higher proportion had a caesarean section and white other ethnicity and a lower proportion were of black ethnicity. There was also a low rate of IAP in the carrier population. The authors suggested that factors beyond the screening programme may have influenced the reduction in EOGBS. In Hong Kong, Ma *et al.* (2017) found that EOGBS incidence did decrease in the era of universal screening (1 per 1,000 births) compared with the era of risk-based prevention (0.24 per 1,000 births, p<0.001).[171]

Findings on the impact of universal GBS screening on the mortality from EOGBS are also inconsistent. The two recent studies in the US found no change in EOGBS mortality between the eras with and without universal screening. Bauserman *et al.* (2013) found a case fatality rate of 4% in both periods while Ecker *et al.* (2013) found that the case fatality rate in all early-onset infections was 11.4% under no prevention, 15.5% under risk-based prevention and 13.6% under screening.[172, 170] By contrast, a study in Hungary reported decreased EOGBS mortality rates from 19.5% (29/149) to 1.6% (1/63) after the introduction of screening.[173]

A concern identified in these studies is their focus on culture-confirmed EOGBS, which may lead to the changes in EOGBS incidence that actually reflect a decrease in the likelihood of GBS being detected by culture due to IAP usage. As a result, this would overestimate the impact of GBS screening and widespread IAP. Internationally, there has been a suggestion for studies to explore all-cause early-onset sepsis, however, the results have been contradictory. One US study found that there was an average annual percent decrease in neonatal sepsis hospitalisations for term infants during 1996 to 2001 when risk-based prevention or universal screening were recommended (-3.6%, 95% CI -5.1 to 2.0%), and this did not change after 2002 when only universal screening was introduced.[174] Using a proxy for early-onset sepsis (term infants diagnosed with sepsis during delivery with admission and discharge within ten days of birth), they found no trend between 1988 and 2006. The first multi-state population study exploring the rates of neonatal sepsis in the US under universal screening found that

rates of early-onset sepsis have remained stable at around 0.76 to 0.77 per 1,000 livebirths.[175] Recently in Brazil, early-onset sepsis incidence decreased from 1.9 to 1.3 cases per 1,000 livebirths after the introduction of screening prevention compared with no prevention, however, this was not statistically significant.[176] Schrag *et al.* (2005) similarly found stable rates of early-onset sepsis in 2005 and 2014 at around 0.77 and 0.79, respectively.[87]

Recently, an expert group assembled by the UK NSC estimated the impact of culture-based screening in addition to risk-based prevention in the UK by developing a model of the best available evidence.[177] They estimated that 30,666 women would receive antibiotics in labour which would prevent 70 cases of EOGBS during risk-based prevention compared with no prevention. Under screening and risk-based prevention, a further 96,260 women would receive IAP and this would prevent an additional 52 to 57 cases of EOGBS, the prevention of three deaths and four cases of severe disability. With screening, an additional 1,675 to 1,854 women would have to receive IAP to prevent one case of EOGBS and 24,065 to 32,087 to prevent one death from EOGBS. This result is similar to Angstetra *et al.* (2007) who found that that 1,191 women would require IAP treatment to prevent one case of EOGBS (95% CI 813–3,272).[178] Although this model uses the best available evidence and expert input, there is a level of uncertainty because of the limitations and associated gaps in the evidence used to develop the model. These gaps, identified throughout this review, are around the natural history of GBS, the test accuracy of culture and the effectiveness of IAP.

Overall, there are increasing numbers of observational studies assessing the effectiveness of their screening programmes compared with their previous prevention strategies. However, these results are not only inconsistent across studies, they are also fraught with serious methodological limitations, which prevents the identification of the impact of universal GBS screening. RCT evidence is required to quantify the impact of screening on GBS outcomes, however, at the time of this thesis, RCT evidence was not looking promising due to feasibility and ethical issues.

2.6.3 Harms from widespread IAP treatment

The authors have raised concerns about the potential harms from the use of IAP and the increase of IAP under screening. However, the occurrence of harmful outcomes from IAP and their clinical significance has not been well explored. Firstly, widespread IAP could increase antibiotic resistance. GBS remains almost universally susceptible to penicillin,[14] although there are recent reports in Ethiopia and Italy with evidence of penicillin resistance in GBS isolated from pregnant women.[179-181] However, these are small studies and/or have

methodological limitations in their susceptibility testing methods, thus, larger studies with robust methods are needed to confirm this in pregnant women and neonates. By 2005 in the US, 0.2% of GBS isolates had reached the upper level of susceptibility for beta-lactams.[182, 18] A number of mutations in the bacterial cell wall have been associated with reduced penicillin susceptibility, but their clinical significance is not known. In Japan, 5% to 15% of GBS isolates are reported to have reduced penicillin susceptibility;[183] approximately half of these are susceptible under European breakpoints and came from populations where chronic antibiotic exposure is likely to be common (due to chronic respiratory disease). The clinical significance of increased minimum inhibitory concentrations close to breakpoint is uncertain.

Resistance to clindamycin and erythromycin has increased in the last 20 years.[81, 14] In countries adopting universal screening, reported rates of resistance have been higher compared with countries with risk-based prevention though no formal comparison has been performed. In the US where universal screening is adopted, EOGBS resistance to erythromycin was reported at 48% and resistance to clindamycin was reported at 27% in 2010,[57] whereas in 2010 in the UK, where risk-based prevention is adopted, erythromycin resistance was reported under 15% in EOGBS[81] and clindamycin resistance was reported at 9% though this included all GBS cases.[184] However, in Australia, where either risk-based prevention or universal screening is recommended, reported resistance rates in invasive GBS strains were low at 6.4% for erythromycin and 4.2% for clindamycin resistance, which did not increase between 1982 to 2001 and 2002 to 2006.[185] In 2014/15, invasive neonatal GBS isolates reached a resistance rate of 15.9% for clindamycin, 23% for erythromycin and 15.7% for both clindamycin and erythromycin in the UK (any pathogen resistant to clindamycin is expected to be resistant to erythromycin, but the converse is not necessarily true).[5] This was an increase from 2000/01, where there were 3% of neonatal GBS isolates resistant to clindamycin, 6% to erythromycin and 2% to both.[80] A recent meta-analysis found that most GBS isolates from pregnant women were susceptible to penicillin, ampicillin and vancomycin, while the pooled resistance to clindamycin and erythromycin was 27% and 25%, respectively.[186] Resistance rates for both clindamycin and erythromycin were highest in Asia (47% and 46% respectively) followed by America (20% and 14% respectively), Africa (18% and 14% respectively), Oceania (15% and 8% respectively) and Europe (11% for both). In general, most countries in Asia and Africa do not have GBS prevention programmes, therefore, it would seem that resistance patterns do not follow IAP prevention. However, the resistance patterns may instead reflect unregulated antibiotic use in these regions. Without knowing the prevention strategy in the specific centres in the meta-analysis, it is difficult to assess their impact. There may also be other factors besides screening and widespread IAP that could be contributing to the differences between regions and across time within countries.

Widespread IAP may also increase neonatal infections caused by gram-negative bacteria as a result of selection pressure on the organisms causing infection, though results are contradictory.[32, 19] *Escherichia coli* (*E. coli)* is the most common gram-negative bacterium causing invasive neonatal disease and there is evidence indicating that neonatal sepsis from *E. coli* may have increased since the introduction of GBS screening. Bizzarro *et al.* (2008) found that early-onset *E. coli* in very low preterm births (<1500g) increased from 2.83 per 1,000 admissions during no prevention to 7.12 per 1,000 admissions during risk-based prevention to 10.22 per 1,000 admissions during screening in the US.[187] Similarly, Stoll *et al.* (2002) reported that the rate of early-onset *E. coli* infections increased from 3.2 to 6.8 per 1000 livebirths over time in low birthweight infants between 1998 to 2000 in the US. Other studies have found that IAP increased the odds of early-onset *E. coli* infection in univariable analyses but not in multivariable analyses when other factors, such as gestational age, were accounted for.[188, 189] On the other hand, more recent studies have found that early-onset *E. coli* has remained stable from the period of 'either prevention' to the period of screening at 1.4 per 1,000 admissions,[172] and during screening only at around 0.2 per 1,000 livebirths.[87] In Australia and New Zealand, early-onset *E. coli* has seen a statistically non-significant decrease from 1992 to 2001 during either or risk-based prevention.[72, 190] Again, any changes in before and after studies are difficult to attribute to screening alone.

There is evidence that antibiotic usage may cause changes in neonatal microbiota that could lead to long-term health problems. Disturbances in the microbiota have been associated, though not causally, with asthma, obesity, diabetes, and autism.[191, 32, 192, 27] There has been evidence showing that intrapartum antibiotics are associated with alterations in the microbiota of women and children.[193, 194] For example, intrapartum penicillin was associated with lower relative proportions of *Lactobacillus spp*. in treated women compared with those who were not treated (13.1% vs 88.1%).[195] However, GBS itself can also affect the microbiota composition.[195]

Antibiotic use in mothers may also predispose them to *Clostridium difficile* infection, a well-recognised complication of antibiotic treatment that is increasingly being observed in the post-partum population.[21] Maternal anaphylaxis may also occur in the pregnant women being treated, which, although very rare, can be fatal for both mother and neonate.[14] Although figures are based on limited evidence, an estimate of anaphylaxis associated with penicillin use in labour is one in 10,000 and fatal anaphylaxis is estimated in one in 100,000 patients.[196] One study between 2003 and 2004 found no maternal anaphylaxis in 2,432 women who were treated with IAP.[197] Since 2008, the CDC has noted one case of maternal anaphylaxis in which an emergency caesarean section was performed. Both mother and child survived, however,

the child suffers from severe neurological damage.[198, 14] A positive screening culture could also increase anxiety for the mother, family and medical staff, increase the medicalisation of labour and reduce the patient choice in birthing options.[32, 13] These harms would be unnecessary in false positive and over-diagnosed cases who would not have had a neonate with EOGBS in the absence of treatment. Studies in prenatal screening indicate that false positive results could have a lasting effect of anxiety.[199, 200] In another study, half of clinicians felt that IAP caused additional stress to mothers and families and anxiety to physicians and the floor staff.[201]

2.6.4 Cost effectiveness of GBS prevention

There have been two key studies about the cost effectiveness of GBS prevention strategies in the UK, both concluding that screening and risk-based prevention are not cost-effective under the NHS willingness to pay threshold. The first was published in 2007 and determined the cost-effectiveness of 14 different prenatal strategies including doing nothing, risk-based prevention, antenatal culture screening, PCR screening and vaccination.[32] The authors concluded that vaccination was the most cost-effective strategy, followed by culture screening of women without risk factors assuming that preterm and high-risk groups (including elective caesarean section) were treated. Risk-based prevention was not cost-effective. They also concluded that all cost-effective options involved treatment of all preterm and high-risk groups. The model has a list of limitations, possibly as a result of being one of the first detailed studies on the topic. GBS experts have commented that there were some concerning assumptions, which could decrease screening cost-effectiveness, such as using culture positive (and not negative) infection, which overestimates IAP effectiveness, potentially overestimating the effectiveness of IAP itself (taken as 99% although, as indicated above, a Cochrane review found that IAP was around 83% effective), using a low rate of GBS maternal colonisation of 12% which would increase the number of women screened with no benefit and excluding the harms of IAP such as that on antibiotic resistance.[167, 23] They have also identified that the authors excluded some fundamental issues, for example, they did not account for the wider costs of observation and testing of babies whose mothers were screen positive but did not receive adequate IAP, the increase in exposure to medical interventions during labour and community midwifery input, which has consequences for staffing and size of units and a reduction in labour choices.[167, 23]

The most recent cost-effectiveness analysis of GBS prevention in the UK, identified that compared with doing nothing, antenatal culture screening costs £23,444 per quality-adjusted life year (QALY).[122] Antenatal culture screening was more cost-effective than risk-based

prevention, however, both approaches were above the NHS willingness to pay threshold of £20,000 per QALY. The cost-effectiveness of screening compared with risk-based prevention was finely balanced with variability in the findings. The sensitivity analysis showed that if you do not administer IAP to all women who deliver before the screening test at 35 to 37 weeks, risk-based prevention is more cost-effective than culture screening. Risk-based prevention is also more cost-effective than culture screening if the costs of a culture test increased by £0.87. However, a key limitation of the cost-effectiveness analyses is that the models are based on uncertain evidence on the clinical effectiveness of GBS screening and IAP treatment (as discussed above), which makes the assumptions and findings on the costs and benefits uncertain.

2.6.5 UK NSC screening recommendations

In 2012, the UK NSC reviewed the evidence for universal screening.[23] There were 22 UK NSC screening criteria at the time, three of which relate solely to genetic mutations and are not relevant to GBS screening. Essentially, the screening criteria examine four things: 1) the seriousness, epidemiology and natural history of the condition, i.e. if the condition moves from asymptomatic to an illness; 2) is the test accurate, acceptable and able to separate those with a problem from those without it; 3) is the treatment available, effective and acceptable; and 4) does the programme do more good than harm at a reasonable cost. The NSC concluded that universal screening should not be offered as there was insufficient evidence to ensure that the benefits of screening would outweigh the harms (see Table 3 for a summary against the key criteria).

In particular, they identified the following important gaps in evidence, which made it difficult to weigh up the benefits and harms of universal screening. Firstly, there was a lack of information about why GBS colonisation status changes from 35 to 37 weeks to birth, which women transmit GBS to their babies and which babies will suffer from GBS disease and why. There was also a lack of evidence on the harms of IAP treatment, limited evidence on the effectiveness of screening on EOGBS, early-onset sepsis and the overall harms from screening and widespread IAP. In 2016, the UK NSC updated their review to fill in some of these gaps. As this thesis was a part of the 2016 NSC review, it will be considered in the discussion chapter of the thesis (Chapter 15). Nevertheless, there continues to be increased pressure to introduce a universal antenatal GBS screening programme in the UK in addition to the current risk-based strategy.

Table 3. Summary of the 2012 UK National Screening Committee review key findings[23]

Criterion	Judgement	Explanation
1. The condition should be an important health problem.	Met	EOGBS is an important health condition, as although it is relatively uncommon, consequences can be long-term and severe and fatal.
2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.	Partly met	The natural history of GBS is poorly understood – we do not understand why GBS colonisation status changes from 35 to 37 weeks to birth, nor do we understand which women transmit GBS to their babies nor which babies will suffer from GBS disease nor why. We also do not fully understand the long-term morbidities of EOGBS. There is evidence to support the association of risk factors with GBS disease, especially with GBS colonisation and intrapartum fever, when other factors were adjusted. However, evidence on how many GBS-colonised or EOGBS babies have maternal risk factors varies.
5. There should be a simple, safe, precise and validated screening test.	Not met	Antenatal culture has a moderate ability to predict GBS colonisation status in labour. There are no post-screening methods to narrow down which mothers, colonised with GBS, are at greatest risk. Screening at 35 to 37 weeks would also miss out preterm deliveries before 37 weeks (who have a higher burden of GBS mortality).
10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.	Partly met	A Cochrane review found RCT evidence that IAP reduces culture-confirmed and probable EOGBS but not neonatal mortality. However, the RCTs were small, of poor quality, and 20 years old. Therefore, the authors concluded that IAP is not supported by conclusive evidence.
13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity.	Not met	There are no RCTs assessing screening effectiveness for reducing EOGBS mortality or morbidity, and sub-optimal observational evidence makes it difficult to quantify the benefits and harms of screening compared with risk-based prevention.
15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).	Uncertain	The literature did not report about the harms from screening and treatment, such as antibiotic resistance and long-term health problems from changes in the neonatal microbiota.
16. The opportunity cost of the screening programme should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money).	Not met	There were no new cost-effectiveness studies since the previous review in 2008.

EOGBS early-onset GBS, GBS group b *Streptococcus*, IAP intrapartum antibiotic prophylaxis, RCT randomised controlled trial

2.7 Conclusions

This review presents an exploration of the literature pertaining to EOGBS disease and the strategies to prevent it. With an incidence of 0.57 per 1,000 livebirths and mortality rate of 5.2%, EOGBS is an important health condition in the UK. There are some well-defined risk factors of EOGBS, with preterm births and low birthweights at particularly high risk. Regarding the natural history of GBS, it is still not fully understood why some mothers, but not all, transmit GBS to their neonates. Nor is it known which neonates will develop the disease.

Risk-based prevention in the UK has not managed to stabilise or decrease the incidence of EOGBS, although risk-based prevention in other countries has shown decreases. The evidence on risk-based prevention is based on observational before and after studies making it difficult to assess its impact. Estimates in the UK show that only 44% of women with risk factors are treated with IAP; 50% receive IAP for less than two hours and only 25% receive IAP for at least four hours before delivery. The low adherence to the risk-based prevention policy makes it difficult to identify its impact, particularly as the reasons for the low proportion of women with indication receiving IAP are unknown. In addition, a substantial proportion of EOGBS cases do not have any risk factors. Estimates show that 63% to 67% of EOGBS cases and 56% to 67% of EOGBS deaths born at term have no maternal risk factors indicative of IAP treatment.

An alternative strategy to increase the detection of women at risk of having a neonate with EOGBS is universal GBS screening in addition to the risk-based prevention. Screening is a precarious tool that can only be powerful through evidence-based and careful implementation. As maternal GBS colonisation is a pre-requisite for EOGBS disease, the selective enriched culture test is recommended to detect maternal colonisation. However, this test which is administered at 35 to 37 weeks of gestation is not an accurate predictor of EOGBS disease, with only somewhere between 0.2% to 0.4% being correctly identified. A more accurate and practical test is required, however, the lack of evidence on the natural history of GBS inhibits this venture.

Despite IAP being the recommended preventative treatment for EOGBS worldwide, the effectiveness of IAP is uncertain due to the high risk of bias in the evidence. Equally, the effectiveness of universal screening is difficult to assess as there is no RCT and the observational evidence is at high risk of bias and inconsistent. However, an RCT for EOGBS prevention would require a large sample size. Furthermore, the harms from screening and

widespread IAP treatment are not well-studied or documented even though over 99% of tested women would be over-treated. As a result, it is difficult to quantify the benefits and harms of screening and treatment.

Despite increasing pressure in the UK to introduce universal GBS screening in addition to risk-based prevention, it is not recommended due to the gaps in the evidence base summarised below. EOGBS infection is an important health problem and more research is required to understand and prevent neonatal invasive disease.

2.8 Key areas requiring further research

- Research is needed to more fully understand the current gaps in the evidence related to EOGBS and the implications this has for whether universal GBS screening meets international standards to introduce a screening programme.
- There is a need to explore why some mothers transmit GBS to their neonates and why some colonised neonates develop EOGBS. This will help to understand if we can reliably predict which mothers colonised with GBS will have a neonate with EOGBS.
- There is also a need to explore the current data on the adverse events from IAP in order to inform the balance between the benefits and harms from GBS treatment.
- To measure the overall benefits and harms of introducing a universal GBS screening programme would require RCT evidence. However, as RCTs would require a large sample size, an alternative method may be needed to assess this.
- Finally, data are needed regarding how different countries assess screening programmes, such as universal GBS screening, and how decisions are made to introduce them.

3. RESEARCH AIMS AND OBJECTIVES

3.1 Research gap and thesis rationale

As discussed in Chapter 2, in 2012, the UK NSC recommended against introducing a universal GBS screening programme after assessing the evidence against their screening criteria. The NSC identified the following three key gaps in the literature: the uncertainty of the evidence on the effectiveness of GBS screening due to a lack of RCT data, a lack of evidence on the natural history of GBS and a lack of evidence on the harms from IAP treatment and the overall screening programme.[23] In this thesis, I address the three research gaps, examine whether the evidence now meets the criteria and recommend whether the UK should introduce a universal GBS screening programme. Each of these research gaps is discussed below.

There is considerable uncertainty about the clinical effectiveness of universal GBS screening as there has been no RCT on the impact of screening on GBS morbidity and mortality. However, when this thesis began, efforts to conduct an RCT to inform the benefits and harms of GBS screening had not been executed due to the large sample size requirements. With increasing political and media pressure for the UK to follow in the footsteps of other countries and implement screening, the NSC may require a method for estimating the effectiveness of GBS screening in the absence of RCT data. GBS is the leading cause of neonatal infections and death, causing severe morbidity and mortality for a small proportion of neonates. It is critical that the UK and other countries apply the most effective, and evidence-based prevention strategies for GBS that will result in more benefit than harm to mothers and their neonates. In the absence of RCT data, it has been suggested that careful data collection and the use of historical and coexisting controls from different regions should be utilised to make screening decisions.[202] The best available data for universal GBS screening have been collected from those countries that have implemented programmes across the world. Using these data could contribute towards our understanding of the beneficial and harmful outcomes associated with GBS screening programmes compared with no prevention and risk-based prevention strategies.

To determine the transferability of a public health intervention such as screening, programme outcomes cannot be crudely transferred from one country with the expectation that they will occur in another. Interventions are dependent on their context or their "social, political and/or organisational setting".[203 p119] Contextual factors describing a country's economy, health system, and population can impact the effectiveness of an intervention.[204-206] To transfer the

evidence on the effectiveness of a GBS screening intervention from one country to another would require an adjustment for the contextual differences. This approach would not only assist in decision-making for GBS screening, but could also provide a broader solution on evidence-based decision-making for other diseases, rare or otherwise, where RCTs may not be possible. Using this approach may also help low and middle-income countries make evidence-based screening decisions without spending resources on screening trials. Screening programmes and their outcomes in high-income countries could be transferred to low and middle-income countries by adapting the approach and estimating the impact for their context. This would allow low and middle-income countries to optimise the allocation of scarce resources.

The second research gap was about the harms from IAP treatment. Treatment harms are crucial to decision-making on whether or not to recommend a screening programme, as they inform the basic information required to determine the balance between the benefits and harms of screening. As indicated in Chapter 2, IAP would be given to over 150,000 pregnant women and their babies every year, of whom over 99% will be unnecessarily treated, therefore, it is particularly important in this context. The 2012 NSC GBS report concluded that this evidence has never been reviewed. It is necessary to synthesise the evidence on the adverse events to mothers and their children after IAP treatment in order to identify the implications this may have for universal GBS screening programmes worldwide.

Likewise, better information is required on the natural history of GBS. As discussed in Chapter 2, we do not know why vertical GBS transmission occurs in approximately 36% of cases or why 1% to 3% of colonised neonates progress to invasive EOGBS disease. Understanding the natural history of a condition is crucial to finding a valid target for testing and detection of individuals at high risk of a disease. This information could allow identification of the best points in time that GBS screening should take place, the GBS carriers at most risk of EOGBS who should be targeted, and whether there are other mechanisms that could be used for GBS screening tests and procedures. Again, the 2012 NSC GBS report concluded that this evidence has never been reviewed. It is important to examine the evidence on the factors associated with GBS transmission and EOGBS in GBS-colonised women in order to identify how they might affect a universal GBS screening programme in the UK and abroad.

Finally, preliminary research for this thesis highlighted that there was a paucity of information about the policy-making processes used to decide on whether, or not, to introduce screening programmes. Within the literature and amongst screening policy-makers, little is known about the screening infrastructure, criteria or decision-making methods used across the world. The

way in which countries make these decisions not only has implications on whether GBS and other screening programmes are introduced in various countries but it also enables us to learn from other countries' practices. This would expand the current understanding on worldwide screening practices and would also contribute insights on the international GBS screening policies and how they were made. As a result, policy-makers can ensure they are operating to the best international standards when making screening recommendations.

3.2 Overall aim of research

The overall aim of this thesis is to investigate whether the UK should commence a universal GBS screening programme by addressing the three key research gaps identified in the 2012 NSC review. To provide a solution for the uncertainty of screening effectiveness from a lack of RCT data, I will analyse international trends on the benefits and harms related to universal GBS screening, to underpin national (UK) and international policies on universal GBS screening programmes. I will investigate whether the trends of the benefits and harms related to universal GBS screening across countries with different prevention programmes, can be adjusted for country-level differences in ecological trend analyses, to make screening decisions. To investigate the natural history and factors associated EOGBS and the adverse events from IAP treatment, I will synthesise the current evidence in the literature. I will also synthesise the current evidence to explore the screening policy-making procedures used in different countries.

3.3 Research questions and objectives

The specific research questions are listed below and the objectives are summarised in Table 4:

1. What are the screening systems and policy processes used in different countries to develop health screening policy for conditions such as GBS?
2. Are bacterial load and/or bacterial molecular markers associated with GBS vertical transmission, and progression from neonatal GBS colonisation to EOGBS?
3. What, and how frequently, are the adverse events experienced by the mother and/or her child after receiving intrapartum antibiotic prophylaxis treatment?

4. Adjusting for country-level differences, what is the international impact of GBS screening on the trend of annual EOGBS incidence across time compared with other prevention strategies?
5. Adjusting for country-level differences, what is the international impact of GBS screening on the trend of all-cause early-onset sepsis incidence across time compared with other prevention strategies?
6. Adjusting for country-level differences, what is the international impact of GBS screening on the trends of annual early-onset *Escherichia coli* and LOGBS incidences, and the percentages of clindamycin and erythromycin resistance in early-onset and neonatal GBS disease across time compared with other prevention strategies?

Table 4. Research objectives

No	Objective	Study design	Chapter
1	To examine how different countries make screening policy for GBS and other conditions.	Systematic review	5
2	To examine the association between bacterial load, bacterial molecular markers and GBS transmission and EOGBS disease	Systematic review and meta-analysis	6
3	To assess the risk of adverse events experienced by women or children after intrapartum antibiotic prophylaxis treatment.	Systematic review	7
4	To examine the trends and estimate the potential impact of GBS screening on annual EOGBS incidence across time compared with other prevention strategies.	Ecological time trend analysis using linear and multi-level regression	11
5	To examine the trends and estimate the potential impact of GBS screening on annual all-cause early-onset sepsis incidence across time compared with other prevention strategies.	Ecological time trend analysis using linear regression	12
6	To examine the trends and estimate the potential impact of GBS screening on potential harmful outcomes such as early-onset *E. coli*, LOGBS, and clindamycin and erythromycin resistance in early-onset and neonatal GBS disease across time compared with other prevention strategies.	Ecological time trend analysis using linear regression	13

E. coli Escherichia coli, EOGBS early-onset GBS, GBS Group B *Streptococcus*, LOGBS late-onset GBS

3.4 Thesis structure

This thesis is broadly composed of two sections of research based on the methodology used: systematic review and meta-analysis (part II) and ecological trend analysis (part III). The remaining sections (part I and IV) cover the thesis as a whole. The thesis includes 15 chapters.

- **Part I** covered the introduction and research aims of the thesis as a whole.

Chapter one introduced the thesis.

Chapter two summarised the review of the current literature related to neonatal GBS and screening.

Chapter three explained the research rationale and listed the research aims and the objectives of the thesis as a whole.

- **Part II** comprises three systematic reviews and meta-analyses of the current evidence.

Chapter four discusses the objectives, rationale and overview of the implemented methodology in part II of the thesis. I provide the methodology of each systematic review in the chapters that follow as the details vary between reviews.

Chapters five, six and seven present the methods, results and discussion of the findings from the systematic review on the systems and processes for health screening policy-making (**chapter five**); the systematic review and meta-analysis on the bacterial load and bacterial molecular markers associated with neonatal GBS (**chapter six**); and the systematic review on the adverse events after IAP treatment (**chapter seven**).

- **Part III** comprises three ecological trend analysis studies on the benefits and harms of GBS screening.

Chapter eight introduces the rationale, aims and objectives of part III of this thesis.

Chapter nine details the methodology of the ecological trend analyses covered in part III of this thesis. As I applied the same methodology for all three ecological trend analysis studies, I present it together in this chapter.

Chapter ten presents the overview and general characteristics of the data collected from the three ecological trend analysis studies, as they were collected from the same survey. This includes a description of the overall outcomes, predictors and covariates collected as well as information about the geographical area from which the data originate.

Chapters eleven, twelve and thirteen present the results and discussion of the findings from the ecological trend analysis studies on the impact of universal GBS screening on EOGBS (**chapter eleven**); early-onset sepsis (**chapter twelve**); and adverse outcomes such as LOGBS, early-onset *E. coli* and clindamycin and erythromycin resistance (**chapter thirteen**) across time, compared with other prevention strategies.

Chapter fourteen presents a summary of the findings from the ecological trend analysis studies covered in part III of the thesis, the strength and limitations of the methodology and the research and policy implications. As the aim, objectives and methodology for each of the three studies were similar, so were the strengths, limitations and implications. Therefore, I present this discussion for all of the studies together in this chapter.

- **Part IV** comprises the discussion and conclusion of the overall thesis.

Chapter fifteen summarises and discusses the findings of this thesis from each review and study, assesses the strength and limitations of the methods of the thesis overall and offers research and policy recommendations for universal GBS screening overall.

PART II. SYSTEMATIC REVIEWS ON CURRENT EVIDENCE

4. RESEARCH AIMS & METHODOLOGY

4.1 Research aims

The research aim for this part of the thesis is to address three of the four key gaps listed in Chapter 3 in order to aid decision-making on whether the UK should introduce GBS screening: the screening systems and policy processes, the natural history of GBS and the harms from IAP treatment. The specific research questions are:

1. *What are the screening systems and policy processes used in different countries to develop health screening policy for conditions such as GBS?*
2. *Are bacterial load and/or bacterial molecular markers associated with GBS vertical transmission, and progression from neonatal GBS colonisation to EOGBS?*
3. *What, and how frequently, are the adverse events experienced by the mother and/or her child after receiving intrapartum antibiotic prophylaxis treatment?*

4.2 Methodology rationale

To address these aims, I carried out three systematic reviews: one to explore the international screening systems and processes (objective 1), one to identify the factors associated with GBS transmission and EOGBS disease (objective 2) and one to identify the harms associated with IAP treatment (objective 3). A systematic review is a "review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyse data from the studies that are included in the review".[207] A meta-analysis is "the process of combining the quantitative results of separate (but similar) studies by means of formal statistical methods".[208 p17]

The many strengths of using systematic reviews and meta-analyses to answer a research question have been well documented.[209] Systematic reviews allow existing information to be efficiently integrated, they involve explicit methods which limit bias and allow information to be scientifically integrated, they increase the power and precision of estimates from meta-analytical methods and they allow the generalisability and consistency of individual study findings to be established.[209] Systematic reviews are useful for clinicians and other practitioners to keep up-to-date with literature in the field, enable economists and decision-analysts to estimate key variables and outcomes and aid policy-makers to develop

guidelines.[209] Another important purpose of performing systematic reviews is that they can be the first step in directing future research and motivating the effort and resources to for primary studies to further the field,[210, 208] as "researcher[s] can use the review to identify, justify, and refine hypotheses".[209] In addition, they can ensure that a research idea does not venture down a path that has already been explored and does contribute to advancing the field.[209]

I specifically chose systematic reviews for objectives 1 to 3 for these theoretical and practical reasons. This is coherent with the recommendation that "research methods should follow research questions in a way that offers the best chance to obtain useful answers".[211 p18] Research questions 1 to 3 were research areas where the literature had not been previously synthesised and the status of the evidence in these fields was not known. Therefore, it was important to answer these questions to move the research in the right direction and avoid replicating previous work and wasting resources. For example, reviewing the harms from IAP could direct future projects and did direct research question 6 in this thesis about some of the harms that need to be measured. Secondly, the systematic reviewing method was the most pragmatic and efficient option. For example, researching the harms of IAP in a primary study would have required a follow up period beyond the scope of a PhD thesis. Likewise, interviewing policymakers on their systems and criteria would have been a waste of resources when this information was available but had not yet been collated.

For the systematic reviews on the natural history of GBS (objective 2) and the harms of IAP treatment (objective 3), I prioritised quantitative methods and meta-analysed the findings as far as possible, since they were the most appropriate to address the research questions. For interventions such as screening, robust estimates are required to indicate the numbers of different groups of patients who would be affected by the condition and the number who would experience a morbidity and mortality harm from the intervention. To estimate the frequency of these outcomes, quantitative methods are the most appropriate. On the other hand, for the systematic review on the screening systems and policy processes (research question 1) I used a narrative synthesis in texts and tables as these data were narrative in their nature.

In the following chapters (5, 6 and 7), I will provide the detailed methodology, such as the search strategies, data extraction and quality appraisal for each systematic review separately, as they differ across the reviews.

5. INTERNATIONAL POLICY-MAKING SYSTEMS AND PROCESSES FOR POPULATION HEALTH SCREENING

5.1 Introduction

As described in Chapter 2, there has been a recognition that, while screening programmes can be valuable in disease prevention, they can also cause harms. The benefits of screening are better prognosis and less radical treatment for some individuals, reassurance for others and more cost-effective care. The potential harms from screening include anxiety, false reassurance, the hazards of the screening and diagnostic tests, the side effects from the treatment, the time and financial burden to the patient and the opportunity cost of spending resources on a screening programme instead of other services.[145] For individuals with false positive results, the anxiety and complications from testing would be unnecessary as they were not actually at risk of the disease. Similarly, screening can also cause over-diagnosis and overtreatment, whereby individuals are true positives for latent conditions, however, without screening, these latent conditions would never become symptomatic. Again, the testing and treatment could be invasive, cause severe adverse physical and psychological harm and would be unnecessary.[146, 142, 144] Many of these harms would also be concealed once a screening programme is implemented.

To ensure that benefits of screening outweigh the harms to an asymptomatic population, it is essential to undertake a robust evaluation of screening programmes prior to implementation.[144] As discussed in Chapter 2, to do so systematically, Wilson and Jungner developed 10 criteria to assess screening programmes for any disease.[147] The criteria were: important condition, with a recognisable latent phase, whose natural history is known, available and acceptable treatment, available facilities, a suitable and acceptable test, agreed policy on whom to treat as patients and screening to be cost-effective and continual. Policy-makers and academics across different countries have accepted these criteria and adapted them to incorporate consumer choice, quality assurance, accountability, evidence-based healthcare, and the advent of genetic screening.[148] For example, at the time of this review, the NSC had developed these principles into a 22-item list of criteria. However, it is not currently known which criteria policy-makers use within other countries or how they are evaluated.

Equally, there has been limited discussion about the systems or structures responsible for screening within countries. For criteria to be valuable, countries need to have screening

systems that encourage the implementation of evidence-based screening decisions. It has been proposed that there should be one national organisation to make national screening decisions and implement national screening programmes.[142, 143, 150] However, the financing and structure of general health systems can affect those for screening.[148, 142, 150] Countries that have decentralised screening policies usually have general health systems that are decentralised, insurance-based, autonomous and less regulated. On the other hand, countries that have national agencies for the decision-making and implementation of screening are more likely to have tax-based general health systems and greater regulation.[151] In the UK, the NSC is nationally responsible for making screening recommendations and for implementing the recommended programmes. Within other countries, we do not know which organisations assess screening programmes for their introduction, decide whether or not to introduce screening programmes or implement screening programmes as a result.

Consequently, we neither know which organisations made the policy for GBS screening within each country nor the processes used to make it. As shown in Chapters 2 and 3, the NSC developed the GBS screening policy in the UK. After assessing GBS against their screening criteria, they recommended against GBS screening as there was little information on the natural history of GBS, poor accuracy of the culture test, a lack of RCT evidence on screening effectiveness and a lack of information about screening harms. As shown in Chapter 2, GBS screening guidelines were made by the CDC for the US.[86, 152, 14] In 1996 they developed guidelines in conjunction with experts from relevant disciplines and provided a report outlining an epidemiologic basis for prevention protocols, summarised results of clinical trials demonstrating IAP efficacy and examined the limitations of different prevention strategies. In 2002, the CDC stated that the recommendation was based on available evidence and expert opinion where evidence was lacking. Similarly, in 2010, clinical and public health representatives revaluated the 2002 guidelines on the basis of available evidence and expert opinion when evidence was insufficient. We do not know whether there are organisations responsible for screening in the US or other countries that may have made GBS screening guidelines.

There has been limited discussion about how screening policy decisions are developed across countries for GBS and other conditions. The structures and processes used to make GBS screening policies may have implications on whether GBS screening is in use or not. This information is useful for later comparison of the impact of GBS policies across countries in part III, Chapters 8 to 13. Beyond GBS, it is also useful to know the systems and processes used to make screening decisions for any condition. Having incomplete policy-making systems or processes that do not examine screening programmes rigorously may lead to the

introduction of disorganised, unsuccessful and expensive programmes, which have physical, financial and emotional harms, with little benefit.[146, 142, 144] There has been no systematic comparison of the screening systems that have been established, nor of the screening criteria developed nor the other policy-making processes used in each country. A better understanding of worldwide practices could help uncover the optimal systems needed to ensure that every person is receiving evidence-based screening care. Therefore, in this chapter I will explore the systems and processes used to make screening policy worldwide.

I will first present the aim and specific objectives of the review, followed by the methods used to search and extract the data and then report the results. Finally, I will discuss the principal findings compared with previous literature, the strengths and limitations of the review and the research and policy implications.

5.2 Aims and objectives

The aim of this chapter is to examine how different countries make population health screening policy for GBS and other conditions. The objectives are to examine the systems responsible for:

a) developing screening policy recommendations;
b) making a decision on whether or not to introduce screening programmes; and
c) implementing screening programmes.

In addition, I will explore the processes used to develop the policy recommendation on population health screening. The policy processes that I will specifically explore are:

d) the screening criteria;
e) the methodologies to synthesise evidence for criteria; and
f) the procedures used to reach a final decision on whether or not to recommend a programme.

The findings of this chapter have been reported to a UK NSC meeting, the Department of Health's triennial independent review of the UK NSC processes and have been provided as written and oral evidence to the UK House of Commons Science and Technology Committee's Inquiry on Health Screening.

5.3 Methods

As discussed in Chapter 3, I chose systematic review methodology to address the objectives of this chapter. The systems and processes used to make screening policies within countries had never been previously synthesised or collated. I decided to find out what is publically available before using a more labour and resource intensive methodology. I planned and reported this systematic review according to recommendations from the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) 2009 statement.[212] However, I adapted the systematic review processes to suit the needs of this review.

In particular, the data for this review were largely from grey literature as opposed to medical and health science journal databases. Although I started the search strategy with these databases, this was primarily to find grey literature sources where I could then find the information about the screening systems and the policy-making processes. I developed a framework that first searched electronic databases for the policies developed for the screening of GBS. I then searched the full-texts and reference lists of abstract-included papers for organisations that made the GBS screening policy. Thereafter, I searched the organisations' websites for information on the structures or processes they used to make screening policy on any health condition or screening programme (see Figure 2). Therefore, I extracted and synthesised the majority of the data from the policy manuals and the webpages of policy-making organisations, in addition to some journal articles containing relevant information addressing my aims. The justification for this decision was that the required information was not available in electronic databases, but electronic databases were needed to find, systematically, the sources where this information was available. Further details are provided below.

Compared with systematic reviews, I did not assess the quality of the data found and extracted for the review. This was not necessary as the data I extracted were descriptive in nature. It was also not feasible to do this as the data were in different formats, ranging from journal articles to policy manuals and website information, for which quality assessment tools were not appropriate.

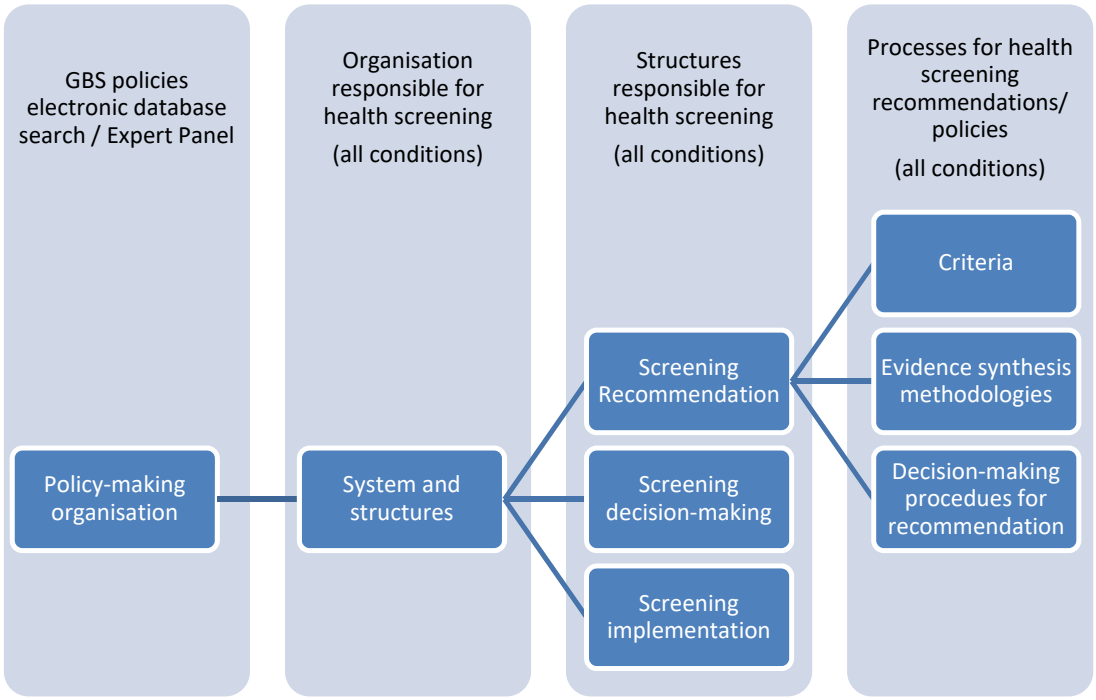


Figure 2. Framework for the methodology used in the health screening policy-making review

5.3.1 Search strategy and selection criteria

I conducted a literature search on electronic bibliographic databases MEDLINE (Ovid), EMBASE (Ovid), Science Citation Index Expanded (Web of Science) and the Applied Social Sciences Index and Abstracts (ProQuest) from 1996 to 29 November 2013, as 1996 was the first year that GBS screening was nationally recommended.[1] I developed a search strategy with input from the authors, an information specialist, and had the strategy reviewed by the 2014 Department of Health working group for the independent review of the UK NSC. To address all aspects of the objectives, in the final strategy, I combined three sets of search terms using both text words and Medical subject headings (MeSH) terms through Boolean operators OR within each set and then AND to combine the sets. The first set was made up of search terms for GBS, the second set was made up of search terms for screening, and the third set was made up of search terms for policy (see Appendix 1 for complete search strategies).

As shown in Table 5, I included titles and abstracts that satisfied the following criteria for full-text and reference list examination: the population was pregnant women or women in labour, the intervention was population-based screening policy for GBS and one of the outcomes likely reported in full-texts or reference lists were policy-making processes or the

organisations that made the screening recommendation. After I searched the full-text and reference lists of the abstract-included articles, I only included full-text articles for synthesis if the outcomes included sufficient information about policy-making processes. However, for articles that provided the screening organisation in the full-text or reference list, but did not have information about policy-making processes, I extracted the name of the screening organisation. After extracting all screening organisations' names, I then searched for these organisations on the Internet. Once I found the screening organisations' websites, I searched the websites to find policy documents with information on the screening structures and processes for assessing any health condition (see Table 6). Overall, I excluded articles published before 1996, articles that did not contain sufficient information on screening processes, were abstracts or letters.

Table 5. Selection criteria for articles in the electronic databases for the review on health screening systems and policy-making processes

Component	Title and abstract electronic database search
Study design	Any study design, to identify as many countries and organisations as possible. The study design would not impact or bias the information on the policy-making organisation or processes.
Participants	Pregnant women or women in labour.
Intervention	Population-based screening policy or recommendation for the prevention of neonatal GBS.
Comparator	None or any comparator used for comparison with the intervention.
Outcome	Description of policy-making processes.
Type, Year, and Language of publication	Any type of report including primary and secondary studies, reviews, opinions and consensus statements. All languages. 1996 onwards.

GBS Group B *Streptococcus*

Table 6. Selection criteria for policy documentation from screening organisation websites for the review on health screening systems and policy-making processes

Component	Policy documentation from screening organisation website
Study design	Any design used to describe policy-making processes.
Participants	Any population or health condition.
Intervention	Population-based screening policy or recommendation for any health condition.
Comparator	No comparator or any comparator.
Outcome	Policy-making processes.
Type, Year, and Language of publication	Any report, policy documentation, manual or guideline. Any language. 1996 onwards.

Finally, I invited screening policy experts from around the world, as identified by the UK NSC and my supervisors, to identify any further useful documentation on policy-making systems

and processes across countries. I also searched websites of the European Commission, European Council, European Observer and the World Health Organization for articles on screening policy-making systems and processes.

5.3.2 Study selection and data management

I downloaded identified, electronic database references to bibliographic management software (Endnote X5) and de-duplicated them. A second-reviewer and I independently screened the titles, abstracts and full-texts of all identified records using the selection criteria in Table 5 (screening level I). I then obtained full-text reports of all potentially relevant records identified at screening level I and, along with the second-reviewer independently examined them for screening organisations as well as information on policy-making processes in the full-text and the reference lists (screening level II). The second reviewer and I independently extracted the names of screening organisations from the abstract-included articles and included full-text articles that had sufficient information about policy-making processes. Finally, the second reviewer and I independently searched for the organisations' websites and searched the websites for policy documentation using selection criteria in Table 6. We resolved any disagreements over inclusion/exclusion at screening level I and II, the inclusion of screening organisations and policy documentation from the organisation websites by discussion, involving a third reviewer where necessary. I documented the study flow and reasons for exclusion of papers in a PRISMA study flow diagram.[212] For any articles or documents that were not in English, I attempted to translate them informally using computer software.

5.3.3 Data extraction

A second reviewer and I independently extracted relevant data using an *a priori* defined extraction sheet piloted and refined before implementation (see Appendix 2). We cross-checked each other's data extraction forms and resolved any disagreements by discussion and, where necessary with the involvement of a third reviewer. The extracted data included: author, title, year, condition, country, level of recommendation, screening organisation and authority, screening criteria and how they are used, evidence synthesis methodologies and how a final recommendation is reached.

5.3.4 Quality assessment

I did not assess the quality of the data found as all of the information was descriptive in nature. The articles did not involve any methodology that would affect the resulting information.

Furthermore, the information was found in various formats, some in peer reviewed journals, some in policy manuals, and some on different webpages of the websites. In addition, some were in English sand some were translated informally. Therefore, it would not have been feasible to judge or compare the quality of information.

5.3.5 Data synthesis

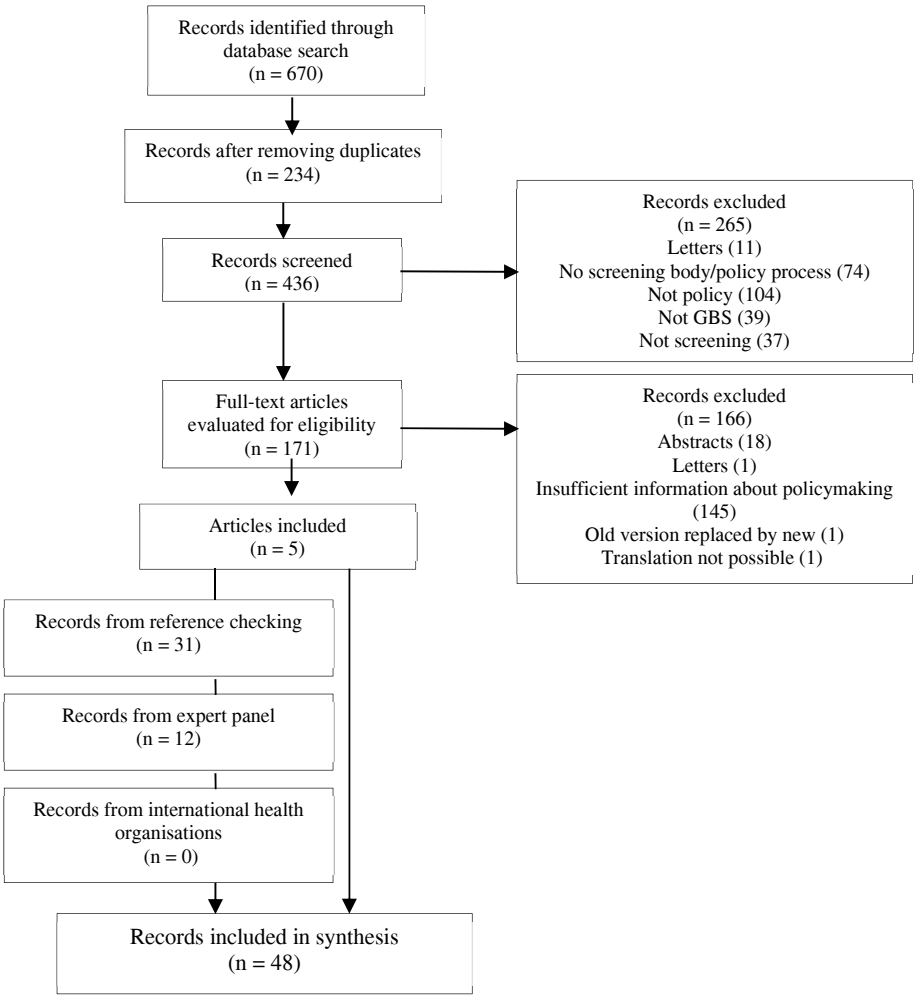
I systematically and narratively synthesised the data in text and tables. To examine health screening systems, I tabulated the screening organisation in each country along with the level of responsibility for making the recommendation, decision-making, and implementation of screening programmes within that country (e.g. national or regional). Based on preliminary work, I anticipated that I would find professional medical bodies that make screening recommendations but that are not screening bodies themselves. I state these organisations in the results but I do not describe their processes. To examine the screening criteria used in different countries, I first tabulated each criterion produced by Wilson and Jungner,[147] and then analysed the use of that item for each country. As I identified those criteria used in countries that were additional to Wilson and Junger's original list, I tabulated the criteria and assessed them for inclusion in the remaining countries. To assist in the comparability of criteria across countries, I only tabulated the information used in the statement of each criterion and did not include the detailed paragraphs describing the criterion or details in the other sections of the manual. Finally, for countries that did not explicitly use criteria, I extracted information from their policy-making processes. I narratively synthesised evidence synthesis methodologies and the procedure for deciding a recommendation in each country in text. A second reviewer also independently tabulated for screening systems and criteria and cross-checked the text synthesis for the evidence review methodologies and the decision-making processes. I discussed any discrepancies with the second reviewer with the involvement of a third author where necessary.

5.4 Results

5.4.1 Study selection

Figure 3 shows the flow of study selection. From the electronic database search, the second reviewer and I identified 436 unique records. After screening, we excluded 265 records and assessed 171 full-text articles for inclusion as well as searching the full-texts and reference

lists for screening organisations. We subsequently excluded 166 articles, which resulted in including five articles. From the reference checking of 171 articles and organisational websites, we identified 31 documents and experts identified 12 further documents. We did not identify any documents from international health organisations. Overall, 48 articles met the inclusion criteria and were included in the synthesis.[14, 11, 156, 157, 155, 159, 213-215, 132, 216-224, 166, 225-227, 22, 138, 228-230, 163, 231-238, 151, 239, 143, 240-246, 150]



GBS group B *Streptococcus*

Figure 3. Flow diagram on the selection of studies for objective 1

5.4.2 Characteristics of documents

There were 42 documents that covered screening policy-making in one country or international organisation alone and six documents that covered more than one country.[238, 151, 143, 240, 246, 150] Ten documents were informally translated into English from Czech,[220] Dutch,[227, 138, 234] German,[214, 233] Italian,[223] Spanish[219, 163] and Swedish.[243] Table 7 shows the screening

policy-making systems and processes found for 17 countries and two international organisations. The screening organisations found in all countries were responsible for developing screening policy for all conditions and were not restricted to GBS. The exception was for Belgium, the Czech Republic and Japan where there were only professional medical societies, thus, we did not extract their processes. Only cancer programmes were covered by the National Screening Observatory in Italy and the Screening Subcommittee in Australia. There was information on screening systems for 15 countries (objectives a, b and c), on criteria for 14 countries and two international bodies (objective d), on evidence synthesis methodologies for 11 countries (objective e) and on decision-making information for eight countries (objective f).

Table 7. Screening policy-making systems and processes found across countries and international bodies

Country or Organisation	Conditions covered	Was a screening organisation found?	Were criteria found?	Were evidence synthesis methodologies found?	Were decision-making processes found?
Countries					
Australia[221, 222, 228, 229, 245, 150]	Cancer, GBS, General	Yes	Yes	Yes, but unclear	No
Belgium[215, 156, 157]	GBS	Yes	No	Yes, but unclear	No
Canada[216, 217, 155, 230, 150]	General, GBS	Yes	Not used	Yes	Yes
Czech Republic[220]	GBS	No	No	No	No
Denmark[238, 239, 143, 241]	General	Yes	Yes	Yes	No
Finland[238, 143, 225]	General	Yes	Yes	Yes	No
France[159, 213, 143]	General, Early neonatal bacterial infection	Yes	Yes	Yes	Some
Germany[214, 143, 233]	General, GBS	Yes	Yes	Yes, but unclear	Yes
Italy[11, 223, 143]	Cancer, GBS, General	Yes	Yes	Yes, but unclear	No
Japan[166]	Obstetrical practice	No	No	No	No
Netherlands[151, 227, 138, 234]	General, Genetic screening, GBS	Yes	Yes	Yes	No
New Zealand[132, 226, 244, 229, 150]	General, GBS	Yes	Yes	Yes	Some
Spain[219, 143, 163]	General, GBS	Yes	Yes	Yes	No
Sweden[238, 151, 143, 243]	General	Yes	Yes	Yes	Yes
Switzerland[237, 232]	General, GBS	Not in use	Not used	Not used	Not used
UK[238, 151, 142, 143, 22, 230, 150, 246]	General, GBS	Yes	Yes	Yes	Yes
US[218, 224, 150, 235, 236, 14]	General, GBS	Yes	Not used	Yes	Yes
International organisation					
WHO[231]	Non-communicable disease	-	Yes	No	No
Council of the European Union[239, 242]	Cancer	-	Yes	No	No

GBS group B *Streptococcus,* UK United Kingdom, US United States of America, WHO World Health Organization consultation group on methodology of non-communicable disease screening

5.4.3 Screening systems (objectives a, b and c)

There was a national organisation in 14 countries that was responsible for providing screening recommendations to varying extents (Table 8). In 2010, officials had been considering the creation of a national screening body in Switzerland, however, there was no screening organisation as yet. With the exception of Spain, all screening bodies made some form of evidence-based screening recommendations at the national level. In Spain, regional authorities were permitted to make screening recommendations that the Ministry of Health had to approve before introduction.

The decision-making and implementation of screening recommendations was at the national level in the UK, New Zealand and the Netherlands. In the other 11 countries, these responsibilities were delegated to the regional, local, or municipal bodies and *via* insurance schemes. In France, Germany and Belgium, decisions to introduce screening programmes were applied at the national level, however, the responsibility to implement them was delegated to lower levels. In Canada, Sweden and Australia, both the decision to introduce and implement screening programmes was devolved to the regional or local level. In Finland, Italy and Denmark, the national screening organisation asked municipal and regional authorities to introduce screening programmes with differing amounts of authority. Lower level authorities were obligated to introduce some screening programmes but did not have to introduce others. In the US, decision-making was previously delegated to the individual states and health insurance plans (including Medicare, Medicaid and the Veterans Health Administration), as guideline recommendations and coverage decisions were separated. Thereafter, under the Affordable Health Care Act, screening programmes that the United States Preventive Services Task Force (USPSTF) recommended with a grade A or B (see Section 5.4.6 below for grading) had to be covered by health insurance plans. Screening programmes not recommended by the USPSTF Health plans could still be covered by insurance plans. Therefore, in the US, Finland, Italy and Denmark, lower level bodies were able to introduce screening programmes not endorsed in national recommendations.

GBS screening recommendations or policies across many countries were not developed by the national screening agencies identified above. In Australia, the Czech Republic, Germany, Italy, Japan, the Netherlands, New Zealand, Spain and Switzerland, GBS screening recommendations were developed by professional medical societies, such as obstetrics and gynaecology societies. The screening organisations in these countries did not have a GBS recommendation on their website. In Belgium, GBS screening recommendations were developed by the Ministry of Health, however, it was not clear whether the Ministry was

responsible for developing other screening recommendations as well. In the US, the CDC, a national disease prevention agency, developed the GBS screening recommendation, however, it is the USPSTF that is nationally responsible for screening. The USPSTF does not have a recommendation on GBS screening. The national screening agency in France did make the GBS recommendation, however, this recommendation was dated before the publication date for the policy manual to assess screening programmes. Only the GBS recommendations in Canada and the UK were made by the national screening organisation.

Table 8. Screening systems across countries

Name	Organisation	Recommendation	Decision-making	Implementation	Other GBS screening recommendations
Australia	The Screening Subcommittee, Australian Population Health Development Principal Committee	National	Regional	Regional	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Belgium	Superior Health Council	National	National	Local	
Canada	Canadian Task Force on Preventative Health Care	National	Regional	Regional	Canadian Strategy for Cancer Control: Screening Working Group Society of Obstetricians and Gynaecologists of Canada (SOGC) Canadian Paediatric Society
Czech Republic					Czech Gynaecological and Obstetrical Society
Denmark	National Board of Health	National/Regional	National/ Regional	Regional	
Finland	National Screening Committee, Ministry of Health and Social Affairs	National/Municipal	National/Municipal	Municipal	
France	Haute Autorité de Santé	National	National	Local through health insurance	
Germany	The Federal Joint Committee	National	National	Regional through health insurance	Working Group of the scientific medical professional societies
Italy	National Observatory Screening	National	National/ Regional	Regional	GBS Prevention Working Group of Emilia-Romagna.
Japan					Japan Society of Obstetrics and Gynaecology Japan Association of Obstetricians and Gynaecologists
Netherlands	The Health Council National Institute for Public Health and the Environment	National	National	National	Dutch Organisation of Obstetrics and Gynaecology Dutch Association of Paediatrics (NVK)

Name	Organisation	Recommendation	Decision-making	Implementation	Other GBS screening recommendations
New Zealand	National Screening Advisory Committee National Screening Unit	National	National	National	Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Spain	Ministry of Health, Social Services and Equality	Regional	Regional	Regional	Spanish society of obstetricians and gynaecology
Sweden	The National Board of Health and Welfare	National	Local	Local	
Switzerland	Considering national recommendation and decision-making organisation				Swiss society of neonatology
UK	UK National Screening Committee	National	National	National	Royal College of Obstetricians and Gynaecologists
US	United States Preventive Services Task Force	National	National coverage for a set of conditions	Regional: health insurance National: Medicare, Medicaid, Veterans Health	Centers for Disease Control and Prevention American College of Obstetricians and Gynaecologists American Academy of Paediatrics

GBS group B *Streptococcus,* UK United Kingdom, US United States of America, Recommendation: geographical coverage for screening recommendation, Decision-making geographical coverage for decisions to introduce screening, Implementation: geographical level that screening programme is implemented.

5.4.4 Screening criteria (objective d)

Not every country used explicitly set criteria. The US and Canada did not use a checklist of criteria but have developed an analytic framework with a set of key and contextual questions. The framework and associated questions can be tailored for each individual review. For the purposes of this review, I extracted the key questions and examples of the contextual questions provided in the manuals. However, other aspects of screening may also be assessed given the tailored approach. Similarly, I only found general guidelines required for the assessment of screening before programmes were introduced for Finland, Germany and Italy. Although I synthesised these areas, it is worth noting they were broader and more general relative to other countries. Finally, the Council of the European Union used the Council of Europe recommendations and Wilson and Jungner's criteria,[239] so I extracted the Council of Europe recommendations.

Specifically for the US, Harris *et al.* (2001) described key questions in more detail than those stated in the 2008 USPSTF manual.[224] Harris *et al.* described some sub-questions within each key question stated in the USPSTF manual. These were:

- what is the prevalence of disease in the target group?
- Is there significant variation between examiners in how the test is performed?
- In actual screening programmes, how much earlier are patients identified and treated?
- Does treatment work under ideal, clinical trial conditions?
- How do the efficacy and effectiveness of treatments compare in community settings?
- How similar are people diagnosed clinically to those diagnosed by screening?
- Are there reasons to expect people diagnosed by screening to have even better health outcomes than those diagnosed clinically?
- Is the test acceptable to patients?

I only extracted the key questions stated in the manual into Table 9, not the sub-questions mentioned in Harris *et al*.

Overall, I classified criteria across 13 countries and two international bodies into 44 items (see Table 9). The criteria that were most frequently used were from Wilson and Jungner's list.[147] All countries used criteria covering test quality and the cost-effectiveness of the screening programme. Countries cited test quality in different ways. The majority stated that the test should be appropriate or suitable but others mentioned that the test should be accurate, efficient, valid, precise, reliable and reproducible, safe, sensitive and specific and have known

predictive values. Other commonly used criteria developed by Wilson and Junger were understanding the natural history and epidemiology of the condition and the condition having a detectable disease marker, early symptomatic stage, latent period or risk factor. Commonly used criteria that were not on Wilson and Jungner's list were that the overall benefits from the screening programme should outweigh the harms and that there should be scientific evidence to provide screening effectiveness.

The differences in criteria across countries often did not mirror the differences in the screening systems. There were two criteria that did differ between countries with national compared with devolved decision-making. Ten countries with devolved decision-making stated scientific evidence to prove screening effectiveness, however, none required RCT evidence. By contrast, only countries with national decision-making stated the need for RCT evidence to prove screening effectiveness: the UK required RCT data, New Zealand 'ideally' required RCT data, and France required RCTs or international consensus. The second criterion only used in countries with devolved decision-making but not in those with national decision-making was 'clarifying organisational aspects (to achieve national equivalence)'.

There were some criteria recommended by the international organisations that were not applied in any country as far as I identified and other criteria that were only used by one or two countries. The most prominent of these criteria were focussed around quality assurance and genetic considerations. The Council of Europe recommended to correct or stop screening programmes if quality assurance standards are not met. They also recommended that early diagnosis outside of organised screening programmes should also be subject to quality control, which was only considered by Italy.

There were some infrequently used criteria addressing genetic screening, which were only applied in the UK and Spain. Both the UK and Spain assessed a criterion used to clarify the subset of mutations to be covered by screening. The UK also stated that genetic mutation programmes should to be acceptable to people identified as carriers and to other family members and that the natural history of people with carrier status should be understood. Relatedly, the Council of Europe stated that neonatal screening could only be justified if the intervention is of direct health benefit to the child but this was not in use in any country I identified.

Other infrequently used criteria were focussed around ethical issues (research consent, managing individuals with high risk of disease, and consequences of test results) and implementation issues (stakeholder involvement, public pressure, accountability).

5.4.5 Evidence synthesis methodologies (objective e)

In all 10 countries, scientific evidence was searched to assess a screening programme against the screening criteria, or the analytical framework and key questions in Canada and the US. An evidence synthesis review of scientific literature was performed in these countries by an expert scientific body that was independent of the screening organisation. In the UK, however, the NSC's information specialist first conducted a knowledge update of the relevant research and, on the basis of that, the NSC director decided whether a full external review was required. Only the full external review would be conducted by an independent expert.[22] In all countries, screening organisations invited stakeholders, such as healthcare professionals, patient and carer groups and groups involved in developing public health or screening guidelines to participate in the evidence synthesis and decision-making process. Stakeholders played a larger role in Canada,[217] the US[236] and Denmark,[241] helping to formulate the framework and questions to be addressed in the evidence review.

Countries that do not always make national decisions (Canada,[216, 217] Sweden[243] and the US[235, 236]) and France,[213] assessed the quality of evidence in reviews using specified tools. Canada and Sweden used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group methodology,[247] where the quality of evidence is graded in two steps. First, a grade is assigned to the quality of evidence for the combination of studies for each important outcome in question, then a grade is assigned for the quality of evidence across all the outcomes, which results in a grade for the evidence base on the whole screening programme. The US has developed similar methods to GRADE, where the quality of each study is examined and then combined to examine the quality of evidence for each key question, then the quality of evidence for the key questions is combined to assess the quality of evidence for the entire screening programme.[235, 236] France stipulated key critical appraisal questions and endorsed appraisal tools to examine specific study types within the criteria for screening tests, programme effectiveness and economic assessment.[213]

In many countries, once evidence reports were completed they would be externally reviewed for quality assurance. Screening bodies consulted peer reviewers and experts in the field (Canada,[217] Denmark,[241] France,[213] the Netherlands,[227] New Zealand[226, 244] the US[236]), partner organisations that were liaising federal agencies and primary care societies (Canada,[217] and the US[236]), public health bodies (Sweden[243] and Spain[219]), stakeholders (Denmark)[241] and the general public (the UK[22] and Sweden[243]).

5.4.6 Decision-making processes (objective f)

Screening organisations in six countries stated that they would hold a meeting where members could discuss the criteria and key issues in the evidence and consultation, to reach a decision on whether, or not, to recommend screening. It was not clear whether there was a structured procedure for this in the UK, New Zealand and Sweden. A vote would be taken in Canada,[217] Germany[233] and the US,[236] with a quorum set for the level of agreement needed in the US and Canada.

Canada,[217, 216] Sweden[243] and the US[235, 236] that do not always make national screening decisions, as well as France[213] that does make national screening decisions, would grade recommendations according to the quality of evidence. For Canada and Sweden, recommendations were graded weak, where benefits of the intervention probably outweigh its harms or *vice versa*, or strong where benefits of the intervention outweigh its harms or *vice versa*, following GRADE methodology. In the US, the grades were from A, B, C, D, and I, and were related to the certainty of the evidence and magnitude of the net benefit (benefits minus harms) for a programme. For example, grade A was a programme with high certainty that there is substantial net benefit while grade D is a programme with moderate or high certainty that the harms outweigh the benefits or the service has no benefit. Grade I is a programme where you cannot determine balance of benefits and harms as the evidence is of poor quality, conflicting or lacking. Where screening recommendations are graded A and B, the USPSTF recommends offering the programme and where recommendations are graded C, the USPSTF only recommends offering the programme if there are other considerations in support of offering services in an individual patient. The USPSTF discourages offering recommendations graded D and advises that, if services graded I are offered, the uncertainty about the balance of benefits and harms should be understood by the patients.

Table 9. Screening criteria used across countries and international bodies

No	Criteria Item	W&J	Aus	Can[a]	Den	Fin	Fra	Ger	Ita	Neth	NZ	Spa	Swe	UK	US[a]	WHO	EU[b]
Condition																	
1	Suitable or well-defined candidate for screening			X		X					X				X	X	
2	Condition should be an important health problem	X	X		X				X	X		X	X	X	X		X
	a. Burden of condition – Incidence & prevalence			X		X	X								X	X	
	b. Mortality and morbidity						X										X
	c. Socioeconomic impact						X										X
3	The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood	X	X	X	X		X			X		X	X	X	X	X	X
	a. Known strength of association between intermediate outcomes and clinically relevant outcomes for the condition			X											X		
	b. Is pseudo disease present in the apparently diseased population?														X		
4	There should be a detectable risk factor, disease marker, latent period or early symptomatic stage	X	X	X	X			X	X	X		X	X	X	X		X
5	All the cost-effective primary prevention interventions implemented as far as practicable.						X					X		X			
6	If the carriers of a mutation are identified as a result of screening, the natural history of people with status should be understood, including the psychological implications.													X			
7	Current clinical practice			X											X		
Test						X											
8	Suitable test or examination	X		X	X			X	X		X		X				X
	a. Test should be simple						X					X		X		X	
	b. Test should be precise													X			
	c. Test should be accurate			X											X		
	d. Test should be sensitive and specific		X	X											X		X
	e. Predictive values of the test should be evaluated		X		X												X
	f. Test should be valid		X		X		X					X		X			
	g. Test should be safe		X									X		X		X	
	h. Test should be reliable and reproducible						X			X		X					
	i. Test should be efficient				X							X					

No	Criteria Item	W&J	Aus	Can[a]	Den	Fin	Fra	Ger	Ita	Neth	NZ	Spa	Swe	UK	US[a]	WHO	EU[b]
9	The test and [further investigation] should be acceptable to the population [Swe]	X	X		X		X			X		X	X	X			X
10	The distribution of test values in the target population should be known and suitable cut-off level defined and agreed on whom to categorize as “screen positive" "screen negative”		X											X		X	
11	There should be an agreed evidence based policy for each group following disclosure of screening results:																
	a. On further diagnostic investigation and support of individuals with a positive test result and on the choices available to those individuals		X		X		X					X		X		X	X
	b. Providing information about negative screening tests		X														
12	If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not tested, that should be clearly set out.											X		X			
13	New technologies for screening/and or intervention														X		
Treatment						X											
14	There should be an effective treatment or intervention to benefit premature mortality, benefit quality of life, or alter the course of the disease for patients identified through early detection		X	X			X	X			X			X		X	X
	a. Evidence of early treatment leading to better outcomes than late treatment						X		X			X	X	X	X		X
	b. Treatments available that make a difference in intermediate outcomes when the disease is caught early, or detected by screening														X		
15	There should be an accepted treatment	X	X		X					X			X				X
16	The treatment must be accessible		X								X						X
17	The treatment must be available		X														X
18	There should be agreed evidence based policies and referral systems covering which individuals should be offered treatment and the appropriate treatment to be offered	X	X		X		X			X		X		X		X	X
	b. There needs to be an established policy for the management of individuals who are identified as being at high risk of developing the disease or condition		X														
19	Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme											X		X			
Programme																	
Effectiveness						X											

No	Criteria Item	W&J	Aus	Can[a]	Den	Fin	Fra	Ger	Ita	Neth	NZ	Spa	Swe	UK	US[a]	WHO	EU[b]
20	Have scientific evidence of screening programme effectiveness (reduces morbidity and mortality)		X	X			X			X	X	X	X		X		X
	a. High quality RCT evidence that screening programme is effective in reducing mortality or morbidity										X			X			
	b. Have evidence from experimental studies																X
	c. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice", there must be evidence from high quality trials that the test accurately measures risk. The information must be of value and readily understood													X			
21	Cost-effectiveness	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	a. All other options for managing the condition should have been considered to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available.						X							X			X
	b. Cost effective to encourage high coverage.		X														
Planning and implementation																	
22	Respond to a recognised need		X							X							
23	Have a clear definition of the objectives of the programme, the roles and responsibilities, the expected health benefits, and the financing required from the outset		X							X							X
24	Identify the target population which stands to benefit from screening		X							X		X					X
25	Patient values and preferences included in screening policy-making			X													
26	Case-finding should be a continuing process and not a 'once and for all' process with a clearly defined and optimal interval	X	X	X	X		X			X					X		X
	a. Ages when screening should be stopped														X		
27	Public pressure for widening the eligibility criteria for reducing the screening interval and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.													X			
Organisation, Infrastructure, Workforce, and facilities																	
28	Relevant programme organisational aspects should have been clarified (to achieve national equivalence)				X	X			X				X				
	a. Staff training				X												X

No	Criteria Item	W&J	Aus	Can[a]	Den	Fin	Fra	Ger	Ita	Neth	NZ	Spa	Swe	UK	US[a]	WHO	EU[b]
	b. Awareness programmes should be organised for target population and health professionals						X										X
	c. The programme should integrate a coherent set of education, training, practice, test, care, clinical services, and programme management		X							X							
29	Adequate workforce, facilities, education, organisation and infrastructure for testing, diagnosis, follow-up assessment, treatment and programme management should be available prior to the commencement of the screening programme	X	X		X		X	X		X			X	X		X	X
30	Registration system - a database capable of providing a population register for people screened and health information collected for the programme		X		X				X								X
	a. A detailed description of test result dissemination				X				X								X
31	Feasible programme within the health system					X					X	X	X				X
32	A detailed description of the steering committee				X												
33	Involve multiple disciplines and professions								X								
34	The governance structure at the government level should be accountable to society, in terms of both the overall performance of the programme and the implications for society																X
	Monitoring and quality assurance																
35	There should be a plan for managing, monitoring and evaluating the screening programme		X	X			X			X		X	X	X			X
36	An agreed set of quality control and assurance standards to minimise potential risks of screening		X				X		X	X		X		X			X
	a. Activity of early diagnosis done outside of organised screening programmes must be subjected to a quality control enabling the assessment of the adequacy and results.								X								
	b. A limited number of the appraisal criteria and indicators should be validated; they should be chosen at the design stage and be based on the results of the literature review or the opinion of a panel of experts						X										
	c. If quality assurance standards are not met in the long term it should be possible for the screening programme to be corrected and, if this is not possible, stopped.																X
	Acceptability and ethical issues																
37	There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically and socially acceptable to health professionals and the public.										X	X		X			

No	Criteria Item	W&J	Aus	Can[a]	Den	Fin	Fra	Ger	Ita	Neth	NZ	Spa	Swe	UK	US[a]	WHO	EU[b]
38	There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is ethically acceptable to health professionals and the public.					X			X		X	X	X	X			
	a. An evaluation of the ethical and psychological consequences for the examinees				X												
	i. An evaluation of the consequences of "false positive" and "false negative" test results/experience of overdiagnosis				X												
	b. An evaluation of stigmatisation				X												
39	Promotion of human rights, including upholding the principles of autonomy and confidentiality		X				X			X							X
40	Promote equity and access to screening for the entire target population, regardless of socio-cultural and economic availability		X	X			X		X	X						X	X
41	Informed choice: Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice		X		X		X		X	X			X	X			X
	a. If the programme is provided as a service and conducted also for research purposes, the decision to make available personal medical data stemming from the screening programme for research purposes should be taken freely, without undue pressure																X
42	Neonatal screening can only be justified if the intervention is of direct health benefit to the child. Otherwise, screening should be postponed until the child can decide for itself.																X
43	If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members													X			
Overall benefits versus harm																	
44	The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment)		X	X			X		X	X	X	X	X	X	X		X
	a. Frequency and severity of harms of workup / screening test			X											X		
	b. Harms of treatment														X		

W&J Wilson & Jungner, Aus Australia, Can Canada, Den Denmark, Fin Finland, Fra France, Ger Germany, Ita Italy, Neth Netherlands, NZ New Zealand, Spa Spain, Swe Sweden, UK United Kingdom, US United States of America, WHO World Health Organization consultation group, EU Council of Europe

a. No screening criteria b. The Council of Europe

5.5 Discussion

5.5.1 Principal findings

In this systematic review, I reported the policy-making systems and processes for population health screening programmes across 15 countries and two international organisations. I found systems and processes for the screening of any conditions in 14 of the 15 countries and only for GBS in one country. The principal findings are as follows. A national body has been established in several countries that assesses the scientific evidence for a screening programme against a list of screening criteria to create screening policy recommendations (objective a). Even Canada and the US, which did not have a list of criteria, resonated the principles of screening criteria in their evidence review processes (objective d). Nevertheless, there were differences in the national screening organisations' degree of influence to implement the screening decisions across countries (objective b and c). As screening decisions could be decided at a local or regional level, there were organisations beyond the screening organisations that made screening recommendations. Crucially, for GBS, few screening recommendations across countries were developed by the organisation nationally responsible for assessing screening. There was also a divergence in countries' choices of the screening criteria used, little of which mirrored the differences in health systems. The important concerns here were that different countries required different levels of scientific evidence, few countries had criteria around genetic conditions, and none had criteria around ceasing programmes, all of which could be valuable.

There were screening bodies across 14 countries that had the responsibility to make national screening recommendations and they did so using systematic policy-making processes that the organisations had developed (objective a). The screening organisations conducted thorough evidence reviews of scientific literature that incorporated screening principles beyond Wilson and Jungner's list to ensure that the recommended programmes offer more benefit than harm.[147] From the information that was available, these reviews also critically appraised the evidence and quality assured their reviews using external input (objective e). The decision on whether or not to recommend a screening programme was reached by discussion amongst the members of the organisation which incorporated the screening principles, evidence available, as well as its quality (objective f). However, once these national screening recommendations were formulated, they were not enforced in all countries. Screening recommendations became regulations that required national implementation only in some countries. In other countries, the recommendations were similar to best practice guidelines, and local and regional health authorities could decide all or some of the screening

programmes for their citizens (objective b and c). Therefore, screening practices may vary within countries.

Relatedly, a key finding was that the screening recommendations for GBS were made by medical professional societies and other organisations not nationally responsible for screening in 15 of the 17 countries. The only countries with GBS screening reviewed by the screening organisation were Canada and the UK. In the majority of the countries, recommendations were produced by professional medical societies while in the US they have been developed by the CDC, which is a preventative health organisation but not the USPSTF which is responsible for screening nationally. Whether these organisations took the key screening principles into account is not known and may have serious implications on whether the critical and likely unseen harms of GBS screening have been considered. Similarly, in France and Belgium, it is not clear whether the national organisation that made the GBS recommendation took screening principles into account. Whether these societies and organisations assessed GBS screening using rigorous and systematic evidence-based methods that are free from bias and incorporated screening principles remains unknown.

There was a divergence in the specific screening criteria that were utilised across countries (objective d). One of the most important criteria was that different countries required different levels of scientific evidence. Unlike the UK, many countries including Australia, Canada, Denmark, Finland, Germany, Italy, the Netherlands, Spain, Sweden and the US did not specifically require RCT evidence to prove screening effectiveness in reducing morbidity and mortality. Canada, Sweden and the US may fulfil this need with the use of GRADE or similar tools for quality appraisal and strength of recommendation. Sweden also mentioned RCT requirements in the detail of their manuals but not explicitly in their statements.[243] The difference in the evidence approach between countries may reflect the decision-making structures for screening within the countries. Requiring RCT evidence may be more appropriate in countries where national screening decisions are similar to regulations, whereas GRADE methodology may be more appropriate in countries where the recommendations are guidelines that regional or local health authorities decide to follow. This is because GRADE provides a clear and comprehensive method to rate the quality of evidence and the strength of recommendation that can be used by authorities to decide the programmes that should be introduced. This difference could have implications on whether a programme is introduced, or not, as organisations in some countries may accept observational evidence as satisfactory and recommend a programme whereas organisations in other countries may not. Evidence other than RCT could overestimate the benefits of screening due to study design biases (see Section 5.5.4).

Another striking criterion that was not utilised in any country was the requirement where, if quality assurance is not met, the screening programme should be stopped. Sweden stated in their manual, that there should be criteria of when to stop a programme but this was not reflected in their criteria statement and how this should be done was not mentioned.[243] Finally, only Spain and the UK covered criteria related to genetic conditions, which have some different considerations to other screening programmes. For example, as identified in these countries, there are ethical implications to wider family members who would not be screened but would be identified as carriers as well as the ethical implications of the unintended revelation of mutations not screened for as part of the programme.

5.5.2 Comparison with previous literature

There has been little literature on the policy-making systems or processes of screening. In particular, there has been no detailed comparison of the screening criteria used in different countries. Andermann *et al.* (2008) summarised 54 screening criteria lists proposed since Wilson and Jungner's criteria and found that, the additional and newer criteria reflected developments in healthcare such as accountability, consumer choice, evidence-based healthcare, quality assurance and the advent of genetic screening.[148] However, Andermann *et al.* did not identify which criteria were used by policy-makers within countries or how the criteria were evaluated. Identifying the criteria actually used for policy-making in countries, I found that they have indeed been extended to cover advances of ethical considerations, implementation issues and scientific effectiveness. However, there was little evidence of genetic criteria utilised in countries. As indicated above, the UK and Spain have progressed in this respect. Following the completion of this review, I found that in the Netherlands, a sub-committee for the Health Council provided a separate list of screening criteria for assessing genetic screening programmes.[248] Likewise, in the US, recommendations for genetic screening are developed by the Evaluation of Genomic Applications in Practice and Prevention initiative, which integrate USPSTF processes for appraisal and evaluation.[249]

Previous literature comparing screening policy-making processes in different countries includes: a literature review on the comparison of decision-making processes for newborn screening in New Zealand, the US, the UK and Australia,[250] a literature review on the links between health technology assessments and health policy for three specific screening programmes in nine European countries,[151] a commentary of screening practices in six European countries[143] and an unpublished summary of screening processes in the UK, the US,

Australia, New Zealand and Canada.[150] Using systematic review processes and covering 15 countries, similar to these reviews, I found that screening decision-making and implementation varied across countries and that, in many countries the decision is made at the regional or local level.[240, 150, 151] As indicated by many authors, this difference in the screening process is often a result of the structure and financing of general health systems.[148, 240, 150] In countries where health systems are insurance-based or heavily decentralised and are autonomous, screening practices are less regulated and are less likely to have national decision-making or national implementation of screening programmes. On the other hand, in tax-based health systems, governments are found to have more control and regulation of screening policies.[151] There has been little discussion about the screening system required to ensure that every person is receiving screening care that reflects the national evidence-based recommendation. There has been a suggestion that there should be one body in each country that is responsible for screening recommendation, decision-making and implementation.[143, 240] Likewise, it is recommended that decision-making to introduce screening programmes should be made at the national level to ensure that only the correct programmes are introduced in the correct way and that there is consistency across the country.[246] However, similar to this review, it is recognised that although a country may have a national recommendation, it may not be utilised adequately or appropriately.[142]

Compared with other reviews, I also found that processes to make screening policy are based on Wilson and Jungner's screening criteria, involve evidence reviews and stakeholder engagement.[151, 250] However, the more detailed comparison in this review showed that there are differences in the way in which criteria are used and the level of evidence required for meeting the evidence requirements. In addition, there are recommendations in the literature that ongoing screening programmes should be regularly evaluated to ensure that the programme is performing and delivering the expected outcomes as this may lead to changes in the programmes or stopping the programmes.[246] In this review, I found that there were no criteria in place on how this should be done.

5.5.3 Strengths and limitations

This systematic review was the first examination on the systems and processes used across countries to develop policies on population health screening. I used innovative methods prioritising the reference list search that resulted in meeting the objectives of the review. By extensively searching the trail of reference lists to the organisation websites and thoroughly

searching them allowed me to capture as much data as possible. I made an effort to translate documents not in English allowing more data capture across countries. Furthermore, I invited a panel of international experts who identified further documentation within their countries of expertise, as well as UK based experts who additionally reviewed the search strategy and the findings of the review, assuring its quality. I also enlisted a second independent reviewer to sift, extract and synthesise all of the information to maintain the quality of the review.

However, findings must be considered in light of some limitations. While I searched extensively to find the correct screening organisations and processes, I may not have found all organisations responsible for screening within countries. For example, countries such as Australia, Belgium, and Italy where only one or few conditions were covered, there may be other screening organisations that I did not find. Similarly, my search strategy may have missed screening organisations outside the countries I did find. My search only resulted in countries from Australasia, North America, and Western Europe. In the literature, there have been no screening organisations reported in Asia, Africa, Eastern and Central Europe or South and Central America.[151, 251] However, like some of the screening organisations that were found in the grey literature, organisations in these countries may also not be in the published domain. Similarly, policy processes and websites are updated constantly and as the search was performed in 2013, systems and processes may already have changed. Finally, although my primary interest was GBS, and I chose it as the condition to use in the search strategy, I may have found more countries and organisations with another condition.

There are also some limitations in the data extraction process. As I only tabulated explicitly mentioned criteria, for the US and Canada that did not use a criteria list approach, I may have missed some information that is not included in the key questions yet may still be included in the review process. However, as a tailored approach was used in the US and Canada, it was difficult to compare them with the remaining countries that did use criteria lists. While I attempted to translate documents not in English, these were informal translations generated by computer software, which means there could have been some errors in the translations. Due to resource constraints, this was the only option to include this information in a meaningful way. Related to this, I did not contact organisations to request further information in English or further documentation that may not have been easily accessible even in English. This may have resulted in further information or documentation. Lastly, I only extracted processes for screening organisations and did not extract the policy-making processes by other organisations, the majority of which made the GBS recommendations. Consequently, the methods used to make the GBS recommendations across countries are not known.

5.5.4 Research and policy implications

Having robust evidence-based policy development processes that critically scrutinise screening programmes and effective systems that allow evidence-based decisions to be applied, is essential to have safe and successful screening programmes. Having incomplete policy-making systems or processes that do not examine screening programmes rigorously may lead to the introduction of disorganised, unsuccessful and expensive programmes, which have physical, financial and emotional harms, with little benefit.[146, 142, 144] The screening systems and policy-making processes I have identified in this review can be adopted by governments in countries with and without screening bodies in order to implement health screening programmes safely within their contexts. Indeed, this review was used in the UK to ensure that the screening processes measure up to international standards and to identify areas for improvement. However, the methods used by committees to make final screening recommendation decisions are not as well understood. To explore this, interviews with key members of the screening organisations could inform the discussions that occur in practice. In addition to international policy-making standards, I have identified specific implications from the key differences across countries, which indicate potential concerns and scope for improvement for screening policy-making.

Firstly, countries need screening systems that encourage evidence-based screening decisions to be implemented. In countries where decision-making is at the local or regional level, citizens in some areas may not be receiving evidence-based screening care as national evidence-based recommendations do not have to be followed. This is especially a risk as screening recommendations are also produced by medical societies, which authorities may choose to adopt. However, these recommendations may not necessarily be developed around screening principles or account for screening harms. Studies have shown differences between expert opinion and research evidence. For example, Antman *et al.* (1992) found that professional recommendations on acute myocardial infarction therapies in review articles or textbooks frequently contradicted the evidence from meta-analyses of trials.[252] Similarly, a systematic review demonstrated that clinicians overestimate the benefits of screening and underestimate the harms[253] possibly based on their clinical experiences.[254] This is a particular risk for the case for GBS screening, where most recommendations have been made by clinical organisations and it is not clear if they have used internationally recognised screening criteria. As the criteria require that all the effects of a programme (harms as well as benefits) are examined, they ensure, as much as possible, that the introduction of a GBS screening programme to hundreds of pregnant women and their children would bring more benefit than harm. The next research step would be to examine the processes used by these organisations

and compare them with the processes used by screening organisations. Future researchers may also wish to examine the screening policy-making processes used by local and regional authorities and the degree to which national recommendations are adhered to in countries with decentralised decision-making.

Secondly, the difference in evidence requirements for RCTs *versus* the use of GRADE between countries is a complex aspect of policy-making process. Countries using GRADE may need to be cautious. Using levels of evidence lower than RCTs to assess screening programmes could overestimate the benefits of screening due to the inherent biases of lead and length time and the healthy screenee effect in observational studies.[144] Equally, RCTs are not always feasible and, therefore, not always applied. For example, in the UK, the NSC decided to recommend new conditions on the newborn inherited metabolic screening programme despite no availability of RCT evidence. The rarity of the conditions prevents RCT data. Therefore, the requirement for RCT is a complicated matter and flexibility may be required. However, agreement is needed on the circumstances where the RCT criterion is necessary and where it is not. In addition, a clear acceptable level of evidence should be set out for when RCT evidence is not available.

Thirdly, criteria to cease screening programmes would be influential and should be considered by countries as a way to assess and stop a programme if the evidence on the balance between benefits and harms changes. It is exceedingly difficult to assess the harms of screening programmes once they have been introduced and even more difficult to stop them, due to the popularity paradox.[144] For example, emerging evidence of reduced benefits and increased overtreatment have been recently identified for mammography screening compared with previous estimates. This means that there is now an uncertainty about the balance between the benefits and harms of the breast cancer screening programme.[255, 256] There is a strong dispute as to whether a sufficient level of evidence is still available to continue the breast cancer programme. Establishing conditions or criteria, such as the level of evidence or quality assurance standards, could provide a systematic method to reconsider or cease screening programmes until there is better evidence or assurance. Such processes could safeguard citizens during times of uncertainty and ensure they are offered robust evidence-based screening. This is a serious policy-making gap that requires process development.

Finally, policy-makers may wish to develop their processes for genetic screening recommendations. Countries need to prepare for the genomic era that is fast approaching and involves additional considerations that differ from those for screening other conditions. For example, implications for family members who may also be identified as carriers and the

identification of mutations related to other conditions not tested for as part of the screening programme. Three approaches have been reported in this chapter: 1) adding genetic criteria in to the list of criteria for all conditions and using all of the same processes to evaluate genetic conditions, 2) using a distinct list of genetic criteria to evaluate genetic programmes separate from the general list, or 3) using a separate organisation to evaluate and make genetic recommendations.

While screening policy-making across countries has undoubtedly come a long way since Wilson and Junger's list of criteria, there are still areas for improvement. Research and policy-makers can use the findings of this review to benchmark the systems and processes in their country against the international processes and invest in the development of reducing the remaining gaps.

5.6 Conclusions for this chapter

- Countries recognise the need for national screening organisations and robust evidence-based processes for making health screening policy. Governments have established systems and evidence-based processes to make screening policy.
- However, there are some concerns about the capacity of these systems to introduce the recommendations. There may be a disconnect between national evidence-based screening recommendations and the requirement to introduce them, which could leave a citizen not receiving the best available screening care.
- This is particularly important as the majority of international GBS screening recommendations have not been developed by the national screening organisations. Devolved health authorities may choose to implement these guidelines although they may not have incorporated screening principles or accounted for screening harms. When comparing GBS across countries in the remainder of this thesis (and generally in research or clinical practice), it is imperative to bear this context in mind.
- To ensure that screening programmes are safely offered on robust evidence, countries should either have minimum study design requirements or apply GRADE methodology, according to their context.
- Countries should incorporate genetic screening considerations into their criteria or processes as they were currently lacking in their preparedness for the genomic era.

- Finally, to ensure that implemented programmes remain safe, countries should address how to correct or stop screening programmes if the evidence becomes uncertain or the quality assurance can no longer be guaranteed.

6. BACTERIAL LOAD AND BACTERIAL MOLECULAR MARKERS ASSOCIATED WITH GBS VERTICAL TRANSMISSION AND EOGBS DISEASE

6.1 Context of this chapter

In 2016, with the help of my supervisors, I enlisted a team of clinical and methodological experts and submitted an application to undertake the UK NSC evidence review on the policy of universal GBS screening.[24] I was successfully commissioned to lead this work and this chapter presents one part of the evidence review on the bacterial load and/or molecular markers predictive of neonatal GBS. While the overarching objectives of the review were set by the NSC, I led the design of the specific research questions and development of the research protocol outlining the methodology for the systematic review reported in this chapter. I also conducted the searches, study selection, data extraction and report writing. My supervisors and other team members contributed their technical expertise by reviewing and advising me throughout. Their expertise included public health screening, infectious diseases, microbiology, obstetrics and gynaecology and systematic reviewing and meta-analyses. Some of the team members also conducted the second-reviewing of the systematic review processes. My supervisor (OU) conducted the initial meta-analysis for the UK NSC review and I then re-ran the meta-analysis and conducted the sensitivity analyses.

6.2 Introduction

As discussed in Chapter 2, little is known about the natural history of GBS colonisation and EOGBS. We know that a prerequisite for EOGBS is maternal GBS colonisation of the gastrointestinal and/or genitourinary tract. There have also been estimates on how many women and neonates are affected in the natural history pathway from maternal GBS colonisation to EOGBS. However, there is, as yet, a poor understanding on the mechanisms influencing the natural history pathway. We do not know why vertical GBS transmission occurs in 36% of cases,[4] or why 1% to 3% of colonised neonates progress to invasive EOGBS disease.[3, 257, 4] In particular, there are limited data about the pathogenic factors associated with GBS vertical transmission and development of EOGBS. While a number of virulence factors have been proposed to be important in the pathogenesis of GBS disease in laboratory studies,[49, 48] their importance in clinical settings is not clear. As shown in Chapter 2, the polysaccharide

capsule of GBS appears to be important, with GBS capsular serotypes Ia, Ib, II, III and V more frequently responsible for GBS disease.[38, 40, 39, 41-43] Clonal complex CC-17 has also been associated with disease in the neonate,[50, 51, 40, 41] as has the maternal GBS bacterial load.[91, 90]

Studying the mechanisms that increase or decrease the risk of vertical transmission and development from neonatal colonisation to EOGBS may have vital implications for GBS screening. As shown in Chapters 2 and 5, understanding the natural history of a condition from a latent to symptomatic phase is one of the key screening criteria across countries. Without knowing the reasons why some women transmit GBS and why some neonates develop EOGBS, it is difficult to find a good target for testing. As shown in Chapter 2, the currently recommended culture test at 35 to 37 weeks is not an accurate predictor of EOGBS in the neonate. As only approximately 0.2% or 0.4% would be correctly identified (see Chapter 2), a large proportion of women would be over-treated with antibiotics. The harms of widespread IAP are not known but could include increases in: gram-negative infections, antibiotic resistance, maternal anaphylaxis, microbiota disruption that could lead to long-term health problems, and the medicalisation of labour.[32, 23, 13] Data on the mechanisms associated with GBS vertical transmission and EOGBS could allow identification of: the GBS carriers at most risk of EOGBS who should be targeted for treatment; the best points in time that screening should take place; and whether there are other mechanisms that could be used for GBS screening tests and procedures.

Consequently, better information on the natural history of GBS could allow more efficient screening strategies. However, this literature has not been previously synthesised and the status of this evidence in not known. This research is critical as part of assessing whether the UK should introduce a screening programme for GBS. Therefore, in this chapter I will explore the evidence about the bacterial load and the bacterial molecular markers associated with GBS transmission and EOGBS, in order to identify the implications this may have for a screening programme in the UK (objective 2).

I will first present the aim and specific objectives of the review, followed by the methods used to search, appraise and analyse the data and then report the results. Finally, I will discuss the principal findings compared with previous literature, the strengths and limitations of the review and the research and policy implications.

6.3 Aims and objectives

The aim of this chapter is to identify, appraise and meta-analyse the evidence on the bacterial load and the bacterial molecular markers associated with GBS transmission and EOGBS disease to inform the natural history of GBS. The objectives are to examine whether there is a bacterial load and/or bacterial molecular markers associated with the transition of GBS from:

a) Maternal GBS colonisation in pregnancy to neonatal GBS colonisation;
b) Maternal GBS colonisation in labour to neonatal GBS colonisation;
c) Maternal GBS colonisation in pregnancy to neonatal early-onset GBS disease;
d) Maternal GBS colonisation in labour to neonatal early-onset GBS disease; and
e) Neonatal GBS colonisation to neonatal early-onset GBS disease.

6.4 Methods

As discussed in Chapters 3 and 4, I chose systematic review and meta-analysis methodology to address the objectives of this chapter. Evidence on the mechanisms associated with the natural history of GBS had not been previously synthesised and the status of the evidence was not known. It was important to answer this question to move research in the right direction and avoid replicating previous work and wasting resources. I reported this systematic review according to recommendations from the PRISMA-P 2015 statement.[210] The protocol is registered at the International Prospective Register of Systematic Reviews (PROSPERO): CRD42016037196.

6.4.1 Search strategy

I conducted comprehensive electronic literature searches in well-known and recommended databases: MEDLINE (Ovid), MEDLINE In-Process & Other Non-Indexed Citations (Ovid), EMBASE (Ovid), Cochrane Library: Cochrane Database of Systematic Reviews, CENTRAL, DARE and HTA databases (Wiley), and Science Citation Index Expanded (Web of Science) from inception to 10th October 2016. I applied an extensive search with no date limit to capture as much data as possible. To address all aspects of the objective, in the final strategy, I combined three sets of search terms using both text words and MeSH terms through Boolean operators OR within each set and then AND to combine the sets. The first set was made up of search terms for GBS, the second set was made up of search terms for neonate or pregnancy, and the third set was made up of search terms for bacterial load or molecular markers. I limited

the strategy to humans as this was the population of interest, and to the English language, as I did not have the time or resources to translate studies in other languages (see Appendix 3 for complete search strategies). Although excluding non-English studies could introduce selection bias,[258-260] the impact of this is not clear in the literature, with some reviews showing that it does not affect results.[261, 262] Furthermore, methodologists have suggested that the impact of language bias has reduced recently as a result of the move towards publishing in English.[263]

To reduce reporting bias, a second reviewer and I searched the reference lists of all included studies and relevant systematic reviews identified from the electronic databases. In addition, I enlisted subject area experts, through the help of the UK NSC, to cross-check the included studies and identify any further references not captured by the search. The team members of this project also cross-checked the included studies.

6.4.2 Study eligibility criteria

Study inclusion criteria

I included studies that satisfied the following criteria:

Study design: prospective or retrospective cohort studies and nested case-control studies. If the search did not result in a sufficient number of these studies, I included case series with ≥50 patients. If a sufficient number of studies was still not found, I included case series with ≥10 patients. I avoided case series as far as possible, as results from such studies do not have control groups, making it difficult to interpret whether the risk factor is associated with GBS transmission or EOGBS compared with those that do not have the risk factor.

Participants: culture-confirmed GBS colonised mothers or colonised neonates across any setting. Mothers had to be tested for GBS after the onset of the third trimester using vaginal or rectal swabs and selective or standard culture. Neonates had to be tested soon after birth using any surface culture. Selective culture inhibits the growth of competing organisms and increases the sensitivity of GBS culture, however, standard culture is also acceptable.[107] I included studies where some participants met the inclusion criteria, and some met the exclusion criteria, if participants meeting the inclusion criteria could be separated or participants that met the exclusion criteria were fewer than 10% of the study population.

Interventions/Exposures: any bacterial load or individual bacterial molecular marker evaluated for association with risk of neonatal GBS colonisation or neonatal early-onset GBS

disease. The criteria for exposures was intentionally wide to capture as many bacterial markers as possible.

Comparators/Controls: any bacterial load or individual bacterial molecular marker used as the reference categories for exposures.

Outcome: occurrence of GBS colonisation in neonates less than seven days after birth confirmed by surface culture, or neonates diagnosed with early-onset GBS disease less than seven days after birth. GBS less than seven days is considered to be passed from mother to neonate, while GBS more than seven days can also be transmitted from other sources.[3] To avoid bias from the contamination of GBS from other sources, I excluded GBS more than seven days.

Type and Language of publication: full-text report in the English language.

Study exclusion criteria

I excluded studies that fulfilled the following criteria:

Study design: To reduce bias, I excluded intervention studies where participants received an intervention that interferes with GBS transmission or transition such as IAP as well as studies conducted in the context of IAP. I also excluded, cross-sectional studies, ecological studies and case-reports.

Participants: pregnant women tested for GBS before the third trimester, as GBS carriage can transition in pregnancy and colonisation before the third trimester is too distant from the outcome.

Outcomes: economic evaluation and/or cost-effectiveness outcomes, diagnostic accuracy outcomes, complications of GBS such as disability and mortality (including case-fatality).

Type and Language of publication: abstracts, reviews (systematic or non-systematic), editorials, letters, books, consensus statements and opinions. I excluded reviews as sources of primary data but I used them to identify the original studies contributing the evidence. I excluded all publications in any other language than English.

6.4.3 Study selection and data management

I downloaded identified references to bibliographic management software (Endnote X7) and de-duplicated them. A second-reviewer and I independently screened the titles and abstracts of all identified records (screening level I). I then obtained full-text reports of all potentially relevant records identified at screening level I and the second reviewer and I assessed them independently using the same study eligibility criteria (screening level II). We resolved any disagreements over inclusion/exclusion at screening level I and II by discussion and involved a third reviewer where necessary. I documented the study flow and reasons for exclusion of full-text papers in a PRISMA study flow diagram.[210]

6.4.4 Data extraction

A second reviewer and I independently extracted relevant data using an *a priori* defined extraction sheet piloted and refined before implementation and cross-checked each other's forms (see Appendix 4). The extracted data included study characteristics (year of publication, country of origin, study design, sample size, sampling strategy, study period), participant characteristics (maternal or neonatal GBS colonised participants, GBS confirmation methods), details of bacterial load or molecular marker (description, reference categories, measurements), outcomes (GBS neonatal colonisation or disease, measurement) and the results for the association between bacterial molecular marker or bacterial load and GBS neonatal colonisation or EOGBS disease. Where data permitted, I estimated any missing statistical parameters of importance (e.g., OR, RR) and variability measures (e.g., 95% CI, p-values). I denoted all calculated or derived data as 'calculated'.

6.4.5 Quality assessment

The second reviewer and I independently appraised the risk of bias for each included study using the Quality in Prognosis Studies (QUIPS) tool.[264] The QUIPS tool is a published and validated quality assessment tool. I chose QUIPS as it is specifically designed to assess the risk of bias in prognostic factor studies, therefore, explicitly accounting for biases that arise from this study design. The QUIPS tool includes assessment of risk of bias for six domains: patient selection, study sample attrition, prognostic factor measurement, outcome measurement, confounding and statistical analysis and reporting. According to responses to prompting items, each of the six domains is rated as high, moderate or low risk of bias.

The second reviewer and I discussed the quality appraisals and resolved any disagreements by discussion, with the involvement of a third reviewer where necessary. I tabulated the individual item-specific quality assessment ratings for each study.

6.4.6 Data synthesis

Meta-analysis was only possible on the serotypes associated with progression from neonatal GBS colonisation to EOGBS, due to the heterogeneity in the definition of bacterial load levels and only one study on the other molecular markers. As there were no summary measures (such as RRs and 95% CIs) reported in the respective studies and only raw numbers and proportions were reported, I calculated the RR along with 95% CIs for each study, which were pooled in the meta-analysis. I compared a separate RR comparison for the proportion of colonised neonates who developed EOGBS for each serotype *versus* another. I only meta-analysed serotypes that were included in at least two studies. Due to anticipated between-study differences in the methods, EOGBS definitions and countries where studies were conducted, I used a random effects model to pool the RRs across individual studies.[265] A random effects meta-analysis assumes that the estimates of the exposure effect can vary across studies because of differences between the studies.[265] Therefore, the pooled result represents the mean RR in a random distribution of true effect sizes. On the other hand, a fixed effects model assumes that there is no heterogeneity and there is one true effect of the exposure, which is the same across all individual studies.[266] Although the studies were reasonably comparable in the exposures used and the patient characteristics, there were slight differences in the definitions of the outcome and the culture media used. Therefore, the random effects model was more appropriate.

I assessed the heterogeneity between the studies by inspecting forest plots and the I^2 statistic where a value of less than 50% was interpreted as low to moderate heterogeneity.[267, 268] I determined the stability of the meta-analysis results in sensitivity analyses. One sensitivity analysis only included the cohort studies, another only included studies explicitly using sterile site culture and another only included studies not explicitly using selective culture. Finally, I performed a leave-one-study-out sensitivity analysis, which evaluates the influence of individual studies by estimating the pooled analyses in the absence of each study.[269]

The meta-analysis on neonatal GBS serotypes were pooled from two cohort studies and one case-controlled study. Meta-analysis on observational studies has been controversial, partly, because of the diversity in study designs. Researchers have cautioned that interpreting a meta-analysis of different study designs can be problematic as the heterogeneity between them

could bias the results.[270-272] However, others have argued that it is useful and possible to pool observational studies if: the different designs are addressing the same question of interest;[273, 271, 270] the heterogeneity is carefully assessed between the studies;[273, 271, 270] and a random effects model is used.[273] For the meta-analysis in this chapter, the studies were answering the same question on the neonatal GBS serotypes in one group of participants that had EOGBS and another group of participants that were only colonised on surface sites. As discussed with clinical and microbiology experts, the studies were similar in their populations, exposures and health conditions, and all of the studies were at equally high risk of bias as none accounted for any confounding variables in their study design. I chose a random effects model because of the anticipated heterogeneity and deemed the heterogeneity as adequate using the methods described above. I also conducted sensitivity analyses to assess the heterogeneity in study design and other methodology. Therefore, a meta-analysis combining the two study designs was appropriate, with the acknowledgment that the findings are not adjusted for confounding variables.

For the remaining studies, where results could not be combined using meta-analysis due to clinical heterogeneity, I synthesised the results narratively. I displayed the results of individual studies in tables and texts as appropriate, to enable a succinct summary of the evidence. For the majority of these studies, summary measures were not reported, therefore, I calculated ORs for case-control studies and RRs for all other study designs. I performed all analyses in Stata version 14 (Stata Corp, College Station, Texas).

6.5 Results

Figure 4 shows the flow of study selection. From the search, there were 1,107 unique records, four of which were from grey literature. Upon screening, we excluded 1,029 records and assessed 78 full-text articles for eligibility. We subsequently excluded 59 studies (see Appendix 5 for excluded full-text studies with reason) and this resulted in seventeen articles that met the inclusion criteria, which were included in the synthesis.[274-278, 103, 279-287, 90, 39]

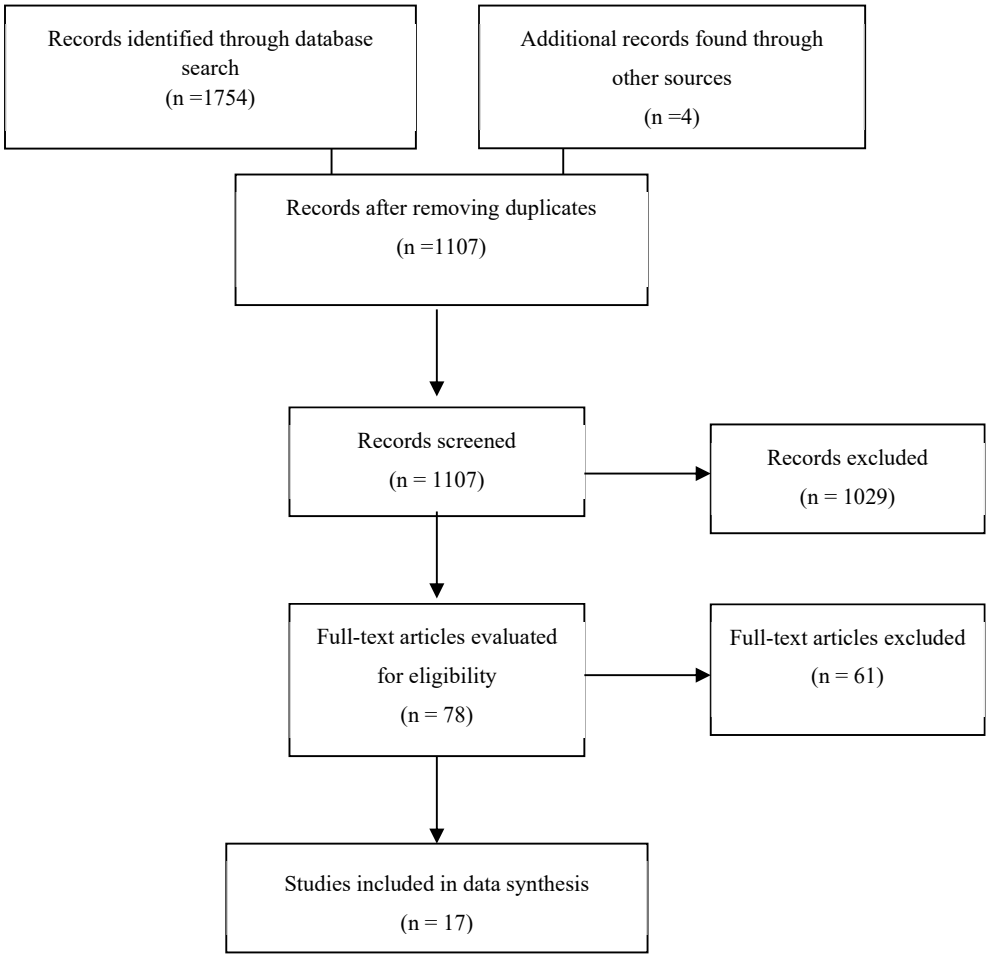


Figure 4. Flow diagram on the selection of studies for objective 2

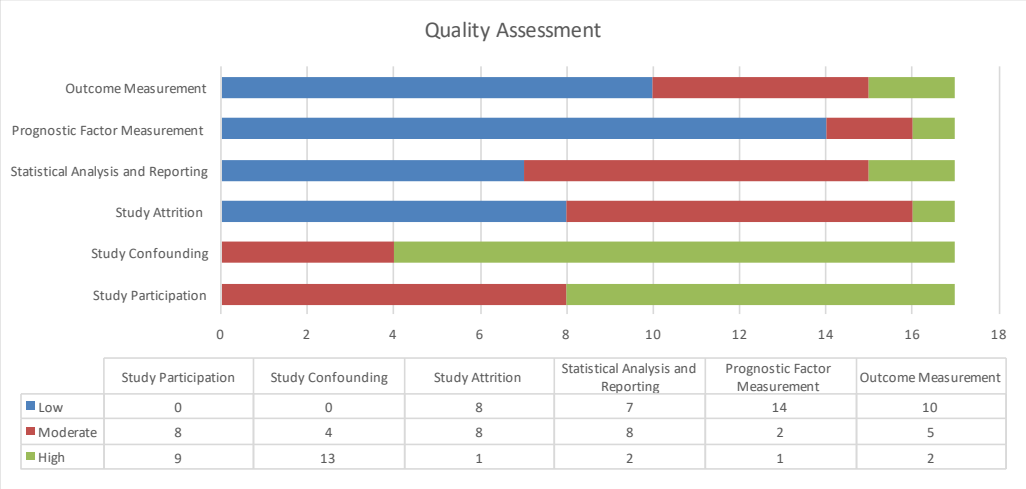
6.5.1 Characteristics of studies

The studies differed in their study designs, bacterial factors, population and the definition and measurement of GBS colonisation and EOGBS (see Table 10). Two studies were case-control studies,[277, 286] and the remaining were cohort studies. Nine studies were on vertical transmission of GBS colonisation (objectives a and b),[274, 280, 103, 281, 284, 285, 276, 282, 283] five were on maternal colonisation to EOGBS disease (objectives c and d),[281, 285, 276, 282, 283] and eight were on transition of neonatal GBS colonisation to EOGBS (objectives e).[275, 277, 286, 287, 90, 279, 278, 39] There were 13 studies published before 1990,[275, 280, 276, 281, 103, 279, 283, 284, 278, 282, 286, 287, 90] two during the 1990s,[277, 285] and two after 2000.[274, 39] There were six studies examining serotype,[274, 275, 277, 286, 287, 39] and 11 examining bacterial load.[276, 278, 103, 279-285, 90] EOGBS diagnostic criteria strictly required a positive culture from a normally sterile site in some studies, whereas in other studies it was also defined as positive urine or surface culture in the presence of symptoms of systemic EOGBS disease.

6.5.2 Risk of bias of included studies

The overall evidence from included studies had poor methodological quality as assessed by the QUIPS tool (see Figure 5).[264] None of the studies were at low risk of bias in all six domains. In 10 of 17 studies (59%), risk of bias was high in two or more domains and in 4 of 17 studies (24%), risk of bias was high in one domain. The domain with the greatest risk of bias was study confounding. Thirteen of the seventeen studies (76%) were at high risk because the study designs did not account for key potential confounders.[274, 279-281, 283, 284, 287, 90, 276, 277, 103, 278, 39] The remaining four studies were at moderate risk as there was information on some, but not all, confounding factors.[275, 282, 285, 286] Nine studies (53%) were at high risk of selection bias [274-277, 280, 281, 90, 278, 103] and the remaining eight studies were at moderate risk,[286, 287, 279, 282-285, 39] as the recruitment methods were not fully stated and/or baseline characteristics were not adequately described. Two studies were at high risk of bias for the statistical analysis and reporting domain[103, 280] and eight were at moderate risk,[275, 286, 277, 278, 281, 282, 285, 276] as there was selective reporting and/or insufficient reporting of the number of cases in each prognostic group.

Of the five studies on the serotypes associated with progression from neonatal GBS colonisation to EOGBS, two were at high,[275, 277] and three were at moderate[287, 39, 286] risk of bias for study participation, while three were at high,[277, 287, 39] and two were at moderate risk of bias for study confounding.[275, 286] One of these studies was also at high risk of bias for prognostic factor measurement,[275] and one for outcome measurement as there was no information provided on the definition and measurement of EOGBS or serotyping procedures.[39] The study on the serotypes associated with GBS transmission from mother to neonate had high risk of bias for study participation and study confounding.[274] Similarly, the risk of bias for study confounding in bacterial load studies was high in nine of the 11 studies[279-281, 283, 284, 90, 276, 278, 103] and moderate in the remaining two studies.[282, 285] Six of these studies were also at high risk of bias for study participation[280, 281, 90, 276, 103, 278] while five were at moderate risk.[279, 282-285] One bacterial load study had high risk of bias for study attrition and outcome measurement[276] and two had high risk of bias for statistical analysis and reporting.[103, 280] Only prognostic factor measurement was at low risk of bias across all bacterial load studies. The study on C-protein antigen was at high risk of bias for study participation and study confounding, as well as moderate risk of bias for statistical analysis and reporting.[277]



	Study Participation	Study Confounding	Study Attrition	Statistical Analysis and Reporting	Prognostic Factor Measurement	Outcome Measurement
Low	0	0	8	7	14	10
Moderate	8	4	8	8	2	5
High	9	13	1	2	1	2

Figure 5. Quality assessment across included studies, according to the QUIPS tool[264]

Table 10. Characteristics of studies

Study Country	Design	Participant characteristics and GBS culture method	Definition and measurement of outcome
Serotype			
Maternal GBS colonisation to neonatal GBS colonisation or EOGBS			
Al-Sweih 2005[274] Kuwait	Prospective cohort study	124 women colonised with GBS on vaginal-anorectal swabs in labour (Selective culture)	Neonates colonised with GBS on surface swabs at unspecified time (Selective culture)
Neonatal GBS colonisation to EOGBS disease			
Baker 1973[275] US	Cohort study	66 neonates: 54 asymptomatic neonates colonised with GBS on surface culture at mean age of 13.8 hours (Selective culture), 13 neonates with EOGBS disease (one patient in both groups)	EOGBS disease: ≤ 10 days (all infants developed symptoms in the first five days of life)
Baker 1974[286] US	Case control study	Neonates (numbers unclear): 53 asymptomatic neonates colonised with GBS on surface swabs at <3 days, 15 neonates with EOGBS meningitis, Not known number of neonates with EOGBS sepsis	EOGBS sepsis (clinical symptoms and pre-mortem blood cultures or post-mortem heart and lung cultures in neonates with pneumonia) or meningitis (CSF culture) ≤5 days
Chun 1991[277] US	Case-controlled study	121 neonates: 74 asymptomatic neonates colonised with GBS at birth on surface swabs, 47 EOGBS sepsis	EOGBS sepsis: < 7 days, blood and CSF culture
Embil 1987[287] Canada	Prospective cohort study	55 strains from 54 neonates: 42 asymptomatic neonates colonised with GBS on surface swabs within 1 hour of birth (Selective culture), 12 symptomatic GBS	Symptomatic EOGBS < 3 days
Madzivhandila 2011[39] South Africa	Prospective cohort study	525 neonates: 389 neonatal isolates colonised on surface swab shortly after birth (Standard culture), 136 neonates with invasive EOGBS	EOGBS: < 7 days, blood and CSF culture
Reaction to C-protein			
Neonatal GBS colonisation to EOGBS			
Chun 1991[277] US	Case-controlled study	121 neonates: 74 asymptomatic neonates colonised with GBS at birth on surface swabs, 47 EOGBS sepsis	EOGBS sepsis: < 7 days, blood and CSF culture
C protein ß antigen gene			
Neonatal GBS colonisation to EOGBS			

Study **Country**	**Design**	**Participant characteristics and GBS culture method**	**Definition and measurement of outcome**
Chun 1991[277] US	Case-controlled study	121 neonates: 74 asymptomatic neonates colonised with GBS at birth on surface swabs, 47 EOGBS sepsis	EOGBS sepsis: < 7 days, blood and CSF culture
Bacterial load: Number of positive sites			
Maternal GBS colonisation to neonatal GBS colonisation and EOGBS			
Hoogkamp 1982[280] Netherlands	Prospective cohort study	46 women colonised with GBS on throat, nose, vagina, cervix, rectum, and midstream urine swabs in labour (Selective culture)	Neonates colonised with GBS on surface swab at < 6 hours of birth (Selective swab)
Neonatal GBS colonisation to EOGBS			
Dillon 1987[278] US	Prospective cohort study	1448 neonates colonised with GBS on surface culture within 1 hour of birth (Selective culture)	EOGBS: < 3 days, symptoms and blood, CSF, urine, and other clinical specimens
Pass 1979[90] US	Prospective cohort study	290 neonates colonised with GBS on surface swabs 1-2 hours after birth (Selective culture)	EOGBS: blood and CSF culture
Bacterial load: Number of colony counts per plate			
Maternal GBS colonisation to neonatal GBS colonisation and EOGBS			
Easmon 1985[103] England	Prospective cohort study	140 women colonised with GBS on vaginal swabs in labour (Selective and standard culture)	38 neonates colonised with GBS on surface culture within 24 hours of birth and/or on discharge from hospital (Selective culture)
		141 women colonised with GBS on rectal swabs in labour (Selective and standard culture)	39 neonates colonised with GBS on surface culture within 24 hours of birth and/or on discharge from hospital (Selective culture)
Hoogkamp 1982[280] Netherlands	Prospective cohort study	46 women colonised with GBS on throat, nose, vagina, cervix, rectum, and midstream urine swabs in labour (Selective culture)	Neonates colonised with GBS on surface swab at < 6 hours of birth (Selective swab)
Neonatal GBS colonisation to EOGBS			
Gerards 1985[279] Netherlands	Cohort study	68 neonates: 47 neonates colonised with GBS on surface swabs immediately after admission to NICU (Selective culture), 21 EOGBS	EOGBS: < 7 days sepsis symptoms with GBS cultured from normally sterile culture
		66 neonates: 47 neonates colonised with GBS on surface swabs immediately after admission to NICU (Selective culture), 19 probable sepsis	Probable sepsis symptoms with surface culture but no culture from sterile site

Study Country	Design	Participant characteristics and GBS culture method	Definition and measurement of outcome
Bacterial load - Colony-forming units (CFU) per ml			
Maternal GBS colonisation to neonatal GBS colonisation and EOGBS			
Jones 1984[281] US	Prospective cohort study	130 women colonised with GBS on vaginal swabs at labour (Selective culture)	Neonates colonised with GBS on surface swabs at unspecified time (Selective culture)
Jones 1984[281] US	Prospective cohort study	130 women colonised with GBS on vaginal swabs at labour (Selective culture)	EOGBS: 2 neonates were blood culture positive, Probable EOGBS: 1 had symptoms and surface culture positive
Persson 1986[284] Sweden	Secondary analysis combined with a prospective cohort study	64 women colonised with GBS on urine swab in labour (Selective culture)	12 neonates colonised with GBS on surface culture < 5 days (Selective culture)
Sensini 1997[285] Italy	Prospective cohort study	260 women colonised with GBS on lower vaginal swabs in labour (Selective culture)	108 neonates colonised with GBS on surface culture before first bath (Selective culture)
			1 neonate with EOGBS sepsis < 24 hours , blood culture and sepsis symptoms
Bacterial load - Other			
Maternal GBS colonisation to neonatal GBS colonisation and EOGBS			
Boyer 1983[276] US	Prospective cohort study	207 women colonised with GBS on vaginal swabs in labour who gave birth to 209 neonates (Selective culture)	Neonates colonised with GBS on surface swabs in the delivery room
			EOGBS
Morales 1986 US[283]	Untreated control group of RCT	128 women colonised with GBS at labour identified by a rapid slide coagglutination test on selective vaginal culture	59 term neonates colonised with GBS on surface swabs at delivery
			3 GBS sepsis in term neonates Positive body fluid
Morales 1987[282] US	Prospective cohort study	48 women colonised with GBS in labour identified by latex agglutination on selective vaginal culture	17 preterm neonates colonised with GBS on surface swabs on admission to NICU

Study Country	Design	Participant characteristics and GBS culture method	Definition and measurement of outcome
		48 women colonised with GBS at labour identified by latex agglutination on selective vaginal culture	13 preterm neonates with GBS sepsis, blood, CSF, or urine culture, and oropharynx cultures with radiographic and clinical signs of infection

CSF cerebrospinal fluid, EOGBS early-onset GBS, GBS group B *streptococcus*, NICU neonatal intensive care unit, NT non-typeable, US United States of America

6.5.3 Serotypes

Maternal GBS colonisation to neonatal GBS colonisation

Only one study reported information on the serotypes associated with GBS transmission from mother to neonate. Al-Sweih *et al.* (2005)[274] found that mothers colonised with serotype Ia (5/11, 45%) and serotype V (13/27, 48%) on vaginal-anorectal swabs were more likely to transmit GBS than colonised mothers with serotype III (11/33, 33%), serotype Ib (1/3, 33%), serotypes not typeable (7/22, 32%), and the remaining serotypes. The calculated RR for the comparison of the proportion of women colonised with serotype V against the proportion of women colonised by all other serotypes who had a neonate with EOGBS, was not statistically significant (13/27 [48%] vs 31/97 [32%] respectively, RR 1.51 95% CI 0.93 to 2.45).

Progression from neonatal GBS colonisation to EOGBS

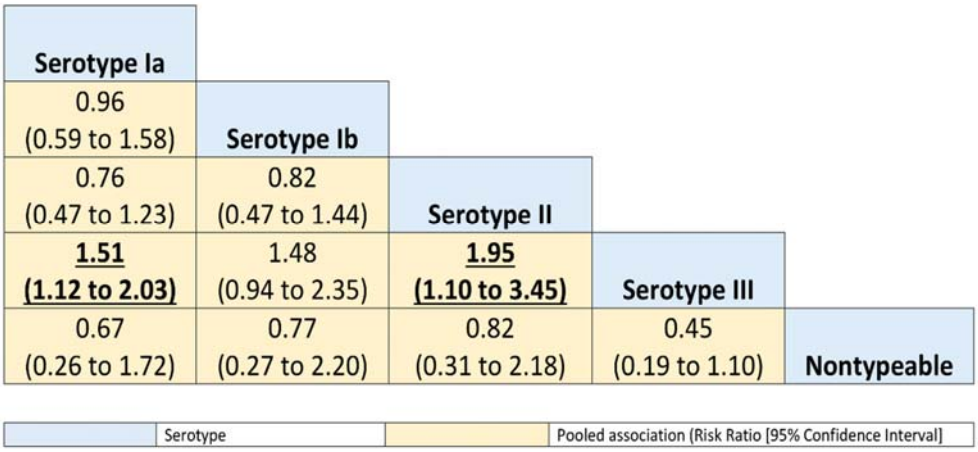
Five studies provided data on the association between serotypes and the development of EOGBS from neonatal GBS colonisation.[275, 277, 286, 287, 39] Two studies could not be meta-analysed as the required data were not available for the following reasons. Baker *et al.* (1973) reported that serotype III was more frequent in EOGBS cases than in asymptomatic colonisation. However, in this study the number of participants in the asymptomatic group was inconsistently reported, therefore, the number with each serotype could not be calculated..[275] Similarly in Baker *et al.* (1974), there was inconsistent reporting in the number of participants with GBS sepsis (reported as 51, 56 and 62 participants), therefore, the findings were unreliable and the numbers with each serotype could not be calculated.[286]

Meta-analysis results

The meta-analysis pooled the data from two cohort studies and one case-controlled study.[277, 287, 39] Figure 6 summarises the pooled RRs for the development of EOGBS by GBS serotype, and Appendix 6 shows the forest plots for the individual and pooled RRs for each GBS serotype comparison. Overall, neonates colonised by serotype III had a moderately higher risk of developing EOGBS. Neonates colonised by GBS serotype III had a higher risk of developing EOGBS than neonates colonised by GBS serotype Ia (pooled RR 1.51 95% CI 1.12 to 2.03, three studies, 439 neonates) (see Figure 6). Of 261 neonates colonised by GBS serotype III, 37.5% (n=98) developed EOGBS compared with 25.3% (n=45/178) colonised by GBS serotype Ia. The results of the individual RRs showed that only one of the three studies had statistically significant results for this comparison (see Appendix 6).[39] The I^2 statistic for

this analysis was 0.0% indicating no heterogeneity in the effect of GBS serotype on the risk of EOGBS. This might have been due to the large contribution from one study.[39]

Neonates colonised by GBS serotype III were twice as likely to have developed EOGBS as neonates colonised by GBS serotype II (pooled RR 1.95 95% CI 1.10 to 3.45, three studies, 355 neonates) (see Figure 6). Among 94 neonates colonised by GBS serotype II, 20.2% (n=19) developed EOGBS compared with 37.5% (n=98/261) colonised by GBS serotype III. Similar to the comparison with serotype Ia, the results of the individual RRs showed that only one of the three studies had statistically significant results for this comparison (see Appendix 6). The I^2 statistic for this analysis was 31.7% indicating low to moderate heterogeneity (i.e. less than 50%). There were no other statistically significant results in the risk of developing EOGBS in neonates colonised by other pairwise comparisons of GBS serotype.



Comparisons must be read from right to left. The pooled estimate is located at the intersection of the column-defining serotype and row-defining serotype. The statistically significant results are underlined and in bold.

Figure 6. Pooled risk ratios of early-onset group B *Streptococcus* by serotypes in neonates

Sensitivity analysis

The sensitivity analysis results are shown in Appendix 7. Including only the two cohort studies and excluding Chun *et al.*'s (1991) case-control study[277, 39] did not change any of the results. Neonates colonised with serotype III were still at increased risk of EOGBS compared with neonates with serotype Ia (pooled RR 1.59 95% CI 1.13 to 2.24, 377 neonates) and II (pooled RR 2.75 95% CI 1.46 to 5.21, 295 neonates). All of the remaining serotype comparisons remained statistically non-significant. Including only the two studies that explicitly required a diagnosis of EOGBS to be sterile culture positive OR did not explicitly require selective

culture (i.e. excluding Embil *et al.*, 1978)[287] showed that neonates colonised with serotype III were still at higher risk of EOGBS compared with serotype Ia (pooled RR 1.49 95% CI 1.09 to 2.02, 416 neonates). However, the difference in the risk of EOGBS in neonates colonised with serotype III compared with serotype II was no longer different (pooled RR 1.87 95% CI 0.86 to 4.03, 332 neonates). All other results remained the same.

Due to the few studies available for meta-analysis, the leave-one-out sensitivity analysis was partly completed in the other sensitivity analyses above. Removing Embil *et al.*'s (1978) study changed the results of serotype III *versus* serotype II but removing Chun *et al.'s* (1991) study did not change the results. When Madzivhandila *et al.* (2011) was removed, all results became statistically non-significant (III vs Ia: pooled RR 1.37 95% CI 0.81 to 2.34; III vs II: pooled RR 1.47 95% CI 0.84 to 2.60; see Appendix 7). This was likely as this study had the largest contributing sample size for neonates colonised with serotype III and had the only statistically significant individual RRs when comparing serotype III with Ia or II.

6.5.4 Bacterial load

Eleven studies reported on bacterial load.[276, 278, 103, 279-285, 90] The definition of bacterial load differed across studies. The number of positive culture sites was investigated in four studies,[278, 280, 90] the number of colonies on a plate in two,[103, 280] a combination of number of colonies and positive sites in one,[279] GBS colony-forming units (CFU) in three,[281, 284, 285] the number of hours by which a rapid slide coagglutination test identified GBS in two[282, 283] and selective *versus* standard culture in one.[276] The statistical results of the studies where they were provided or calculated, are shown in Table 11.

Number of colonies per plate

There were three studies reporting on the numbers of colony counts on a plate and, generally, they found that the risk of GBS transmission and EOGBS rises with the number of colonies per plate. Hoogkamp-Korstanje *et al.* (1982) found that heavy colonisation (87% transmission) was associated with GBS transmission more often than moderate (50% transmission) or light (30% transmission) colonisation.[280] Heavy colonisation was defined as greater than 50 colonies, moderate as 10 to 50 colonies, and light as less than 10 colonies using selective culture on nose, throat, vagina, cervix, rectum and mid-stream urine swabs in labour. Easmon *et al.* (1985) defined presence of GBS colonies only on enriched culture medium, fewer than 10 colonies on direct plating, 10 to 50 colonies on direct plating, and greater than 50 colonies on direct plating.[103] They reported the bacterial load results separately for rectal

and vaginal swabs. However, the labelling of the data in the paper was unclear and could not be interpreted.

Gerards *et al.* (1985) combined the number of colony counts with the number of sites and created the following criteria: heavy colonisation as three or more sites with more than 50 colonies per plate, moderate colonisation as fewer than three sites with more than 50 colonies or three or more sites with less than 10 or 10 to 50 colonies and light colonisation as fewer than three sites with less than 10 or 10 to 50 colonies.[279] Among the eight infants with heavy colonisation, 50% (n=4) had EOGBS, 50% (n=4) had probable sepsis (but no confirmatory culture from a normally sterile site), and none had asymptomatic colonisation. Among the 35 neonates with moderate colonisation 42.8% (n=15) had EOGBS, 31.4% (n=11) had probable sepsis and 25.7% (n=9) had asymptomatic colonisation. Among the 44 neonates with light colonisation 4.5% (n=2) had EOGBS, 9.1% (n=4) had probable sepsis, and 86.4% (n=38) were asymptomatically colonised. They also found that neonates colonised with more than 50 colonies of GBS were more likely to have EOGBS than neonates with less than 50 colonies. Sites swabbed were nose, throat, external auditory meatus (canal), eyes, umbilicus, skin and rectum immediately after admission to NICU.

Number of colonised sites

Equally, there were three studies reporting the number of colonised sites, and they found that the risk of GBS vertical transmission and EOGBS increased with a larger number of colonised sites. Hoogkamp-Korstanje *et al.* (1982) compared the risk of GBS vertical transmission in women with one (light) *versus* two or more (heavy) colonised sites.[280] The sites swabbed were vagina, cervix, rectum, midstream urine, throat and nose. Women with heavy colonisation were 2.5 times more likely to have a neonate with GBS than women with light colonisation (91% vs 36%, RR calculated from percentages given 2.53 95% CI 1.93 to 3.31). Two studies compared the association of one to two colonised sites (light) *versus* three to four (heavy) colonised sites in neonates, and all found a higher risk of EOGBS in neonates with heavy compared with light colonisation (Pass *et al.*, 1979: 8% [n=7/91] vs 0.5% [n=1/199], RR 15.31 95% CI 1.91 to 122.60; Dillon *et al.*, 1987: 5% [n=20/403] *versus* 0.4% [n=4/1045], RR 12.97 95% CI 4.46 to 37.70).[278, 90] Sites swabbed in these studies were external canal, umbilicus, throat and anus within one to two hours of birth[90] and external canal, umbilicus, oropharynx and rectum within an hour of birth.[278]

Colony forming units (CFU)

There were three studies that investigated the CFU of GBS agreeing that the risk of vertical GBS transmission and EOGBS increases with CFU of GBS.[281, 284, 285] Jones *et al.* (1994) plotted the CFU of GBS in mothers' vaginas against CFU of GBS in neonates' rectum and found a linear correlation ($p<0{\cdot}001$).[281] In addition, the authors found that mothers' swabs had to contain a minimum of 10^2 GBS for the neonate's swab to yield a positive result. Finally, they found that neonates colonised with $\geq 10^5$ GBS on a rectal swab were delivered by mothers colonised with $\geq 3 \times 10^4$ GBS on a vaginal swab. On the other hand, the CFU of GBS of mothers' vaginal swabs correlated poorly with neonates' umbilical and nasopharyngeal cultures. In this study, three neonates developed EOGBS: two had blood culture positive sepsis and one was rectal culture positive and had respiratory distress. All three of the neonates had mothers that were heavily colonized (7.70×10^6, 6.62×10^7, and 2.5x106), however, only two of the neonates were heavily colonized themselves (7.02×10^5, 5.25×10^6). One neonate with blood culture positive sepsis was lightly colonised ($<10^1$). The authors explained that this neonate may have been cleaned before culture.

Similarly, Sensini *et al.* (1997) defined light colonisation as 10^2 to 10^6 CFU/GBS ml and heavy colonisation as $\geq 10^6$ CFU/GBS ml, finding that mothers with heavy colonisation were more likely to transmit GBS to their neonates (50% [n=74/148] vs 30% [n=34/112] RR 1.65 95% CI 1.19 to 2.28).[285] Only one neonate developed EOGBS and the mother had light colonisation. Likewise, Persson *et al.* (1986) investigated CFU/GBS ml in the urine of mothers, and found that those with $\geq 10^4$ CFU/GBS ml were six times more likely to transmit GBS to their neonates compared with mothers with $<10^4$ CFU/GBS ml (67% [n=6/9] vs 11% [n=6/55] RR 6.11 95% CI 2.52 to 14.81).[284]

Other definitions

Morales *et al.* (1986, 1987) examined bacterial load by a rapid slide coagglutination test and categorised colonisation as heavy if agglutination with GBS antigens was detectable within five hours of swab or light if agglutination was negative at five hours but positive at 20 hours.[282, 283] They found that heavily colonised mothers in labour who gave birth to term infants were twice times as likely to transmit GBS to their neonates as mothers who were lightly colonised (80% [n=24/30] vs 36% [n=35/98] RR 2.24 95% CI 1.63 to 3.09). Heavily colonised mothers who gave birth to preterm infants were thrice as likely to transmit GBS to their neonates as lightly colonised mothers (73% [n=8/11] vs 24% [n=9/37] RR 2.99 95% CI 1.52 to 5.87).[282] In 1986, Morales *et al.* found three cases of neonatal GBS sepsis (positive

body fluid culture) in term births, all of whom had heavily colonised mothers. In 1987, the group found that GBS sepsis in preterm neonates (including culture of blood, cerebrospinal fluid [CSF], urine and oropharynx cultures with radiographic and clinical signs of infection) was four times more likely in mothers colonised with heavy bacterial load compared with mothers colonised with light load (64% [n=7/11] vs 16% [n=6/37] RR 3.92 95% CI 1.66 to 9.25).

Finally, Boyer *et al.* (1983) categorised degree of colonisation as heavy if intrapartum vaginal culture was positive on direct plate as well as selective culture, moderate if intrapartum vaginal culture was positive on selective culture only, and light if intrapartum vaginal culture was negative but postpartum rectal or vaginal culture was positive.[276] Neonatal colonisation was 3.29 times more likely in heavily compared with light or moderately colonised mothers (64% [n=69/107] vs 20% [20/102] RR 3.29 2.17 to 4.99). Of the women who transmitted GBS to their infants, heavily colonised women were more likely to have neonates colonised at multiple sites (55%) compared with moderately or lightly colonised women (30%, p=0.04). Neonatal sites swabbed were throat, umbilicus, rectum, external ear and nasogastric aspirate. The authors reported four neonates with EOGBS, and all of the mothers were heavily colonised.

6.5.5 C-protein antigen

One study investigated whether asymptomatic GBS and EOGBS (blood and CSF culture) strains reacted to C-protein antiserum and four antigens: α, β, γ, δ.[277] Chun *et al.* (1991) found that GBS isolates reacted to C-protein antiserum in 87% (n=41/47) of neonates with EOGBS and 73% (n=54/74) of asymptomatically colonised individuals, however, this difference was not statistically significant. The authors found that antigen δ was expressed more frequently in isolates from neonates with EOGBS (29%, n=12/41) compared with asymptomatically colonised neonates (19%, n=10/54). The other three antigens were found less often in EOGBS (α = 68% n=28/41, β = 17% n=7/41, and γ = 36.5%, n=15/41) compared with healthy neonates (α = 81% n=44/54, β = 28% n=15/54, and γ = 37% n=20/54). For these data, I did not calculate summary measures as more than one antigen can be expressed in one strain. When comparing antigen distribution among septic EOGBS and LOGBS with healthy neonates, the authors found a higher expression of α in healthy neonates, and of δ in septic neonates. However, this association was not independent of serotypes in multivariable analysis.

Table 11. Findings of bacterial load associated with GBS

Reference Country	Outcome reported	Definitions of bacterial load	Number/ % with no event	Number /% with event	Summary measure (95% CI)	Factor adjusted for in analysis
Number of positive sites						
Hoogkamp 1982[280] Netherlands	Neonatal GBS colonisation	Maternal GBS colonisation Light: 1 site Heavy ≥2 sites	 64% 9%	 36% 91%	*RR of heavy: 2.53 (1.93-3.31) (calculated from %)*	None
Dillon 1987[278] US	EOGBS	Neonatal GBS colonisation Light: 1-2 sites Heavy: 3-4 sites	 1041 383	 4 20	*RR of heavy: 12.97 (4.46- 3.70)*	None
Pass 1979[90] US	EOGBS	Neonatal GBS colonisation Light: 1-2 sites Heavy: 3-4 sites	 198 84	 1 7	*RR of heavy: 15.31 (1.91-122.60)*	None
Number of colony counts per plate						
Hoogkamp 1982[280] Netherlands	Neonatal GBS colonisation	Maternal GBS colonisation Light: <10 colonies Moderate: 10-50 colonies Heavy: >50 colonies	 70% 50% 13%	 30% 50% 87%	Not calculated for heavy *versus* light/moderate as no raw numbers	None
Gerards 1985[279] Netherlands	EOGBS – culture proven	Neonatal GBS colonisation Light: <3 sites positive that were <10 or 10-50 colonies per plate Moderate: <3 sites positive that were >50 colonies per plate OR ≥3 sites positive that were <10-50 colonies per plate; Heavy: ≥3 sites positive that were >50 colonies per plate	 38 9 0	 2 15 4	Moderate and heavy *versus* light: p<0.0005	None
	Probable sepsis	Neonatal GBS colonisation Light (as above) Moderate (as above) Heavy (as above)	 38 9 0	 4 11 4	*RR of heavy versus light and moderate: 3.13 (2.06-4.76)*	None
BACTERIAL LOAD - Colony-forming units (CFU) per ml						
Jones 1984[281] US	Neonatal GBS colonisation	Continuous variable of maternal GBS colonisation from 10^2 to 10^8 colony counts	See text	See text	Correlation between CFU/GBS ml in mothers' vagina and neonates' rectum: P<0.001	None
Persson 1986[284] Sweden	Neonatal GBS colonisation	Maternal GBS colonisation Light colonisation: <10^4 CFU/ml in urine Heavy colonisation: ≥10^4 CFU/ml in urine	 49 3	 6 6	*RR of heavy: 6.11 (2.52-14.81)*	None
Sensini 1997[285] Italy	Neonatal GBS colonisation	Maternal GBS colonisation Light: 10^2-10^5 CFU/ml Heavy: 10^6 or greater	 78 74	 34 74	*RR of heavy: 1.65 (1.19-2.28)*	None
	EOGBS	Maternal GBS colonisation Light: (As above) Heavy: (As above)	 111 148	 1 0	-	-
BACTERIAL LOAD - Other						
Boyer 1983[276] US	Neonatal GBS colonisation	Maternal GBS colonisation Light: Negative intrapartum vaginal culture but positive postpartum rectal/vaginal culture Moderate: Positive intrapartum vaginal culture on selective broth enrichment only	 47* 35	 10* 10	*RR of heavy versus light and moderate: 3.29 (2.17-4.99)*	None

Reference Country	Outcome reported	Definitions of bacterial load	Number/ % with no event	Number /% with event	Summary measure (95% CI)	Factor adjusted for in analysis
		Heavy: Positive intrapartum vaginal culture on direct plate as well as enrichment	38	69		
	EOGBS	Maternal GBS colonisation Light: (As above) Moderate: (As above) Heavy: (As above)	 57* 45 103	 0 0 4	-	-
Morales 1986[283] US	Neonatal GBS colonisation	Maternal GBS colonisation Light colonisation: Agglutination with GBS antigens was negative at 5 hours but positive at 20 hours Heavy colonisation: Agglutination with GBS antigens was detectable within 5 hours	 63 6	 35 24	*RR of heavy: 2.24 (1.63-3.09)*	None
	GBS sepsis	Maternal GBS colonisation Light colonisation: (As above) Heavy colonisation: (As above)	 98 27	 0 3	-	-
Morales 1987[282] US	Neonatal GBS colonisation	Maternal GBS colonisation Light colonisation: Positive latex agglutination identification at 20 hours but not at 5 hours Heavy colonisation: Positive latex agglutination identification at 5 hours	 28 3	 9 8	*RR of heavy: 2.99 (1.52-5.87)*	None
	GBS sepsis	Maternal GBS colonisation Light colonisation: (As above) Heavy colonisation: (As above)	 31 4	 6 7	*RR of heavy: 3.92 (1.66-9.25)*	None

% Percentage, CI confidence interval, EOGBS early-onset GBS, GBS group B *Streptococcus*, US United States of America
*Two extra births: 57 infants from 55 mothers
Numbers in italics calculated

6.6 Discussion

6.6.1 Principal findings

This is the first systematic review that has investigated whether bacterial load or bacterial molecular markers are associated with GBS transmission from mother to neonate, or progression from neonatal colonisation to EOGBS disease. The systematic review findings suggest that the natural history of maternal GBS colonisation, GBS transmission to neonates, and early-onset GBS disease has not been extensively researched and the study is still in its infancy. In addition to bacterial load, only three bacterial markers have been investigated. Furthermore, most of the evidence was considered at a high risk of bias and was relatively old (pre-2000). Bacterial load was consistently associated with GBS vertical transmission, and progression from asymptomatic colonisation to EOGBS regardless of its definition and measurement. The pooled comparison of serotypes in GBS colonised neonates showed that serotype III is more associated with EOGBS than other serotypes.

Of all the evidence in this review, the finding with most confidence was the association between high bacterial load and GBS transmission or EOGBS. Although the studies were at high risk of bias and did not control for confounding variables, the association was evident across different definitions of bacterial load and across study settings. Women colonised with heavy GBS bacterial load (>50 colonies, >1 site, $>10^6$ CFU/GBS ml or identification at five hours on rapid test) were approximately two to three times more likely to have a neonate with GBS colonisation compared with mothers with lighter GBS bacterial load (<50 colonies, 1 site, $<10^6$ CFU/GBS ml identification at 20 hours on rapid test). Neonates colonised with heavier compared with lighter GBS bacterial load (all definitions) were also at higher risk of developing EOGBS. While the association between bacterial load and vertical transmission of GBS colonisation (objectives a and b) and neonatal colonisation *versus* invasive EOGBS (objective e) was consistent, the evidence on the association between bacterial load and transition from maternal GBS colonisation to EOGBS was not as clear (objectives c and d). I was only able to perform statistical analysis on the data from Morales *et al.* (1987)[282] where the risk of EOGBS was almost four times higher in infants who had mothers with heavy colonisation. However, the methods of measuring bacterial load in this study were non-standard and the definition of EOGBS was not limited to sterile site culture. In other studies, neonates with EOGBS had mothers with light GBS colonisation. The uncertainty in these results is likely due to the small number of EOGBS cases in each study.

With respect to serotype, data were pooled between studies using an appropriate meta-analytic model, which resulted in quantitative estimates for the increased risk of serotype III. Neonates colonised with serotype III were approximately 1.5 to two times more likely to develop EOGBS than neonates with serotype Ia and II, respectively (objective e). However, the results of the sensitivity analysis showed that some studies may have undue influence on the pooled estimates. In particular, removing data from Madzivhandila *et al.* (2011) led to statistically non-significant results for both serotypes Ia and II compared with III. It is important to point out that there are many possible explanations for this finding. Madzivhandila *et al.* (2011) had the largest sample size of 525 neonates and this was the only study that showed a difference in the risk of EOGBS by serotype in the individual RR results.[39] Therefore, it may be that the remaining studies lacked statistical power to detect any changes. As confounding variables were not adequately adjusted for, it may also be that specific population characteristics in the study setting for Madzivhandila *et al.* (2011) contributed to the findings. Similarly, removing the data from Embil *et al.* (1978) made the difference in the risk of EOGBS in neonates colonised by serotype III compared with serotype II lose statistical significance.[287] In addition to a reduction in power (as a result of losing one out of three studies) explaining this finding, it may also be due to methodological differences between the two remaining studies from Chun *et al.* (1991)[277] and Madzivhandila *et al.* (2011),[39] as the I^2 for this comparison was high at 63%.

Finally, results on the reaction to C-protein antiserum and four antigens was only available from one study. Chun *et al.* (1991) found that reaction to C-protein was not associated with EOGBS compared with asymptomatic colonisation in neonates, neither was antigen type when serotype was accounted for (objective e).[277]

Despite the evidence on the potential value of these factors to predict GBS colonisation or EOGBS, the risk of bias across the evidence was high or moderate. No study was at low risk of bias for all domains. The most serious limitation of the evidence was the potential bias from confounding factors. None of the seventeen studies adequately adjusted for all important confounding variables in their studies and the majority did not account for any confounding variables. Similarly, as I calculated the majority of the point estimates and confidence intervals (RRs, ORs, and 95% CIs) using unadjusted statistical analyses that did not control for potential confounders, the overall relationships identified in this systematic review could be partly, or completely, a result of confounding factors. The majority of the evidence was also published before the year 2000 and may have limited applicability to today's context. For example, the standardisation of microbiological testing may be more robust in comparison with the settings in these studies and the distribution of the bacterial markers may have also changed.

Following confounding variables, selection bias was another substantial concern, as none of the studies were at low risk of selection bias. Selection bias occurs when there is a systematic error in the two groups being studied.[288] Many studies did not provide information on how participants were selected or any demographic or clinical details about the participants in the exposed or unexposed groups. Therefore, the results from this review on the association between risk factor and GBS transmission or EOGBS could potentially be distorted by the inadequate selection of participants. Related to this, some studies did not report on the number or characteristics of patients who dropped out of the study or refused to participate. Although this is less likely to affect pathogenic factors studied in the review, there is the possibility that there are differences between those who participated, and those who did not, and the impact of this is not known.

Overall, heavy bacterial load was the most convincing factor increasing the risk of GBS transmission or EOGBS because of the consistency across the studies. However, the evidence on bacterial load (and other bacterial markers) contains important uncertainties due to the high risk of bias.

6.6.2 Comparison with previous literature

Previous literature shows that serotype III, along with Ia, Ib, II and V is one of the most commonly identified invasive neonatal serotypes.[3, 289, 38, 40, 290] Globally, it was found in a review that serotype III was indeed the most commonly identified invasive neonatal serotype in all world regions.[6] It has been estimated that 80.3% of EOGBS disease is attributed to serotypes III and Ia.[39] I found that, compared with serotype Ia and II, serotype III may actually be more frequently associated with EOGBS. A Gambian study recently found that serotype V was a predominant GBS colonising serotype in women/infant pairs.[257] The authors also found that mothers with serotype III or V were more likely to have infants with the same serotype. In this review, I was unable to compare serotype V against serotype III or any other serotypes as only one study reported on serotype V.

Within serotype III strains, studies excluded from this review because of antibiotic use or no asymptomatically colonised participants found that invasive strains were more likely to be ST-17 than colonising or non-invasive strains and more likely to give rise to early-onset disease than other sequence types.[291, 292] The increased virulence of ST-17 has been demonstrated in laboratory experiments. Compared with other strains, ST-17 is more likely to

attach and invade decidual cells. [293] In particular invasive ST-17 strains are more likely to invade decidual cells than colonising strains, which are more likely to attach without invasion.[293] Likewise, a CC-17 clone specific surface protein of GBS, which promotes attachment to intestinal and meningeal cells has been indicated as an important determinant of the hypervirulence of CC-17.[52] Despite these findings, the complete mechanisms that make these serotypes of GBS hypervirulent are not fully understood. Furthermore, the invasive GBS serotype and sequence types may not be fixed and can change over time.[44]

Similarly, the finding that heavy bacterial load is associated with GBS vertical transmission and EOGBS is in line with evidence on women with GBS bacteriuria. GBS bacteriuria is a surrogate for heavy maternal colonisation, and many studies have shown that women with GBS bacteriuria have a higher risk of EOGBS.[294, 295, 86, 88] For example, Heath *et al.* (2009) found that GBS bacteriuria increased the odds of neonatal GBS disease by 5.55 (95% CI 1.47 to 20.96). However, this was not statistically significant in multivariable analyses when other factors were adjusted for, therefore, it is important to account for confounding variables. There is also a more recent study (excluded as it was conducted in the context of IAP treatment) showing that heavy neonatal colonisation defined by the number of sites is more strongly associated with EOGBS than light load (25/1000 versus 4/1000, $p<0.001$).[296] Lastly, other virulence factors such as resistance to antimicrobial peptides, factors for immune evasion, and pore-forming toxins have been suggested from laboratory studies.[47] However, I did not find these mechanisms investigated in experimental or clinical studies, which are required to confirm their role.

6.6.3 Strength and limitations

This is the first attempt to systematically review whether bacterial load or bacterial molecular markers are associated with GBS transmission from maternal colonisation, or progression from neonatal colonisation to EOGBS. I applied an extensive search with no date limit to capture as much data as possible. I included microbiology, infectious disease and obstetrician and gynaecology expert input to review the methodology and the findings to ensure that the review made clinical sense. I also involved methodologists and meta-analysts to ensure the systematic review and meta-analysis processes were performed correctly according to best practice. Furthermore, I enlisted second reviewers to duplicate study selection and quality assessment as well as cross-check data extractions, in order to maximise the quality of the review and minimise any errors.[210]

However, there are some limitations that must be noted. I excluded studies in languages besides English, therefore, there is a possibility that I may have missed prognostic studies in non-English speaking countries. Examining the studies included in this review highlights that the majority of the studies were from the US followed by Europe. Only one study was from the Middle East and one was from Africa. Many countries were not represented and it is not clear whether such studies exist in these countries. This could increase selection bias and question the external validity of the findings. Similarly, as I did not contact the authors directly, I may have missed some information where it was missing or unclear in the study report. Doing so could have clarified the data and the study could have added more value to the review.

As part of the exclusion criteria, I removed studies where participants were given IAP or studies conducted in the context of IAP. This decision was to reduce the bias from IAP interfering with the natural history of GBS transmission or progression to EOGBS. An unintended trade-off might have been that more recent studies were excluded as a result. As IAP is the recommended prevention for GBS, it may now be less feasible to study untreated women only. In the full-text sift, however, there were only four such studies. As there was a sufficient amount of data, I also excluded case-series and case-reports. Again, this could have increased the amount of data included from the literature as there are laboratory studies on small numbers of EOGBS cases. However, these studies have few conclusive implications for clinical practice as the exposed groups with disease cannot be compared with controls.[272]

6.6.4 Research and policy implications

The stubbornness of EOGBS combined with the harms from IAP underscore the need for more effective screening and/or prevention. With a large number of women who would be over-treated with IAP (due to the poor accuracy of antenatal culture) in addition to the growing list of potential harms associated with IAP, a more refined approach might be required. Antibiotic resistance, in particular, is a major international threat and, while GBS remains almost universally susceptible to penicillin,[86] 0.2% of GBS isolates had reached the upper level of susceptibility for beta-lactams in the US in 2005,[182, 18] and 5% to 15% of GBS isolates were described to have reduced penicillin susceptibility in Japan.[183] Clindamycin and erythromycin resistance has also been increasing in the last 20 years.[14, 18, 297] Bacterial factors such as bacterial load, serotype, sequence type and the more precise isolate characterisation through genome sequencing, could provide innovative opportunities to target patients with

only the hypervirulent strains of GBS, limiting the risk of harmful outcomes from widespread IAP and potentially reducing under-treatment . Bacterial load is the most promising of the factors as, despite the dissimilar measurements, it was consistently associated with GBS transmission and EOGBS. However, the current evidence has important drawbacks as discussed in Section 6.6.1.

To better understand the mechanisms of GBS and confirm that heavier bacterial load or GBS serotype III is determinant of EOGBS, larger and better-controlled studies are required. The identified factors could be studied separately and, in combination through risk factor models (possibly along with other clinical and demographic risk factors), in order to more accurately predict the mothers that will transmit GBS and have a neonate with EOGBS. Such a study may be challenging as IAP is the recommended treatment, however it may be possible that these risk factor associations and models could be investigated in prospective cohort or case-control studies in contexts where IAP prevention is not adopted, for example, in Africa or Asia. It may also be possible to conduct retrospective studies on databases from countries that already have a GBS screening policy for pregnant women at 35 to 37 weeks. Analyses could be performed on women who were screen-positive but not treated. This may be a small proportion of all screen-positive women (treated mothers would have to be excluded) but combining data across years and across countries could potentially provide a large enough database for the statistical power required. It might also be worth systematically reviewing whether serotype, bacterial load and other factors are associated with the risk of GBS transmission and EOGBS in the presence of IAP.

Although the findings from this review, particularly on heavy bacterial load but also on serotype III, could possibly be involved in guiding future prevention interventions, they cannot be currently used for clinical practice. Due to the uncertainties in the evidence, the factors identified can only be used as a starting point to guide future research on the mechanisms predictive of GBS vertical transmission and EOGBS. For now, it is still not known why some women transmit GBS to their neonates and why some neonates develop EOGBS, while others do not. Consequently, the screening criterion on the understanding of a condition's natural history, which needs to be fulfilled for the introduction of GBS screening, remains unmet.

6.7 Conclusions for this chapter

- Findings from this chapter have highlighted that serotype III and heavy bacterial load may be important factors associated with GBS transmission and EOGBS. However, most of this evidence is at high risk of bias, therefore, confounding variables might be distorting these associations.
- Furthermore, most of the evidence has been published before the year 2000 and it is not clear whether findings are as applicable today as they were around 20 years ago.
- More effective prevention and therapy are needed to combat the persistence of EOGBS and the harms from IAP treatment. Future prevention interventions could target particular serotypes or sequence types and high bacterial load.
- In particular, there is good evidence to investigate the association of the bacterial load as it was strongly and consistently associated with GBS vertical transmission and EOGBS.
- Beyond these bacterial factors, wider research on the mechanisms that underlie the natural history of GBS vertical transmission and EOGBS is essential for the development of new interventions. The factors identified here, in addition to other pathogenic, clinical and demographic risk factors (individually and in risk models) could be studied in large and robust cohort and case-control studies.
- In the meantime, the screening requirement that the natural history of a condition should be known before a screening programme is introduced is not currently met, limiting the ability to find a more efficient screening programme.

7. ADVERSE EVENTS IN WOMEN AND CHILDREN WHO RECEIVE INTRAPARTUM ANTIBIOTIC PROPHYLAXIS TREATMENT

7.1 Context of this chapter

Similar to Chapter 6, this chapter presents another part of the 2016 NSC evidence review that I led to assess the harms from IAP treatment.[24] My contribution, along with the contribution of the NSC and the other team members was the same as Chapter 6. While the overarching objectives of the 2016 review were set by the NSC, I led the design of the specific research questions and development of the research protocol outlining the methodology for the systematic review reported in this chapter. I also conducted the searches, study selection, data extraction and report writing. My supervisors and other team members contributed their technical expertise as required and conducted the second-reviewing of the systematic review processes.

7.2 Introduction

As discussed in Chapter 2, IAP is the internationally recommended treatment of EOGBS prevention. The current recommendation for IAP is intravenous penicillin (or ampicillin in the US) given as soon as possible after the onset of labour and then every four hours until delivery.[13, 14] Second-line treatment for mothers allergic to penicillin varies across countries.[13, 14] In the UK, until September 2017, intravenous clindamycin was recommended.[13] However, in the latest guideline published in September 2017, the recommendations have been modified due to the evidence of increasing clindamycin resistance.[15] If a woman has a history of allergy to beta-lactams that is not severe, i.e. does not have a history of anaphylaxis, angioedema, respiratory distress or urticaria, a cephalosporin is recommended. If a woman has a history of severe allergy to beta-lactams, vancomycin is recommended instead. Similarly, in the US since 2010, intravenous Cefazolin is the first alternative, followed by clindamycin if there is a history of anaphylaxis, respiratory distress, urticaria or angioedema after penicillin or cephalosporin.[14]

A Cochrane review summarised that the clinical practice of IAP treatment is not supported by valid evidence, due to the high risk of bias in small RCTs more than 20 years ago. While the

use of IAP did reduce the incidence of culture-proven EOGBS (RR 0.17 95% CI 0.04 to 0.74) and probable EOGBS (RR 0.17 95% CI 0.03 to 0.91) compared with no treatment, it did not reduce the incidence of all-cause mortality, mortality from GBS or mortality from other infections.[10] On the other hand, the evidence on the adverse events from IAP has not been previously reviewed. As discussed in Chapter 2, a range of harms have been suggested,[23, 13, 32] including maternal anaphylaxis, which although very rare, can be fatal for mother and neonate,[14] neonatal infections caused by gram-negative bacteria,[298, 19, 32] antibiotic resistance,[299, 298, 86] neonatal microbiota changes that could lead to short and long-term health problems,[191, 32, 192] *Clostridium difficile* infection in mothers,[21] anxiety for the mother, family and medical staff and the medicalisation of labour.[32, 13]

The harms from IAP treatment have been poorly documented and understood. This evidence is crucial to decide whether, or not, to recommend a screening programme, as it informs whether the programme does more good than harm. As indicated in Chapters 3 and 4, the 2012 NSC GBS report concluded that, as this evidence has never been reviewed, this major screening criterion was not met. As stated in Chapter 2, in the case of GBS, IAP would be given to over 150,000 pregnant women and their babies every year, of whom over 99% will be over-treated, making the harms from treatment even more important in the context of GBS screening. Therefore, in this chapter, I will explore the evidence about the adverse events to mothers and their children after IAP treatment, in order to identify the implications this may have for a GBS screening programme in the UK (objective 3).

I will first present the aim and specific objectives of the review, followed by the methods used to search, appraise and analyse the data and then report the results. Finally, I will discuss the principal findings compared with previous literature, the strengths and limitations of the review and the research and policy implications.

7.3 Aims and objectives

The aim of this chapter is to identify, appraise and meta-analyse the evidence on the adverse events experienced by women or children after intrapartum antibiotic prophylaxis treatment. I took a broad definition of adverse events to mean any adverse or harmful event experienced after IAP treatment. This included the impact on the microbiome as well as clinical outcomes.

The research objectives are to:

a) Quantify the incidence of each reported adverse event in women who received intrapartum antibiotic prophylaxis compared with women who did not receive it;
b) Quantify the incidence of each reported adverse event in neonates whose mothers received intrapartum antibiotic prophylaxis compared with neonates whose mothers did not receive it;
c) Quantify the overall incidence of any reported adverse events in women who received intrapartum antibiotic prophylaxis compared with women who did not receive it;
d) Quantify the overall incidence of any reported adverse events in neonates whose mothers received intrapartum antibiotic prophylaxis compared with neonates whose mothers did not receive it; and
e) Identify any important gaps in the evidence on adverse events after intrapartum antibiotic prophylaxis.

7.4 Methods

As discussed in Chapter 4, I applied systematic review methodology to address the objectives of this chapter. Evidence on the harms of IAP treatment had not been previously synthesised. It was important to understand the status of the evidence, identify research gaps and determine the direction required for future research to avoid replication and wasting of resources. Furthermore, the harms from IAP could be experienced long-term and a primary study to investigate this was beyond the scope of my thesis. To ensure high standards were maintained, I reported this systematic review according to recommendations from the PRISMA-P 2015 statement.[210] The protocol is registered at PROSPERO: CRD42016037195.

7.4.1 Search strategy

I conducted comprehensive electronic literature searches in well-known and recommended databases: MEDLINE (Ovid), MEDLINE In-Process & Other Non-Indexed Citations (Ovid), EMBASE (Ovid), Cochrane Library: Cochrane Database of Systematic Reviews, CENTRAL, DARE and HTA databases (Wiley) and Science Citation Index Expanded (Web of Science) from inception to 16th October 2016. I initially piloted scoping searches and then iteratively adapted them with input from the team and recommended search filters to inform the development of the final search strategy.[300, 301] In the final strategy, I combined three sets of

search terms using both text words and MeSH terms through Boolean operators OR within each set and then AND to combine the sets. The first set was made up of search terms for antibiotic prophylaxis, the second set was made up of search terms for labour and the third set was made up of search terms for adverse events. I limited the search to antibiotics for prophylactic purposes during labour. In preliminary searches, I discovered that some relevant articles did not include terms for adverse events. Therefore, I included terms for known adverse events from IAP such as antibiotic resistance or maternal anaphylaxis. To do this systematically, I used adverse events that are known from the literature,[32, 28, 13, 23] and I enlisted subject area experts as suggested by the UK NSC for further terms. I applied an extensive search strategy with no date limit to capture as much data as possible, but I limited the strategy to humans as this was the population of interest, and to the English language, as I did not have the time or resources to translate studies in other languages (see Appendix 8 for search strategies). Although excluding non-English studies could introduce selection bias,[258-260] the impact of this is not clear in the literature, with some reviews showing that it does not affect results.[261, 262] Furthermore, methodologists have suggested that the impact of language bias has reduced recently as a result of the move towards publishing in English.[263]

I also searched grey literature to reduce reporting bias. Along with a second reviewer, we hand-searched reference lists of all included studies and relevant systematic reviews that were identified from the electronic searches. In addition, subject area experts cross-checked the included studies to identify any further references not captured by the search. The team members of this project also cross-checked the included studies.

7.4.2 Study eligibility criteria

Study inclusion criteria

I included studies that satisfied the following criteria:

Study design: prospective or retrospective cohort studies, case-control studies and randomised controlled trials. If the search resulted in an insufficient number of these studies, I included case series with ≥50 patients. If the search still resulted in an insufficient number of studies, I included case series with ≥10 patients. I avoided case series as far as possible, as results from such studies do not have control groups for comparison, making it difficult to interpret whether an adverse event is actually more common in those who have undergone treatment.

Participants: intrapartum women and their children.

Intervention: intrapartum antibiotics given to asymptomatic women for a prophylactic purpose only. The antibiotics could be for any prophylactic purpose and not GBS prevention alone, however, it had to be administered in labour. I also included studies in which women received antibiotics in labour, and then continued to receive antibiotics after labour for prophylactic purposes, so long as women remained asymptomatic.

Comparator: placebo, no treatment or an alternative treatment for prophylactic purposes (comparison of one treatment to another). No comparator for case-series.

Outcome: any adverse outcomes experienced by the mother or child. The criteria for outcomes was intentionally wide to capture as many outcomes as possible.

Type and Language of publication: full-text primary studies published in the English language in medical and healthcare journals or in grey literature, such as organisational websites.

Study exclusion criteria

I excluded studies that fulfilled the following criteria:

Study design: ecological studies, cross-sectional studies, case reports, before and after studies across different participants (e.g. population-level resistance studies). I excluded before and after studies as control participants are not contemporaneous, therefore, adverse outcomes could be a result of other factors other than IAP treatment.

Participants: I excluded the following participants as they were not considered clinically similar enough to the population of women and children who would be receiving IAP for GBS prevention: a) pregnant women given antibiotics before labour; b) neonates given antibiotics after birth; c) pregnant women undergoing elective or emergency caesarean sections; and d) women with symptoms of infection such as intrapartum fever or prolonged rupture of membranes before IAP administration. I excluded women with symptoms of infections as this would contaminate the findings, making it unclear whether the adverse events are a result of the treatment or the infection. I included studies where some participants met the inclusion criteria, and some met the exclusion criteria, if participants meeting the inclusion criteria could be separated or participants that met the exclusion criteria were fewer than 10% of the study population.

Intervention: antibiotics given for any other purpose than prophylaxis. I excluded prophylaxis for caesarean sections as women and their children who have undergone caesarean sections may also contaminate the findings making it unclear whether the adverse events were a result of the caesarean section or the antibiotics.

Outcomes: Studies reporting only economic evaluation and/or cost-effectiveness outcomes.

Type and Language of publication: abstracts, reviews (systematic or non-systematic), editorials, letters, books, consensus statements and opinions. I excluded reviews as sources of primary data but used them to identify the original studies contributing the evidence. I excluded all publications in any language other than English.

7.4.3 Study selection and data management

I downloaded identified references to bibliographic management software (Endnote X7) and de-duplicated them. A second reviewer and I independently screened the titles and abstracts of all identified bibliographic records (screening level I). I then obtained full-text reports of all potentially relevant records identified at screening level I and the second reviewer and I assessed them independently using the same study eligibility criteria (screening level II). We resolved any disagreements over inclusion/exclusion at screening level I and II by discussion and involved a third reviewer where necessary. I documented the study flow and reasons for exclusion of full-text papers in a PRISMA study flow diagram.[210]

7.4.4 Data extraction

A second reviewer and I independently extracted relevant data using an *a priori* defined extraction sheet that I piloted and refined with team members before implementation (see Appendix 9). We crossed-checked each other's data extraction forms and resolved any disagreements by discussion, involving a third reviewer if necessary. The extracted data included study characteristics (year of publication, author, country of origin, study design, study setting, sample size, sampling strategy, follow-up duration), participant characteristics (socio-demographic characteristics, study eligibility criteria, co-morbidities), details of the intervention (antibiotic type, route of administration, treatment dose, treatment duration, treatment indication), adverse outcomes (name and definition of outcomes, methods and measurements of adverse outcome, timings of measurements) and the result for the association between IAP and the adverse events, (numbers with event in treatment and control, OR, RR, RD, mean difference) and variability measures (e.g. 95% CI, p-values). Where data permitted,

I estimated any missing statistical parameters of importance (e.g., OR, RR) and variability measures (e.g., 95% CI, p-values) using methods outlined in the Cochrane Handbook for Systematic Reviews of Interventions.[263] I denoted all calculated or derived data as 'calculated'.

7.4.5 Quality assessment

The second reviewer and I independently appraised the risk of bias for each included study using the Cochrane Risk of Bias (RoB) tool for studies in which participants were randomised to either receive IAP or placebo/no treatment,[302] and the Risk of Bias Assessment Tool for Nonrandomised Studies (RoBANS) for non-randomised studies.[303] Both tools are published and validated. The RoBANS tool is based on the Cochrane RoB tool but adapted with questions and probes specific to observational study types. Therefore, the biases assessed across both tools are consistent. The tools include information on selection bias (sequence generation, allocation concealment OR sample population, confounding variables), performance bias (blinding, outcome assessment), detection bias (blinding, outcome assessment), attrition bias (incomplete outcome data) and reporting bias (selective outcome reporting). Each of these domains is assessed and classified as low, high or unclear risk of bias.

The second reviewer and I discussed each quality appraisal and resolved any disagreements by discussion, involving a third reviewer where necessary. I tabulated the individual item-specific quality assessment ratings for each study.

7.4.6 Data synthesis

I intended to perform meta-analyses using a separate random effects model for the likelihood of each adverse event and then a random effects model for the likelihood of any adverse events if data were sufficiently similar. However, as there was a range of different adverse events reported in the literature, using different drug regimens for different durations, on different populations, I concluded that there was too much clinical heterogeneity in the studies to pool the results. Instead, I narratively synthesised the results of the individual studies in tables and text to enable a succinct summary of the evidence. I used Stata 14 (Stata Corp, College Station, Texas) to calculate the summary measures such as RRs, ORs, and RDs, along with 95% CI where they were not calculated but data were available to do so.

7.5 Results

7.5.1 Study selection

Figure 7 shows the flow of study selection. From the search, there were 2,364 unique records. Upon screening, we excluded 2,102 records and there were 262 full-text articles for eligibility. Subsequently, we excluded 232 studies (see Appendix 10 for excluded full-text studies with reason) and this resulted in 30 articles that met the inclusion criteria, which were included in the synthesis.[304-322, 296, 194, 323-328, 20, 329, 330]

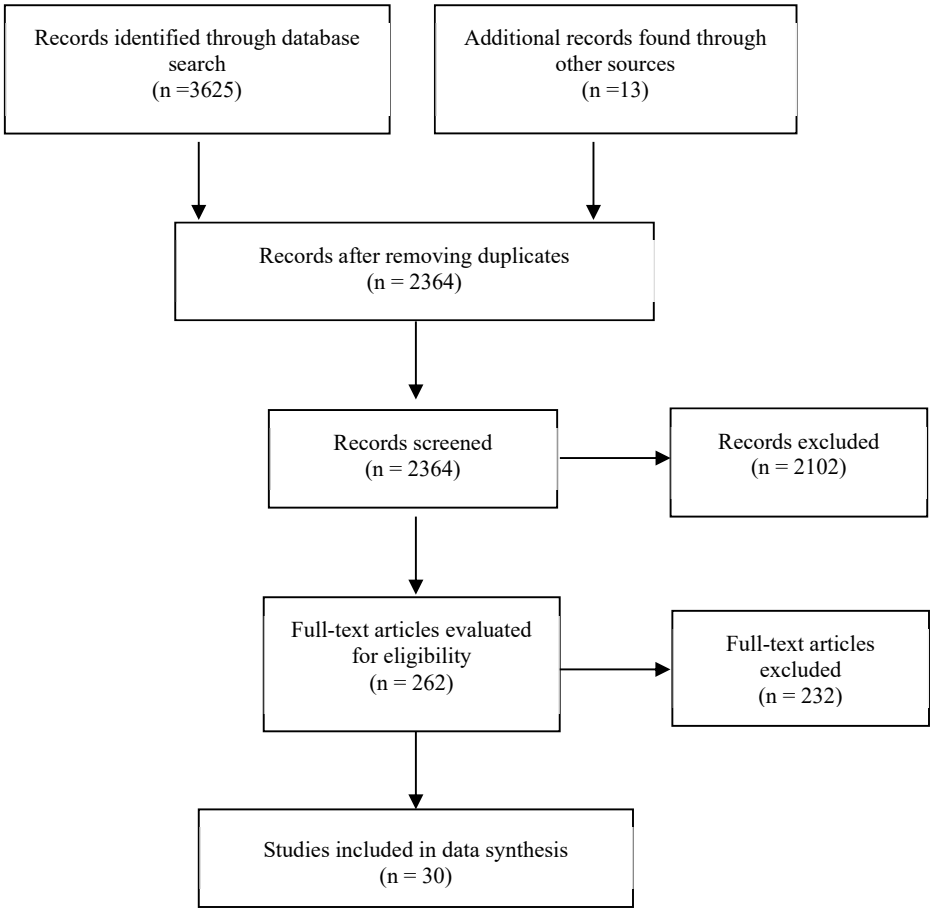


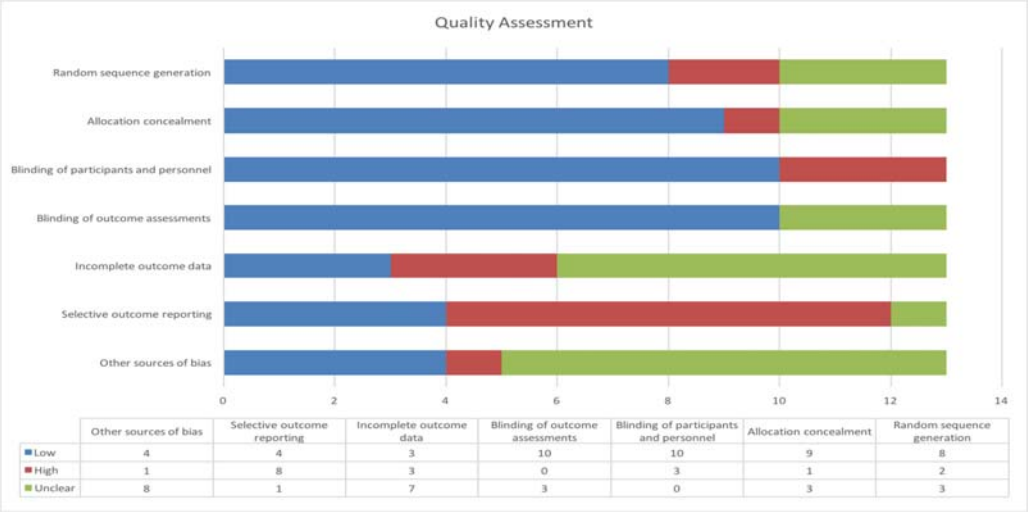
Figure 7. Flow diagram on the selection of studies for objective 3

7.5.2 Characteristics of studies

There were 14 cohort studies, [304-307, 309-311, 313, 316, 321, 296, 194, 20, 330] three case-control studies,[308, 314, 328] 12 RCTs[312, 315, 317-320, 322-326, 329] and one sub-study[327] of an included RCT.[326] There were nine studies investigating IAP for GBS prevention,[304, 305, 308-311, 316, 194, 328] two for GBS prevention and other indications,[313, 321] three for post-partum infection prevention,[317-319] eight for preterm labour,[312, 315, 320, 322-325, 329] two for neonatal sepsis prevention[326, 327] and six that did not state the indication (see Appendix 11 for study characteristics).[306, 307, 314, 296, 20, 330] There were some trials that reported outcomes of interest, such as neonatal and maternal infection, however, the aim of the trials was to investigate the benefit of IAP on these outcomes. If IAP was successful, it could decrease the outcomes. On the other hand, IAP could inadvertently increase these outcomes as it could lead to changes in the organism causing infections and/or antibiotic susceptibility.[20, 298] In order to prevent reporting bias, I reported these outcomes irrespective of whether they were identified as harms or as benefits by the study. I have reported these outcomes separately at the bottom of the table in Appendix 11.

7.5.3 Risk of bias of included studies

Figure 8 shows the methodological quality of the included RCTs, as assessed by the Cochrane Risk of Bias tool.[302] None of the RCTs were at low risk of bias across all domains. The largest risk of bias amongst the RCTs was in the selective outcome reporting domain; eight of 13 RCTs (62%) were at high risk partially or exclusively because they did not pre-specify the definition and measurement of side effects in the methods but only reported them in the results.[312, 315, 317-319, 325, 327, 329] The second greatest risk of bias was in the incomplete outcome data domain, where seven out of 13 (54%) RCTs were at unclear risk of bias as there were substantial missing data, for example, on the adverse events in the control group.[317-319, 322, 325, 326, 329] Finally, I found a number of other sources of bias across RCTs. This included data not presented,[318, 319] relatively small sample sizes,[323, 327] a lack of information on treatment regimens,[315] a lack of details of intention to treat analysis[329] and inaccuracies in the numbers provided for participant flow.[325]



	Other sources of bias	Selective outcome reporting	Incomplete outcome data	Blinding of outcome assessments	Blinding of participants and personnel	Allocation concealment	Random sequence generation
Low	4	4	3	10	10	9	8
High	1	8	3	0	3	1	2
Unclear	8	1	7	3	0	3	3

Figure 8. Quality assessment across included randomised controlled trials, according to the Cochrane risk of bias tool[302]

Similar to RCTs, there were no observational studies at low risk of bias across all domains of the RoBANS tool (see Figure 9).[303] Confounding variables was the domain with the highest concern. Four of 17 (24%) studies were at high risk,[304, 309, 313, 20] none at low risk and 13 (76%) at unclear risk of bias.[305-308, 310, 311, 314, 316, 321, 296, 194, 328, 330] In these studies, some variables were reported or accounted for in the study design, but others such as prenatal antibiotics, caesarean sections and maternal risk factors were not. Similarly, the selection of participants domain was also unclear across nine out of 17 (53%) studies,[305-307, 316, 296, 194, 328, 20, 330] as important baseline characteristics and/or how participants were selected, was not reported.

	Selective outcome reporting (Reporting bias)	Incomplete outcome data (Attrition bias)	Blinding (Detection bias)	Measurement of exposure (Performance bias)	Confounding variables (Selection bias)	Selection of participants (Selection bias)
Low	9	11	14	11	0	7
High	2	1	0	0	4	1
Unclear	6	5	3	6	13	9

Figure 9. Quality assessment across included observational studies, according to the RoBANS tool[303]

7.5.4 Adverse events associated with IAP (objectives a to d)

The 30 studies assessed a range of neonatal and maternal outcomes (see Appendix 11). Below I present the findings on the key results of gut microbiota, antibiotic resistance, neonatal respiratory distress, neonatal bacterial infections, candidiasis, anaphylaxis and long-term adverse events. Findings on the remaining outcomes such as necrotising enterocolitis, *Clostridium difficile* bowel problems, Apgar scores, seizures, maternal infection, bleeding abnormalities and the impact on management and care can be found in Appendix 11. There was no evidence on anxiety, asthma, autism, obesity, supra-infections, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, extended spectrum beta-lactamase-producing organisms or carbapenem-resistant organisms.

Gut microbiota

Gut microbiota changes in babies have been associated with long-term health problems, including respiratory and metabolic conditions. Seven cohort studies compared the colonisation levels of various microbial groups at different points in time in infants whose mothers were treated with IAP with those who were not.[304-307, 311, 316, 194] None of the studies were at low risk of bias for confounding variables and one was at high risk of selection bias.[304] The studies consistently revealed that IAP alters infant microbiota. There were differences in the relative composition and the colony forming units per gram (log CFU/g) of organisms in the gut of newborns whose mothers were treated with IAP with those who were not, at day 2, 3, 6 to 7, 10, 30 and 90 (see Table 12 and Table 13 for results).[305-307, 311, 316, 194] Two studies reported sample richness and biodiversity and found that, compared with controls, at days 6 to 7 and day 30, infants whose mothers were treated with IAP had a less diverse microbial profile.[305, 194] There was also a clear separation between the microbiota profiles of infants whose mothers were treated with IAP, compared with control infants, when plotted on principal coordinate analysis plots at days 6 to 7. However, these disappeared by day 30.[305, 194] Related to gut microbiota, Keski-Nisula *et al.* (2013) found that, compared with controls, neonates whose mothers were treated with IAP had a lower transmission of vaginal *Lactobacillus*-dominant mixed flora on oral surfaces (1 vs 13, OR 0.08 95% CI 0.007 to 0.80).[321] While the evidence of gut microbiota alterations was consistent, it is not clear if any of the alterations are related to clinical adverse events.

Antibiotic resistance

Six studies reported antibiotic resistance. Four were observational studies and two were RCTs. Of the RCTs, Gordon *et al.* (1995) reported zero out of 58 cases of multi-resistant bacterial

infections in mothers who were treated with IAP for preterm labour, however, they did not report on the number of cases in the control group.[315] In another RCT, Roca *et al.* (2016) investigated resistance of GBS, *Streptococcus pneumoniae* (*S. pneumoniae*), and *Staphylococcus aureus* (*S. aureus*) to azithromycin in 829 mothers and 843 infants treated with azithromycin for neonatal sepsis prevention.[326] They found azithromycin-resistant *S. aureus* in maternal breast milk at day 3, in newborn nasopharynx and maternal breast milk at day 6, in vaginal swabs at day 8 and in newborn and maternal nasopharynx and maternal breast milk at day 14 and day 28. They also identified azithromycin-resistant *S. pneumoniae* at day 28 in the maternal nasopharynx only. This study was at an unclear risk of bias for incomplete outcome data.

Of the four observational studies, two were case-control studies,[308, 314] one was a prospective cohort study[316] and one was a retrospective cohort study.[20] There was a high risk of selective outcome reporting bias in Ashkenazi-Hoffnung *et al.* (2011),[308] and a high risk of bias for confounding variables in Stoll *et al.* (2011).[20] There was also an unclear risk of bias in the remaining studies for confounding variables,[308, 314, 316] unclear risk of selection bias and bias in the measurement of exposures in Jaureguy *et al.* (2004)[316] and Stoll *et al.*,[20] as well as an unclear risk of bias for incomplete data in Stoll *et al.*[20]

Glasgow *et al.* (2005) found that 39% (n=24/62) of infants whose mothers were treated with various IAP drugs (indication not stated) had ampicillin-resistant organisms compared with 11% (n=13/120) of infants whose mothers were not treated (OR 5.7 95% CI 2.3 to 14.3).[314] They also found a difference in the ampicillin-resistant bacteria only causing urinary tract infections (OR 4.3 95% CI 1.6 to 11.7). Similarly, Stoll *et al.* (2002) found a higher number of mothers who received intrapartum ampicillin in the group of infants with ampicillin-resistant strains of *E. coli* compared with the number of mothers who received IAP in the ampicillin-sensitive group (93% [n=26/28] vs 20% [n=1/5] p=0.01).[20] In the two studies, it was not clear if the infants were treated with antibiotics before or after they were tested for antibiotic resistance. Ashkenazi-Hoffnung *et al.* (2011) did not report any differences in first generation cephalosporin resistance in *E. coli* (60% vs 22.7% p=0.21) or any bacteria causing late-onset serious bacterial infections (57% vs 26% p=0.19) when comparing 17 infants born to mothers treated with IAP for GBS prevention and 178 infants who were not.[308] However, they did report a higher development of first generation cephalosporin-resistant urinary tract infections (75% vs 23.5% p=0.04). They also found no difference of ampicillin resistance in *E. coli* (100% vs 54.5% p=0.14) or any bacteria causing late-onset serious bacterial infections (85% vs 63% p=0.19) Finally, Jaureguy *et al.* (2004) investigated colonisation of amoxicillin-resistant *Enterobacteriaceae* and aerobic and anaerobic gram-positive bacteria in the gut of

neonates whose mothers were and were not treated with IAP for GBS. They did not find any difference in the number of infants colonised with amoxicillin-resistant *Enterobacteriaceae* (40% [n=10/25] vs 48% [n=12/25], RR [calculated] 0.83 95% CI 0.44 to 1.56) and amoxicillin-resistant *E. coli* (24% [n=6/25], vs 44% [n=11/25] RR [calculated] 0.55 95% CI 0.24 to 1.25).[316]

Neonatal respiratory problems

There were four RCTs investigating respiratory problems in infants whose mothers were, and were not, treated with IAP for preterm labour.[312, 320, 322, 329] Two were at high risk of bias in selective outcome reporting and/or incomplete data.[312, 329] None of the trials found a difference between the two groups for medication for chest problems, admission for chest problems, wheezing, ventilation or respiratory distress syndrome.

There was one observational study investigating the risk of respiratory distress and discharge diagnosis of a respiratory disorder.[296] The study had no domains at high risk of bias and accounted for a number of confounding variables, including comorbidities during labour. However, it was at unclear risk of selection and detection bias, and because it was observational, there could be other factors that are related to the risk of respiratory distress. The authors found a higher risk of respiratory distress (21% [n=44/213] vs 7% [n=95/1378] RR 2.62 95% CI 1.79 to 3.83) and discharge diagnosis of a respiratory disorder (7% [n=12/213] vs 3% [n=39/1378] RR [calculated] 1.96 95% CI 1.04 to 3.69) in the group treated with IAP for GBS.

Candidiasis

Two studies reported the relationship between IAP and candidiasis. Cox *et al.*'s (1996) RCT showed that 27 of 39 (69%) participants had symptomatic vulvovaginitis caused by *Candida albicans* after treatment for preterm labour with ampicillin and sulbactam followed by ampicillin-clavunate for five days.[312] The authors did not report on the number of cases present in the control group, therefore, the RCT was at high risk of bias for the selective reporting and incomplete data domains.

In a retrospective cohort study, Dinsmoor *et al.* (2005) studied neonatal and maternal candidiasis after IAP for GBS prevention and other indications.[313] This study was at high risk of bias for confounding variables, as variables such as the administration of antenatal antibiotics were not accounted for. The measurement of exposure, blinding and selective outcome reporting domains were at unclear risk of bias. Finally, the diagnosis of thrush was

not confirmed by an examination but based on participant report and whether treatment was prescribed. The authors did not find a difference in neonatal thrush between the neonates whose mothers were and who were not treated (12% [n=21/173] vs 7% [n=18/262], OR 1.87 95% CI 0.97 to 3.63). However, they did find a higher risk of maternal thrush in the treated group (13% [n=22/173] vs 6% [n=17/262], OR 2.1 95% CI 1.08 to 4.08).

Neonatal infections

As mentioned earlier, although the purpose of IAP is to reduce neonatal infection, an adverse event is that IAP can potentially increase neonatal infection because of the changes it can cause in the organism producing infections.[20, 298] Here, I report all studies that investigated neonatal infection (whether as a benefit or adverse event). There were four RCTs,[315, 322, 324, 329] three case-control studies[308, 314, 328] and one cohort study.[20]

The four RCTs investigated neonatal infections when assessing the benefit of IAP in preventing preterm labour. Two of the trials had a high risk of bias,[315, 329] and three had an unclear risk of bias,[322, 324, 329] in one or more domain. Nadisauskiene *et al.* (1996) found a lower proportion of neonatal infections in infants whose mothers who were treated with IAP compared with those who were not (9% [n=4/44] vs 21% [n=38/58], RR [calculated] 0.14 95% CI 0.05 to 0.36).[324] The remaining RCTs did not find a difference in neonatal sepsis, meningitis, pneumonia, all infections or positive cultures between treated and untreated groups.

Of the four observational studies, two had a high risk of bias in selective outcome reporting[308, 328] and one had a high risk of bias in confounding variables.[20] Glasgow *et al.* (2005) found a higher proportion of late-onset bacterial infections in infants whose mothers were treated with IAP (indication not stated) compared with those who were not (60% [n=37/62] vs 44% [n=53/120], OR 1.96 95% CI 1.05 to 3.66).[314] The relationship between IAP and infections was attributed to broad spectrum IAP as opposed to penicillin IAP, as when compared separately, only those treated with broad spectrum IAP compared with no broad spectrum IAP had a higher risk of infections (OR 4.95 95% CI 2.04 to 11.98). There was also a higher number of late-onset meningitis, omphalitis and bacteraemia without UTI in the treated group (OR 25.00 95% CI 1.8 to 346).

By contrast, Stoll *et al.* (2002) found no difference in all-cause sepsis (2% [n=63/3,554] vs 1% [n=21/1,893], OR 1.1 95% CI 0.6 to 1.8]) or *E. coli* early-onset sepsis (2% [n=58/3,554] vs 1% [n=26/1,893], OR: 1.0 95% CI 0.6 to 1.6) between neonates whose mothers were treated with IAP (indication not stated) and those who were not.[20] There was a difference in early-

onset *E. coli* sepsis with ampicillin when comparing IAP given within 72 hours of delivery compared with no IAP within 72 hours, however, this lost statistical significance when controlling for gestational age and the interval between membrane rupture and delivery. Total early-onset sepsis was also not associated with IAP use. Likewise, Sinha *et al.* (2003) did not find a difference in the proportion of bloodstream infections (RR 0.20 95% CI 0.011 to 3.6), pneumonia (RR 2.5 95% CI 0.43 to 14.0) or any infection syndrome (RR 1.0 95% CI 0.38 to 2.9),[328] and neither did Ashkenazi-Hoffnung *et al.* (2011) in late-onset serious bacterial infections (47% [n=8/17] vs 10% [n=17/178] OR per dose of IAP 5.1 95% CI 0.01 to 93.11).[308] The treatment in the both studies was IAP for GBS prevention.

Anaphylaxis and other short-term side effects to antibiotics

Eight RCTs investigating the effectiveness of IAP in preventing preterm labour or post-partum infection reported on anaphylaxis and other immediate side effects to antibiotics.[317-319, 322, 323, 325, 326, 329] There were five RCTs at high risk of bias[317-319, 325, 329] and one at unclear risk of bias for selective reporting,[323] and seven at unclear risk of bias for incomplete outcome data[317-319, 322, 325, 326, 329] and other sources.[318, 319, 322, 323, 325, 329]

There were no differences in the side effects between treated and control groups in two RCTs. McGregor *et al.* (1986) found no differences in the number of women who suffered from nausea or vomiting (1/29 in each group)[323] and Rajaei *et al.* (2006) reported no differences in nausea, vomiting, hot flushes, decreased deep tendon reflexes, emotional disturbances and drug intolerance between groups.[325] Keuchkerian *et al.* (2005) and Svare *et al.* (1997) found more palpitations, flushes, nausea and vomiting[322] and undefined side effects[329] in treated compared with control groups, but these did not reach statistical significance (2/47 [4%] vs 0/49 [0%][322] and 4/59 [7%] vs 1/51 [2%][329]).

Keettel *et al.* (1949, 1950), Kampikaho *et al.* (1993) and Roca *et al.* (2016) reported side effects in the treatment group only. Kampikaho *et al.* (1993) reported zero undefined side effects from streptomycin or penicillin (0/330 women).[322] Keettel *et al.* (1949) reported seven mild urticaria (2%), two general urticaria (0.4%), five local allergic manifestations (1%) and no abscess formations (0%) in 465 treated participants, as well as relatively uncommon discomfort at the site of injections which was never severe or persistent.[319] In 1950, Keettell *et al.* found one general urticaria (0.3%), one local allergic manifestation (0.3%) and no abscess formations in 382 treated participants.[318] Roca *et al.* (2016) found one case of moderate urticaria and zero ‘adverse event/serious adverse events’ in 419 newborns whose mothers were treated.[326]

Long-term adverse events

A factorial RCT by Kenyon *et al.* (2008) was the only study that specifically assessed the long-term effects of IAP. Kenyon *et al.* (2008) compared children aged seven whose mothers received any erythromycin (alone or with amoxicillin-clavulanate) with no erythromycin, and any amoxicillin-clavulanate with no amoxicillin-clavulanate (alone or with erythromycin).[320] This RCT had a low risk of bias in all main domains, however, there were key limitations in the 'other biases' domain. A large number of statistical analyses were conducted on a relatively small sample size, which could increase the probability of getting a statistically significant result due to chance. Outcomes were also parent-reported and children were not individually assessed.

The authors found that IAP might be associated with serious consequences of cerebral palsy, functional impairment and bowel problems. The risk of cerebral palsy was higher in infants whose mothers were treated with any erythromycin compared with no erythromycin (3% [n=53/1,611] vs 2% [n=27/1,562], OR 1.93 95% CI 1.21 to 3.09) and in mothers who received any amoxicillin-clavulanate *versus* no amoxicillin-clavulanate (3% [n=50/1,587] vs 2% [n=30/1,586], OR 1.69 95% CI 1.07 to 2.67). A higher number of children who developed cerebral palsy were born to mothers who received both antibiotics (35/735) than to mothers who received erythromycin alone (18/785), amoxicillin-clavulanate alone (15/763) or double placebo (12/735) (OR 2.91 95% CI 1.50 to 5.65). Any erythromycin also increased the risk of bowel problems (4% [n=64/1,611] vs 2% [n=38/1,562], OR 1.66 95% CI 1.10 to 2.49) and functional impairment (42% [n=658/1,554] vs 38% [n=574/1,498], OR 1.18 95% CI 1.02 to 1.37) compared with no erythromycin; amoxicillin-clavulanate did not. None of these effects were found for erythromycin or amoxicillin-clavulanate alone compared with placebo, which may be a result of insufficient power. The authors also investigated attention deficit hyperactivity disorder, behavioural problems, diabetes, education attainment and other development problems, but they did not find any differences between any of the treatment *versus* control groups.

There was one observational study that followed children up to two years of age and investigated the relationship between IAP (indication not stated) and atopic dermatitis in a retrospective cohort study. This study was at unclear risk of selection bias as the response rate was only 43% and not all confounding variables were accounted for. Compared with no treatment, only participants whose mothers were treated with more than 24 hours of IAP were at a higher risk of atopic dermatitis (55% [6/11] vs 27% [100/364], RR 1.99 95% CI 1.13 to 3.49). IAP for 0 to 4 hours, 4 to 12 hours or 12 to 24 hours was not associated with dermatitis.

Table 12. Qualitative composition of gut microbiota in IAP-treated and untreated infants

Organisms	**Reference Year**	**Infants (n) in each group**	**Relative amount in microbiota composition (%) or infants (*n*) colonised**					
			Day					
			1	**2**	**3**	**6/ 7**	**10**	**30**
Phyla level								
All	Arboleya 2016	IAP n=14 Control n=13	No difference					
Actinobacteria	Aloisio 2016*	IAP n=10 Control n=10				IAP: 0.4% Control: 3.8% p<0.05		
	Arboleya 2016	IAP n=14 Control n=13						Lower % in IAP, p<0.05
	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7				IAP: 0% Control: 17% p<0.001		
		Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 1% Control: 8% *RR 0.13 (CI 0.02-0.98)*		IAP: 7%
Bacteriodetes	Aloisio 2016*	IAP n=10 Control n=10				IAP: 16% Control: 47.7% p<0.05		
	Mazzola 2016*	Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 21% Control: 36% *RR 0.59 (CI 0.3-0.93)*		IAP: 34% Control: 26% *RR 1.31 (*CI *0.85-2.01)*
Proteobacteria	Aloisio 2016*	IAP n=10 Control n=10				IAP: 54.7% Control: 15.5% p<0.05		
	Arboleya 2016	IAP n=14 Control n=13						Higher % in IAP, *p*<0.001
	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7				Higher % in IAP, p<0.062		

Organisms	**Reference Year**	**Infants (n) in each group**	**Relative amount in microbiota composition (%) or infants (*n*) colonised**					
			Day					
			1	**2**	**3**	**6/ 7**	**10**	**30**
		Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 37% Control: 17% *RR 2.18 (CI 1.32-3.60)*		IAP: 28%
Firmicutes	Arboleya 2016	IAP n=14 Control n=13						Lower % in IAP, p<0.01
	Mazzola 2016*	Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 41% Control: 29% *RR 1.14 (CI 0.96-2.08)*		IAP: 30%
Family level								
Bifidobacteriaceae	Aloisio 2016*	IAP n=10 Control n=10				IAP: 0.02% Control: 6.47% p<0.05		
	Arboleya 2015	IAP n=14 Control n=13						Lower % in IAP, p<0.05
Comamonadaceae	Arboleya 2015	IAP n=14 Control n=13						Lower % in IAP, p<0.05
Enterobacteriaceae	Arboleya 2015	IAP n=14 Control n=13						Higher % in IAP, p<0.05
	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7				Higher % in IAP, p=0.044		IAP: 44% Control: 16% *RR 2.75 (CI 1.67-4.54)*
		Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 35% Control: 17% *RR 2.06 (CI 1.24-3.42)*		IAP: 28%
	Jaureguy 2004*	IAP n=25 Control n=25			IAP *n*=13 Control *n*=16 p=0.58	IAP: 0%		
Lachnospiraceae	Mazzola 2016*	IAP n=14 Control n=13						IAP: 4%

Organisms	**Reference Year**	**Infants (n) in each group**	**Relative amount in microbiota composition (%) or infants (*n*) colonised**					
			Day					
			1	**2**	**3**	**6/ 7**	**10**	**30**
Leuconostaceae	Arboleya 2015	IAP n=14 Control n=13		Lower % in IAP, p<0.05				
Micrococcaceae	Arboleya 2015	IAP n=14 Control n=13					Lower % in IAP, p<0.05	
Propionibacteriaceae	Arboleya 2015	IAP n=14 Control n=13					Lower % in IAP, p<0.05	
Staphylococcaceae	Arboleya 2015	IAP n=14 Control n=13						Lower % in IAP, p<0.05
Streptococcaceae	Arboleya 2015	IAP n=14 Control n=13						Lower % in IAP, p<0.05
Veillonellaceae	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7						Lower % in IAP, p=0.035
Unclassified *Actinobacteria*	Arboleya 2015	IAP n=14 Control n=13						Lower % in IAP, p<0.05
Unclassified *Bacilli*		IAP n=14 Control n=13						Lower % in IAP, p<0.05
Unclassified *Lactobacillales*		IAP n=14 Control n=13						Lower % in IAP, p<0.05
Genera level								
Bacteroides	Jaureguy 2004*	IAP n=25 Control n=25			IAP *n*=13 Control *n*=7 p=0.15			
	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7				IAP: 7% Control: 20% p=0.078		
		Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 13% Control: 32%		

Organisms	**Reference Year**	**Infants (n) in each group**	**Relative amount in microbiota composition (%) or infants (*n*) colonised**					
			Day					
			1	**2**	**3**	**6/ 7**	**10**	**30**
						RR 0.41 (CI 0.23-0.73)		
Bifidobacteria	Jaureguy 2004*	IAP n=25 Control n=25			IAP *n*=6 Control *n*=12 p=0.18			
	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7				IAP: 0% Control: 16% p=0.001		IAP: 6% (compared with day 7, p=0.025) Control: 6%
	Mazzola 2016*	Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 1% or 0% Control: 5%		IAP: 6% (compared with day 7, p=0.013) Control: 19% *RR: 0.32 (CI 0.13-0.76)*
Clostridia	Jaureguy 2004*	IAP n=25 Control n=25			IAP *n*=3 Control *n*=10 p=0.04			
Enterococci	Jaureguy 2004*	IAP n=25 Control n=25			IAP *n*=15 Control *n*=17 p=0.73			
Escherichia	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7				IAP: 52% Control: 14% *RR 3.71 (CI 2.21-6.25)*		
Staphylococci	Jaureguy 2004*	IAP n=25 Control n=25			IAP *n*=21 Control *n*=22 p=1.00			
Streptococci	Mazzola 2016*	Mixed-fed IAP n=6 Mixed-fed Control n=6				IAP: 32% Control: 10% *RR 3.2 (CI 1.66-6.15)*		IAP: 8% (compared with day 7, p=0.042)
Other microbial genus	Aloisio 2016*	IAP n=10 Control n=10				No differences		

* Group B *Streptococcus* prophylaxis; *Numbers in italics are calculated*

CI confidence interval, IAP intrapartum antibiotic prophylaxis, *p* probability value, RR risk ratio,

Table 13. Quantitative composition of gut microbiota in IAP-treated and untreated infants

Organism	**Reference Year**	**Infants in each group (n)**	**Log colony forming units per gram (CFU/g)**			
			Day			
			3	**6/7**	**30**	**90**
Family level						
Staphylococcaceae	Arboleya 2015	IAP n=14 Control n=13			Lower log cells/g in IAP, $p<0.05$	
Enterobacteriaceae	Arboleya 2015	IAP n=14 Control n=13			Higher log cells/g in IAP, $p<0.05$	
	Jaureguy 2004*	IAP n=25 Control n=25	IAP: Med 8.4 (R 3.3-9.5) Control: Med 9.2 (R 3.3-9.8) p=0.18			
Genera						
Bacteroides	Jaureguy 2004*	IAP n=25 Control n=25	IAP: Med 8.0 (R 6.3-10.3) Control: Med 7.9 (R 3.6-9.6) p=0.12			
Bifidobacteria	Jaureguy 2004*	IAP n=25 Control n=25	IAP: Med 8.2 (R 4.3-9.5) Control: Med 8.5 (R 6.9-10.3) p=0.10			
	Arboleya 2015	IAP n=14 Control n=13				Lower log cells/g in IAP, $p<0.05$
Bifidobacterium spp.	Aloisio 2014*	IAP n=26 Control n=26		IAP: Mn 5.85 (R 3.24-7.79) Control: Mn 7.29 (R 4.12-10.95) $p=0.001$		
	Corvaglia 2016*	IAP n=35 Control n=29		IAP: Med 6.01 (IQR 5.51-6.98)	IAP: Med 8.41 (IQR 7.71-8.80)	

Organism	**Reference Year**	**Infants in each group (n)**	**Log colony forming units per gram (CFU/g)**			
			Day			
			3	**6/7**	**30**	**90**
				Control: Med 7.80 (IQR 6.61-8.26) *p*=0.000	Control: Med 8.39 (IQR 7.96-8.86) *p*=0.363	
	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7		IAP: Med 5.86 Control: Med 8.16 *p*=0.005	IAP: Med 7.72 (compared with day 7, *p*=0.035) Control: Med 8.62 NS	
		Mixed-fed IAP n=6 Mixed-fed Control n=6		IAP: Med 5.81 Control: Med 7.19 p=0.03	IAP: Med 8.50 (compared with day 7, p=0.036) Control Med 8.55 (compared with day 7, p=0.028)	
Clostridia	Jaureguy 2004*	IAP n=25 Control n=25	IAP: Med 5.3 (R 4.3-5.8) Control: Med 6.2 (R 3.6-8.1) p=0.01			
Enterococci	Jaureguy 2004*	IAP n=25 Control n=25	IAP: Med 8.3 (R 3.6-10.3) Control: Med 7.3 (R 3.3-9.5) p=0.78			
Lactobacillus spp.	Aloisio 2014*	IAP n=26 Control n=26		IAP: Mn 6.69 (R 5.40-8.93) Control: Mn 6.73 (R 5.45-8.20) NS		
	Corvaglia 2016*	IAP n=35 Control n=29		IAP: Med 5.56 (IQR 4.94-6.14) Control: Med 5.45 (IQR 4.81-6.14) p=0.872	IAP: Med 5.29 (IQR 4.68–6.01) Control: Med 5.25 (IQR 4.60-6.15) p=0.932	
Staphylococci	Jaureguy 2004*	IAP n=25 Control n=25	IAP: Med 6.5 (R 3.6-8.0) Control: Med 7.0 (R 4.0-9.3) p=0.53			
Species						

Organism	Reference Year	Infants in each group (n)	Log colony forming units per gram (CFU/g)			
			Day			
			3	6/7	30	90
Escherichia coli	Aloisio 2014*	IAP n=26 Control n=26		IAP: Mn 8.18 (R 4.09-12.70) Control: Mn 9.03 (R 5.61-11.78) NS		
Bacteroides fragilis	Aloisio 2014*	IAP n=26 Control n=26		IAP: Mn 8.17 (R 4.68-11.99) Control: Mn 8.53 (R 5.22-11.16) NS		
	Corvaglia 2016*	IAP n=35 Control n=29		IAP: Med 7.71 (IQR 5.80-9.33) Control: Med 7.75 (IQR 5.87-9.61) p>0.05	IAP: Med 7.36 (IQR 5.80-9.09) Control: Med 8.51 (IQR 5.86-9.37) p>0.05	
Clostridium difficile	Aloisio 2014*	IAP n=26 Control n=26		IAP: Mn 3.89 (R 3.12-4.80) Control: Mn 3.70 (R 2.85-5.46) NS		
Total bacteria	Mazzola 2016*	Breast-fed IAP n=7 Breast-fed Control n=7 Mixed-fed IAP n=6 Mixed-fed Control n=6		All groups: R 9.38-9.71	All groups: R 9.53-9.83	
	Arboleya 2015	IAP n=14 Control n=13			Higher log cells/g in IAP, p<0.05	

* Group B *Streptococcus* prophylaxis

IAP intrapartum antibiotic prophylaxis, Mn mean, Med median, R range, IQR interquartile range, p probability value

7.6 Discussion

7.6.1 Principal findings

This is the first systematic review investigating the evidence on adverse events experienced by the mother and/or her child after treatment with intrapartum antibiotic prophylaxis. There were a wide range of adverse events reported in 17 observational studies and 13 RCTs. However, there was little high-quality evidence to quantify the frequency of adverse events from IAP for neonatal GBS disease prevention (objectives a to e). There were three key findings. First, there was a substantial evidence gap around the long-term effects of IAP (objectives e). There was only one RCT, which reported a moderate effect of cerebral palsy, functional impairment and bowel problems at the age of seven, in infants whose mothers were treated with IAP. However, this RCT had limited applicability to GBS prevention. Second, there was consistent observational evidence that IAP for GBS prevention altered infant microbiota from 0 to 90 days of life. However, the clinical significance of this is not known. Thirdly, some observational evidence showed increased antibiotic resistance in infants whose mothers were treated with IAP. However, the evidence was at high or unclear risk of bias due to confounding variables, and these findings were not replicated in other studies.

The best quality evidence was Kenyon *et al.'s* (2008) RCT which found the increased risk of bowel problems, cerebral palsy and functional impairment from IAP. This study had a low risk of bias across major domains. However, the applicability of these findings is uncertain, as the drugs investigated were erythromycin or amoxicillin-clavulanate given for 10 days or until birth to a population in preterm labour.[320] The drug recommendation for GBS IAP treatment is penicillin followed by a cephalosporin or clindamycin,[13, 15] given for shorter durations, at or near, term labour. The effect sizes of the findings were also moderate, and with multiple statistical comparisons on the same population, the probability of a chance result is increased. Furthermore, the plausible biological mechanisms through which IAP can cause the development of cerebral palsy are not known. Despite the relatively higher methodological quality of this study, the findings may not be applicable to GBS prevention.

On the other hand, studies with improved applicability that explicitly included IAP for GBS prevention found that IAP could alter gut microbiota, increase maternal thrush and increase neonatal respiratory distress. However, all of these studies were observational and at high or unclear risk of bias. These studies did not account for all of the important confounding variables, thus, results could be due to other factors. In addition, although there were consistent observational results on gut microbiota, populations in these studies were not followed to

clinical outcomes. Therefore, whether microbiota alterations from IAP are associated with short or long-term health problems is not known.

The remaining evidence in all treated populations (GBS and/or other prevention) was inconsistent, as some studies showed differences while others did not. There was unclear risk of developing antibiotic resistance, *Clostridium difficile* bowel problems, necrotising enterocolitis and neonatal infections. Generally, the RCTs that investigated the effectiveness of IAP found no increase in the treated, compared with control groups, whereas the observational studies did find an increase.

The results may have lacked consistency as the overall evidence across the studies was at high or unclear risk of bias in more than one domain. Eight (64%) of the RCTs were at high risk of bias for selective reporting, as many of the outcomes were not pre-specified or only reported in the treated group, while seven studies had unclear but serious risks of other biases. Furthermore, all but one of the trials aimed to investigate the effectiveness of IAP and might have contained investigator bias. None of the observational studies had a low risk of bias for confounding variables as 13 (76%) studies controlled some, but not all, important confounding variables, while the remaining four (24%) were at high risk. In these studies, key variables such as the proportion of women with maternal risk factors for infection or who were administered antibiotics during pregnancy, were not stated.

As a result of the inconsistent and fairly inapplicable evidence base that was at high risk of bias, it is difficult to quantify the incidence of any one, or all, of the potential adverse events associated with IAP treatment for GBS prevention.

7.6.2 Comparison with previous literature

Below I discuss the previous literature in relation to the three key findings on the adverse events of microbiota changes, long-term severe consequences and antibiotic resistance.

It has been suggested in the literature that early gut bacterial colonisation plays an important role in infants' mucosal and immune system development.[191, 32, 321, 192] Infant microbiome changes from antibiotics have been linked to autism, metabolic problems such as diabetes and obesity and respiratory problems such as asthma.[191, 32, 192] For example, Cox *et al.* (2014) demonstrated gut microbiota changes that led to permanent abnormalities in the immunity and metabolism in mice as a result of low dose penicillin delivered after birth.[331] Antibiotic

exposure repeatedly during infancy or before six months of age was also recently associated with an increase in body mass and height in healthy children.[192] Although these studies relate to gut microbiota from antibiotic exposure, the antibiotics in these studies were not administered during labour. Therefore, it is not clear whether gut microbiota changes from intrapartum antibiotic exposure have these or any other clinical significance.

In this systematic review, I found inconsistent evidence on the increase of antibiotic resistance after IAP. As shown in Chapter 2, the literature shows increases in the rates of antibiotic resistance in countries offering IAP for neonatal GBS prevention. In the last 20 years, both clindamycin and erythromycin resistance have increased.[14] In the US in 2010, EOGBS resistance to erythromycin was reported at 48% and resistance to clindamycin was above 27%.[332, 18, 297] In the UK, in 2014/15, EOGBS resistance to erythromycin was reported at 23% and resistance to clindamycin was 16%; an increase from 6% and 3% respectively, since 2000/01.[5] However, clindamycin and erythromycin resistance in GBS strains isolated from pregnant women have shown increased rates across the world. The highest rates have been reported in Asia, followed by the Americas and Africa. Most Asian and African countries do not administer IAP, however, without knowing the contributing centres and their IAP policies, it is difficult to reach conclusions. While GBS remains almost universally susceptible to penicillin,[86] 0.2% of GBS isolates in the US in 2005 had reached the upper level of susceptibility for beta-lactams.[182, 18] Similarly, in Japan, 5% to 15% of GBS isolates were reported to have reduced penicillin susceptibility;[183] approximately half of which are susceptible under European breakpoints and came from populations where chronic antibiotic exposure is likely to be common (due to chronic respiratory disease). As discussed in Chapter 2, there have been some studies showing penicillin resistance in GBS isolated from pregnant women including one in Italy where IAP is offered,[181, 179, 180] however, these studies were small and had methodological limitations, therefore, require further research. All of the observations are also ecological in nature with no comparators, making it difficult to attribute them to IAP for GBS prevention as they could be due to other factors that occurred during those periods.

The previous literature on the association between IAP and cerebral palsy (shown in Kenyon *et al.*, 2008)[320] is uncertain. The plausible biological mechanisms by which IAP can cause cerebral palsy are not known within the extant literature.[333, 320] Further complicating this result is a second trial administering IAP for pregnant women with preterm rupture of the membranes (which was excluded because of potential confounding effects due to signs of infection) that showed no difference in the number of children who developed cerebral palsy in treated *versus* untreated women.[334] Overall, the reason why cerebral palsy occurred in the

first study and if it would occur because of IAP for neonatal GBS prevention involving different drug durations and regimens, is uncertain.

7.6.3 Strengths and limitations

This was the first systematic assessment of the literature on the adverse events from IAP. I applied an extensive search strategy with no date limit to capture as much as data as possible. I also included reference checking of all included papers and relevant systematic reviews to capture grey literature. Furthermore, I had expert input from clinical microbiology, infectious disease and obstetrics and gynaecology to find any further literature I had not identified, and to review the methodology and the findings to ensure I maintained clinical significance. I also enlisted a second reviewer to duplicate and cross-check reviews processes including study selection, data extraction and quality appraisal to minimise any errors and maintain a high-quality standard for the review. For quality appraisal, I considered and piloted different validated tools to ensure they met the needs of the review. Finally, where summary measures were not available for comparisons of outcomes, I calculated them to improve the understanding of the results.

However, there are some limitations to the review around the search strategy, study eligibility and data extraction. Firstly, as my search strategy was broad and focused heavily on harms or adverse events search terms, I may not have found studies investigating outcomes that would potentially be adverse events, but were not indexed as such. This was the most efficient and effective strategy to find the appropriate studies, and I made an effort to find these studies by including the terms for IAP harms known to experts and known in the literature, searching reference lists and asking experts for any studies missed. Secondly, I only included studies for which full-texts were available in English, therefore, I might have missed adverse events reported in other languages. The majority of the studies included in this review were from the US and Europe, with one study from the Latin America, three from Africa and two from the Middle East. I did not identify studies from many countries, and it is not clear whether there are such studies in other countries. This could potentially increase selection bias and put the generalisability of the review findings at risk.

Secondly, with respect to the study eligibility criteria for the study inclusion, I excluded studies on the adverse events from caesarean section prophylaxis due to differences in the regimens for caesarean prophylaxis compared with GBS prophylaxis and the potential confounding of the surgery itself. Similarly, I excluded studies where more than 10% of

women had risk factors for infection due to the confounding effect. Thus, I may have excluded harms in such studies that may also be relevant to GBS prophylaxis. However, I excluded these studies as it would have been difficult to form conclusions on whether the cause of the adverse event was IAP or the confounding variables. Similarly, I excluded case reports even though they might have identified more adverse events that occurred after IAP, for example from medical records. However, with no control group, it would have been difficult to reach conclusions about whether IAP would increase adverse events.

Thirdly, regarding the data extraction process, there were some studies in which information was not provided, was unclear or numbers did not add up. In these cases, I did not contact the authors directly to clarify the data or provide the information required. Contacting them could have clarified the data and the study could have been more valuable to the review. Finally, I was unable to pool data that were extracted using meta-analysis due to the heterogeneity across the adverse events and the populations investigated. This meant that I could not meet the objectives of this review to quantify the incidence of each adverse event separately or combined. Instead, I narratively synthesised the data which identified that there are still important gaps in the evidence on the adverse events from IAP.

Despite the limitations of this review, I used a broad search strategy, searched reference lists and experts did not identify further studies. Therefore, the findings of this review are representative and reflective of the evidence base on adverse events from IAP available at this time.

7.6.4 Research and policy implications

This review has implications on the widely recommended clinical practice of IAP treatment for maternal GBS carriage to prevent neonatal GBS disease.[1] A Cochrane meta-analysis concluded that despite an 83% reduction in EOGBS incidence from IAP, IAP for maternal GBS colonisation is not supported by conclusive evidence due to a high risk of bias across RCTs.[10] Given this uncertainty, and the uncertainty of the results in this review, it is increasingly difficult to determine whether the benefits of IAP for EOGBS prevention outweigh the harms to mothers and their children. To answer this question, well-designed and large RCTs are needed. However, as IAP is now the recommended treatment, it may no longer be possible for such a trial. An alternative might be to perform large, longitudinal and high-quality observational studies across countries with widespread IAP that comprehensively control for confounding variables. In particular, long-term follow-up investigations are

required on the health consequences of early microbiota alterations from IAP. Without this information, it is not possible to understand the importance, if any, of early gut microbiota changes.

In the meantime, expanding EOGBS prevention from risk-based strategies to universal GBS screening may increase the number of low risk women exposed to IAP. As identified in Chapter 2, the literature indicates that up to 30% of mothers positive in pregnancy become negative by birth, and only up to 3% of mothers colonised in labour have a neonate with EOGBS.[4, 104] In the UK, approximately 150,800 pregnant women would be eligible for IAP every year, and without treatment, over 149,300 (99%) would not have a neonate with EOGBS. These women and their babies would be unnecessarily exposed to potential harm. This review indicates that the adverse events from IAP and their clinical significance are not well investigated. As a result, the balance between the benefits and the harms from expanding IAP administration in a GBS screening programme cannot be calculated. As observational evidence on universal GBS screening effectiveness is limited because of inherent biases,[16, 14, 17] an RCT on the effectiveness of GBS antenatal culture screening could inform on both the effectiveness and the harms of screening and IAP treatment. However, as indicated in Chapters 2 and 3, such an RCT would require a large sample size due to the low positive predictive value of the GBS antenatal culture test (0.2% to 0.4%). For now, the screening criterion that the benefits of screening and treatment should outweigh the harms remains unmet, as it is not clear if widespread IAP is safe to undertake.

7.7 Conclusions of this chapter

- The evidence on the adverse events from IAP treatment revealed a range of potential adverse events. However, the evidence base for IAP treatment specifically for neonatal GBS disease prevention is unclear, inconsistent and/or at risk of bias.
- The key findings were consistent evidence from observational studies that IAP for GBS alters infant microbiota, and some inconsistent evidence that IAP increases antibiotic resistance. However, this evidence was at risk of bias and the clinical consequences of the microbiome alterations are not known.
- There was also evidence from one long-term RCT which showed that IAP in preterm labour is associated with potentially severe consequences such as cerebral palsy. However, it has applicability concerns, unclear biological plausibility and was not duplicated in a similar RCT.

- These limitations prevent accurate conclusions on the frequency of adverse events from IAP treatment for neonatal GBS disease prevention. It is uncertain whether large scale IAP as a result of a universal GBS screening programme would be safe to undertake. Consequently, the requirement that the benefits of screening should outweigh the harms is not met.
- Larger, longer and higher quality studies across countries with widespread IAP are needed to quantify adverse events from IAP treatment for neonatal GBS disease prevention.

PART III. TIME TRENDS AND THE IMPACT OF GBS SCREENING

8. RESEARCH RATIONALE, AIMS AND OBJECTIVES

As discussed in Chapters 2 and 3, one of the reasons the UK NSC recommended against universal GBS screening was that there was no RCT evidence to prove the clinical effectiveness of GBS screening on EOGBS morbidity and mortality. However, as EOGBS is a relatively rare condition with an incidence of 0.57 per 1,000 livebirths,[5] a large sample is required to power an RCT. In such circumstances, an alternative to RCT evidence may be required to make screening decisions. Therefore, in this part of the thesis, I will investigate whether an alternative approach can inform the clinical effectiveness and harms of universal GBS screening. As demonstrated in Chapter 2, there has been growing evidence from countries that have implemented universal GBS screening, on the benefits and harms from screening. However, due to a high risk of bias from confounding variables, the same results may not necessarily be expected in the UK as there are different population, economic and health system contexts. Therefore, to estimate the benefits and harms of universal GBS screening, I will explore whether international data on GBS related outcomes in GBS screening programmes compared with other prevention strategies can be adjusted for country-level differences in ecological trend analyses.

The primary purpose of a universal GBS screening programme, or any EOGBS prevention strategy, is to reduce the morbidity and mortality of EOGBS disease in neonates. Therefore, in Chapter 11, I present an ecological trend analysis study that combines international data to explore the impact of universal GBS screening on the trends of annual EOGBS incidence compared with other GBS prevention strategies, adjusted for country differences. Despite EOGBS being the primary outcome of interest in screening programmes, there is a fundamental limitation of its use in research investigations. Focussing on cultured-confirmed EOGBS can overestimate the reduction in EOGBS incidence under screening. This is because antibiotics present in neonates' blood increase the chance of false negative test results in the presence of infection.[275, 18] Consequently, it has been suggested that early-onset sepsis (less than 7 days) must be assessed to investigate GBS screening. However, as indicated in Chapter 2, the findings from different studies have shown conflicting results. Some studies show that neonatal sepsis incidence does not decrease under screening, whereas others show that it decreases under IAP prevention (screening or risk-based prevention) but not necessarily specific to screening.[174, 175] It is vital to investigate the impact of GBS screening in reducing early-onset sepsis as this would result in a true and unbiased understanding of the effectiveness of GBS screening. In Chapter 12, I present an ecological trend analysis study that combines international data to explore the impact of universal GBS screening on the trends of annual

early-onset sepsis incidence compared with other prevention strategies, adjusted for country-level differences.

As identified in Chapters 2, 3 and 5, a necessary part of assessing screening in the UK and according to international screening standards is to examine the harms of screening in order to ensure that the benefits of screening outweigh the harms. As discussed in Chapter 2, in the case of GBS, IAP would be given to approximately 150,800 pregnant women and their babies every year, of whom over 99% will be over-treated, making the harms from the screening programme even more important in the context of GBS screening. Therefore, in Chapter 13, I present a final ecological trend analysis study that combines international data to explore the impact of universal GBS screening on the trends of some harmful outcomes of GBS screening and IAP treatment compared with other prevention strategies, adjusted for country-level differences. In Chapter 7, I summarised the current literature on the harms of IAP treatment. There were a range of potential harms identified in the literature, although most of the evidence is from small studies that are at high risk of bias. One of the key adverse events identified in this chapter was antibiotic resistance. The evidence about these outcomes was inconsistent across the literature and did not always involve GBS prophylaxis or GBS organisms. Another key adverse event was neonatal infection, where there was also inconsistent evidence that IAP treatment increases early- and late-onset neonatal infections. Although this is counterintuitive, widespread IAP can cause selection pressure and change the organisms causing infection. Therefore, it can lead to an increase in antibiotic resistant strains causing infection, particularly for late-onset disease, and can increase early-onset gram-negative bacteria, of which *E. coli* is the commonest, as shown in Chapters 2 and 7. Therefore, in Chapter 13, I investigate the following potential harms of a GBS screening programme and the expansion of IAP treatment: LOGBS incidence, early-onset *E. coli* incidence, the percentage of EOGBS cases resistant to clindamycin and erythromycin and the percentage of all neonatal GBS cases resistant to clindamycin and erythromycin. I did attempt to investigate maternal anaphylaxis as it is a serious adverse event from IAP, however, due to the rarity of the condition the analyses would not have been possible.

8.1 Research aims and objectives

The research aim of this part of the thesis is to investigate whether the international data on the benefits and harms of universal GBS screening, compared with other prevention strategies, can be adjusted for country-level differences using ecological trend analysis, to inform the clinical effectiveness of universal GBS screening.

The research questions for each of the ecological trend analysis studies are:

1. *Adjusting for country-level differences, what is the international impact of GBS screening on the trend of annual EOGBS incidence across time compared with other prevention strategies?*
2. *Adjusting for country-level differences, what is the international impact of GBS screening on the trend of annual all-cause early-onset sepsis incidence across time compared with other prevention strategies?*
3. *Adjusting for country-level differences, what is the international impact of GBS screening on the trends of annual early-onset E. coli and LOGBS incidences, and the percentages of clindamycin and erythromycin resistance in early-onset and neonatal GBS disease across time compared with other prevention strategies?*

The specific research objectives are to:

a) Describe the frequency of the GBS prevention strategy as well as the mean or frequency of the country-level covariates in general (irrespective of outcome);
b) Describe the mean incidence of each outcome across time, geographical areas, world regions and GBS prevention strategies;
c) Investigate the unadjusted relationship between universal GBS screening and the trend of each annual outcome (as stated above) across time compared with other prevention strategies, using linear regression;
d) Investigate the unadjusted relationship between each country-level covariate and the mean outcome across time, using linear regression;
e) Investigate the relationship between universal GBS screening and the trend of each annual outcome across time compared with other prevention strategies, using linear regression adjusted for the country-level covariates;
f) Examine the stability of the adjusted relationship between universal GBS screening and the trend of each annual outcome across time compared with other prevention strategies in a range of sensitivity analyses, if the relationship was statistically significant; and

g) Investigate the multi-level unadjusted relationship between universal GBS screening and the trend of annual EOGBS incidence across time compared with other prevention strategies, using a multi-level growth curve model to account for the structure of the data.

In Chapter 9, I describe the detailed methodology used to address the research questions and objectives. In Chapter 10, I summarise the data collected for all three studies, and in Chapters 11, 12 and 13, I explore the results and discuss the key findings and how they compare to previous literature for each study separately: Chapter 11 EOGBS, Chapter 12 early-onset sepsis, and Chapter 13 harmful outcomes (early-onset *E. coli*, LOGBS, and clindamycin and erythromycin resistance in early-onset and neonatal GBS disease). Finally, in Chapter 14, I summarise the results of all three studies, discuss the strength and limitations of the methods used in these studies and the research and policy implications.

9. METHODS

I have reported the three ecological trend analysis studies using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[335]

9.1 Study design and rationale

To address research questions 4 to 6, I used quantitative methodology. For interventions such as screening, robust estimates are required to indicate the numbers of different groups of patients who would experience a morbidity and mortality benefit or harm from the intervention. To estimate the frequency of these outcomes, I chose quantitative methods as they are the most appropriate. This is coherent with the recommendation that "research methods should follow research questions in a way that offers the best chance to obtain useful answers".[211 p18]

To formulate a screening decision, the UK currently fills out a screening flowchart to assess the trade-off between the benefits and harms of a screening programme to the population (see Figure 10). This flowchart, recommended in the literature,[336] is filled in to show the numbers and percentages of people who would experience each possible consequence from a screening programme. This part of the thesis focuses on the intervention phase of the flowchart as the target population and the diagnostic accuracy of the sieve phase of the flowchart has already been researched and can be estimated from current literature while the sort phase of the flowchart does not exist in the case of GBS screening as there is no currently available diagnostic test. The UK NSC usually uses RCT evidence to fill in the flow chart. For GBS, most of the flowchart is based on evidence that is partly of fully biased and uncertain or unknown. However, in the absence of RCTs, I proposed to use adjusted international data from epidemiological ecological studies to estimate the benefits and harms of screening in the flowchart.

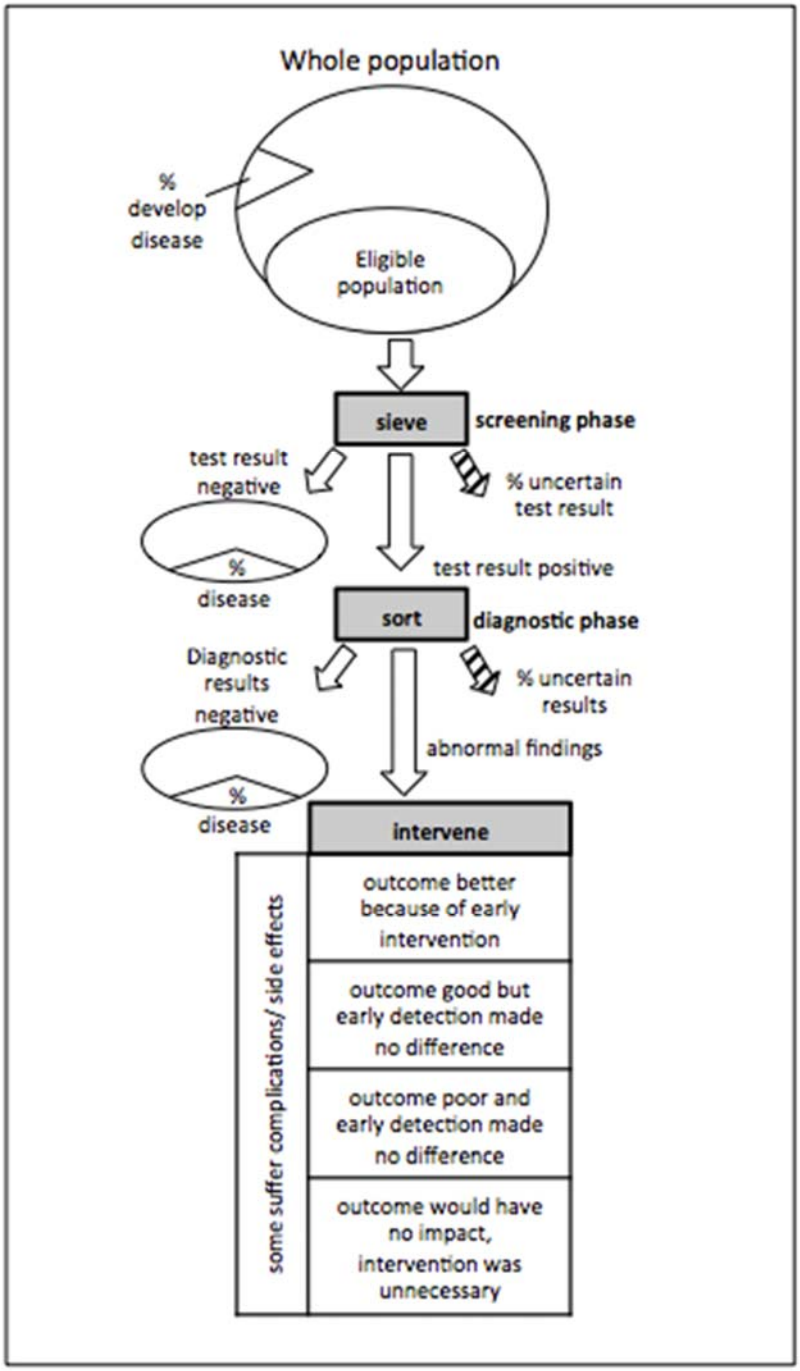


Figure 10. Screening flow chart developed by Raffle and Gray,[336 p78] and used by the UK National Screening Committee

I measured the benefits (objective 4 and 5) and harms (objective 6) of universal GBS screening to populations across countries in three epidemiological ecological trend analysis studies. Epidemiology is defined as "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems".[337] Various methods exist to carry out epidemiological investigations; some studies are descriptive and are used to study disease distribution while others are analytical and used to study determinants of diseases.[338] As epidemiological methodology enables assessment and control for the determinants of diseases at a group level, and allows the researcher to investigate groups of people,[339] it was an appropriate alternative to RCT data to meet the objectives.

Specifically, within epidemiological study designs, I chose the ecological study design. An ecological study is an observational study investigating the disease burden (exploratory design) or the association between diseases and exposures (analytic design at the level of the population or community).[340, 341] Therefore, the unit of measure is the population and the disease rates and exposures are measured at the population level. Ecological studies not only allow geographical correlations between disease incidence rates and the prevalence of risk factors (multiple-group design), they also allow the analysis of trends in incidence rates over time (time-trend design), or a combination of place and time (mixed design). Although validating trends and changes in trends is difficult as it depends on observations made years ago, ecological studies can reveal the reality of trends with reasonable certainty.[341] Indeed, the aim of research questions 4 to 6 were to assess the exposure of different population GBS prevention programmes on a population level over time and across countries. Furthermore, screening programmes are population level interventions or policies where the target level of inference is ecologic. Therefore, an analytical mixed design ecological study was appropriate. Furthermore, ecological analyses can enable the identification of unintended consequences of population interventions, which are not likely to be identified in individual level studies.[340]

I decided to include a time-trend element in the comparison of the outcomes between populations as opposed to a cross-sectional snapshot comparison, as trends enable a more dynamic and reliable estimate of changes related to the effect of exposures such as a GBS prevention strategy. Time trend analyses are useful in describing long-term changes in a population, comparing one geographical area with another and studying effects specific to groups, such as public health interventions offered to the group.[342, 343] These trends reveal facts related to the disease that can aid in making health-related public policies to decrease the population's risk of the disease.[344] Trends also help to monitor the progress made in a particular disease and evaluate the effectiveness of current intervention methods.[344] By

assessing whether the slope of the incidence rates is increasing or decreasing, one can assess the progress of intervention methods such as GBS prevention strategies.[344] While a comparison of one point in time estimates of diseases between countries that have different GBS prevention strategies can provide some indication about the differences in the effectiveness of the prevention strategies, it is not clear whether the rate in one year would be reflective of every year or how the rates might fluctuate between the years. By examining the trends over time, one can achieve a more accurate indication of the changes that can be expected under different prevention strategies, allowing predictions of future outcome rates.[343] This, in turn, can help assist in the planning of population prevention interventions.

In planning for research questions 4 to 6, I also explored the feasibility of a retrospective, quasi-experimental study comparing the outcomes from maternity centres in England that implement different GBS prevention policies. A 2014 RCOG audit on practice to prevent EOGBS in hospitals in the UK identified four English hospitals that were reported to offer universal screening while the remaining offered risk-based prevention.[345] I intended to match these 'screening' hospitals to hospitals that are similar in size and geographical location, but which did not offer universal screening. I would have compared the EOGBS rates between the 'screening' and 'non-screening' hospitals using the PHE's GBS surveillance. However, when I contacted the hospitals that claimed to screen for GBS for more information, I found that they did not have a universal screening programme: one had provided an incorrect answer to the GBS audit, one only screened if a patient requested it and one only screened women with a clinical indication. With only one 'screening' hospital, there was insufficient power to proceed with this study.

9.2 Study setting and participants

As this was an ecological study, the unit of analysis was the geographical area that the institutions collecting data on the disease rates of interest were from. The study setting was international and I made an attempt to sample institutions from as many countries as possible so that the sample of geographical areas used in these studies could be representative. Initially, I attempted to collect national figures on any of the GBS screening benefits and harms (see Section 9.3 below). However, as data collection began, it became clear that, across many countries data were not available at this level. Therefore, I made the pragmatic decision to include data from institutions across countries at the regional, city or centre level. In addition, some of the regional and national data from the different institutions were based on mandatory

surveillance while others were voluntary. Due to these differences, I accounted for the geographical level and type of data in the analyses (see Section 9.3 below). For some countries or regions, multiple institutions provided data that covered the same outcomes for overlapping regions. To avoid double counting, I selected the institution with the widest coverage and/or the best quality data (i.e. mandatory instead of voluntary surveillance) for the area. If the main results were statistically significant, in one of the sensitivity analyses, I replaced the selected data source with the unselected data source to test the stability of the model (see Section 9.8).

9.3 Variables and measurement

The data for these studies included three components: the outcomes, the predictor, and the country or compositional covariates.

9.3.1 Outcomes

Altogether, there were eight outcomes across research questions 4 to 6. The primary outcome for this project was the average annual change in the incidence rate of culture-confirmed EOGBS per 1,000 livebirths addressed in research question 4. I chose EOGBS as the primary outcome because this is the disease of interest that screening aims to prevent. The secondary outcome I addressed in research question 5 was the annual change in early-onset sepsis incidence per 1,000 livebirths. Early-onset sepsis was selected because measuring culture-proven GBS alone may underestimate the incidence of sepsis due to GBS as IAP in the blood may prevent GBS from being isolated.[275, 18]

The secondary outcomes I addressed in research question 6 to assess the harms from GBS screening and widespread IAP treatment were the annual changes in: early-onset *E. coli* incidence per 1,000 livebirths, LOGBS incidence per 1,000 livebirths, the percentage of EOGBS cases resistant to erythromycin, the percentage of EOGBS cases resistant to clindamycin, the percentage of all neonatal GBS cases resistant to erythromycin and the percentage of all neonatal GBS cases resistant to clindamycin. I chose early-onset *E. coli* because widespread IAP can lead to selection pressure on the organism causing disease and increase the rates of neonatal infection caused by gram-negative bacteria.[32, 19] Investigating *E. coli*, which is the most common gram-negative bacterium causing neonatal infection,[346] could provide an indication about whether GBS screening increases the rates of gram-negative bacteria causing early-onset infection compared with other prevention strategies. I chose

LOGBS because there is a suggestion that widespread IAP and resulting selection pressure may increase LOGBS cases.[347, 348, 172] Finally, a concern of widespread IAP particularly in the era of antibiotic resistance, is that it can increase antibiotic resistance rates.[32, 86, 23, 14] I chose clindamycin and erythromycin as they were the second-line drugs offered for IAP treatment for women who are allergic to penicillin.[13, 86, 14] I did not investigate penicillin resistance as GBS remains almost universally susceptible to penicillin.[86]

I collected annual rates of these outcomes from institutions across geographical areas and calculated an average annual change in the analyses as described in Section 9.8 below. Incidence rates per 1,000 livebirths were defined differently across institutions with respect to the number of days at onset of disease. EOGBS, early-onset sepsis, and early-onset *E. coli* varied between zero to two days and zero to seven days (zero to eight days for early-onset *E. coli*) and was also defined as vertical-onset or mother acquired. LOGBS ranged between two days onwards and five days onwards. Neonatal GBS varied between less than 28 days to less than 132 days. I accepted all definitions and accounted for the differences in definitions by adjusting for a categorical variable in the analysis that indicated the definition for the outcome.

9.3.2 Time variable

For each outcome, I recorded time as the year in which each observation was provided for each geographical area, i.e. for each observation, there was an associated year. In a minority of cases, some institutions provided the year as a range, in which case I chose the most recent year as arbitrary to avoid bias in choosing a specific year. I used this approach unless the months were provided for the data and it was known that the majority of the data came from an earlier year. In that case, I used the earlier year. For example, I used the year 2000 if the survey reported February 2000 to February 2001, however, I used 2001 if the survey only reported 2000 to 2001.

9.3.3 Predictor variable

The primary predictor variable of interest for research questions 4 to 6 was the most recently reported GBS prevention strategy recorded during the period for which outcome data were provided in each geographical area (country, region city or centre). It was a categorical variable with four categories: universal GBS screening (antenatal and/or rapid testing), risk-based GBS prevention (based on any GBS risk factors), either a GBS screening or risk-based prevention strategy or no GBS prevention strategy.

Many institutions provided information about their prevention strategy, however, not all reported annually. As I did not have data on the GBS prevention strategy by year, I could not analyse them by year. I also considered categorising the variable into the change in prevention strategy that occurred for each geographical area, e.g. no prevention strategy to a risk-based prevention strategy for the UK, and no prevention strategy to 'either prevention' strategy to universal screening for the US. Again, because previous strategies were not always reported, this could not be accurately recorded. Finally, based on the dates reported by some institutions, as well as the dates reported on national GBS prevention guidelines, I created a variable for the most frequently reported GBS prevention strategy for each geographical area. This was not the most accurate predictor as many guidelines were provided at the country level and it was not possible to ascertain when the geographical area or individual hospitals within the geographical area may have begun implementing the guideline. For these reasons, the most recently reported GBS prevention strategy was the most reliable and least biased method of the available options. I used the most frequently reported strategy in the sensitivity analysis if the adjusted relationship between the most recently reported GBS prevention strategy and the outcome was statistically significant.

9.3.4 Compositional variable

In the epidemiological literature, inter-context differences in outcomes that are attributable to differences in group composition are referred to as compositional effects.[349] Therefore, from this point forward in the thesis, I will refer to the country-level or contextual differences as compositional variables or covariates. In these ecological trend analysis studies, I adjusted the association between the GBS prevention strategy and the annual change in each of the eight outcomes for 17 compositional covariates. I selected each covariate, *a priori,* for a specific reason to account for geographic, economic, health system and population characteristics (related to EOGBS) as well as the methodological differences (see Table 14).

The frequency of each compositional covariate varied as shown in the frequency column in Table 14. For some compositional covariates, there was only one value which was the same for each observational year for each geographical area, meaning that for each geographical area the data values did not differ across the years. This is because these data were either the most recently recorded data for the geographical area (e.g. prolonged rupture of membranes, intrapartum fever) or these data did not vary naturally across the years (e.g. world region, age of onset definition). For other covariates, data were available across years, therefore, the data values for each geographical area differ across years.

Table 14. Compositional covariates adjusted for in the ecological studies

Category	Covariate	Variable type and categorical levels	Frequency
Geographic	Region	Categorical: Asia, Europe, Latin America, North Africa and the Middle East, North America, Oceania, Sub-Saharan Africa	Constant over time
Economic	Human development index	Continuous	Annual rates
Health System	% of skilled attendance at delivery	Continuous	Annual rates
	Per capita government expenditure on health (PPP int $ [purchasing power parity international dollars)	Continuous	Most recent rate
Population	% preterm births	Continuous	Annual rates
	% low birthweights	Continuous	Annual rates
	% caesarean section	Continuous	Annual rates
	Fertility rate	Continuous	Most recent rate
	Average maternal age	Continuous	Most recent rate
	Multiple or twin births /1000 births	Continuous	Most recent rate
	% maternal GBS colonisation	Continuous	Most recent rate
	% prolonged rupture of membranes	Continuous	Most recent rate
	% intrapartum fever	Continuous	Most recent rate
	Most prevalent GBS serotype	Categorical: Ia, Ib, III, V	Most recent rate
Methodological	Type of surveillance	Categorical: Mandatory/enhanced population surveillance, Voluntary population surveillance, Multiple centres, One centre	Constant over time
	Geographical coverage	Categorical: National, Regional, City/town wide, One centre	Constant over time
	Age of onset definitions	Categorical:	Constant over time
		EOGBS: 2/3 days or less, 5/6/7 days or less, vertical onset, not stated	
		Early-onset *E. coli*: 2/3/4 days or less, 5/6/7/8 days or less, mother-infected, not stated	
		Early-onset sepsis: 2/3/4 days or less, 5/6/7 days or less, vertical onset or mother infected, not stated	
		LOGBS: 2/3/4 to 28/90 days, 5/6/7/8 to 13/27/28/30/60/90/365 days 48 hours to 6 days, not stated	

EOGBS early-onset group B *Streptococcus*, *E. coli Escherichia coli*, GBS group B *Streptococcus*, LOGBS late-onset group B *Streptococcus* disease, % percentage

9.4 Data collection

To access data for the outcomes within 194 countries recognised by the United Nations, I identified healthcare institutions in each country through systematic Internet and literature searches as well as the research team and the UK NSC's international knowledge of all surveillance and public health institutions. I approached key institutions involved in surveillance related to maternity and childbirth, paediatric conditions, infectious disease and health-care in each country. To recruit institutions, I used the snowball sampling technique, in which a researcher makes initial contact with a group of participants relevant to the research topic and then uses these to establish subsequent contact with other relevant participants.[350]

Once institutions were identified, I initially contacted them by email, explained the studies, their aims and the processes involved. I informed the institutions that participation was voluntary and that, because aggregate figures were required, the information was anonymous. If the institution was interested in participating, I sent the study information leaflet (see Appendix 12) to the relevant person in the institution. If they agreed to participate, I contacted the individual by telephone to discuss the project and asked them to email the annual aggregate data by filling in the survey questionnaire which was sent *via* email or in whichever software it was available. Once the data were received, I thanked the individual and addressed any questions.

9.5 The questionnaire

The self-completion questionnaire was available in Microsoft Word or Microsoft Excel format (see Appendix 13). Respondents were to complete the tables with data for the outcomes, predictor variable and compositional covariates available to them across different years for their geographical area. Questions included the following outcomes: the geographical area covered, the type of surveillance, the number of livebirths in the area, the number of early-onset, late-onset, all neonatal GBS, *E. coli*, all-cause sepsis cases and their definitions and the number of GBS antibiotic resistance cases. The questionnaires also included the number or percentage of various risk factors such as preterm births, low birthweights, caesarean section, multiple births, prolonged rupture of membranes, intrapartum fever, maternal GBS colonisation as well as the GBS prevention strategy and the most prevalent GBS serotype.

9.6 Data collection for wider compositional covariates and variables unavailable from survey questionnaires

I acquired data describing the wider aspects of each country's economy, health system and population through international websites, including the World Health Organization, United Nations, the World Bank as well as through existing literature. In addition, if data on the compositional covariates were not provided in the surveys, I also collected these from the international websites. This means that for some lower level geographical areas such as regions, cities or centres, I assigned the data for the covariates from national level data obtained through international websites and literature. Table 15 shows the exact data source used for each covariate.

Table 15. Data sources for compositional covariates

No.	Covariate	Data source
1	GBS maternal colonisation prevalence	Kwatra *et al.* (2016)[30]
2	Most prevalent GBS serotype	Kwatra *et al.* (2016)[30]
3	Preterm births	Blencowe *et al.* (2012)[351]
4	Low birthweights	UNICEF & WHO (2004)[352]
5	Caesarean section	WHO[353-356]
6	Multiple/twin births	Developed countries: Pison *et al.* (2015)[357] Developing countries: Smits *et al.* (2011)[358]
7	Fertility rate	UN[359]
8	Average maternal age	UN[360]
9	Skilled attendance at delivery	WHO[353-356]
10	Human development index	UNDP[361]
11	*Per capita* government expenditure on health (PPP int $)	WHO[362]

GBS group B *Streptococcus*, UN United Nations, UNDP United Nations Development Programme, UNICEF United Nations United Nations Children's Fund, WHO World Health Organization

I used a similar process for the GBS prevention strategy (predictor variable) when it was not provided in the survey questionnaire. I assigned the GBS prevention strategy from the national GBS prevention guideline for geographical areas that did not provide information. Consequently, while this GBS prevention strategy might have been the national guideline for the country, it is not necessary that every hospital in the geographical area was administering the guideline.

For the five compositional covariates where data were available across years (human development index, preterm births, low birthweights, fertility rate and skilled delivery), data were available for some years and were missing at random for other years. I statistically

imputed the years with missing data using multiple imputation (see Section 9.8). For example, for preterm births data were only available for years 2000, 2005 and 2010, so I, statistically, imputed data for the years in between. For some geographical areas, compositional covariate data were not available for any of the years, from either the survey or international websites. In these cases, I imputed data for the covariate from the closest neighbouring geographical area (sometimes another region and other times another country) in the dataset. This was the best approach as the variables that I statistically imputed using multiple imputation utilised data from the context of the geographical areas for which data were being inputted. In Chapter 10, I have summarised the compositional variables and geographical areas for which I had to impute data from other countries and in Table 17, I have detailed them.

9.7 Study size

I calculated the sample size based on my aim to analyse the difference in the average change of annual EOGBS incidence per 1,000 livebirths (the primary outcome of interest) in geographical areas implementing one GBS prevention strategy compared with geographical areas implementing another. I calculated that a sample size of 63 incidence rate observations per GBS prevention group provided an 80% chance of detecting an average annual difference of 0.1 per 1,000 livebirths between two GBS prevention groups with 95% confidence.

9.8 Data management and statistical analysis

I manually entered data from the institutions into a Microsoft Excel sheet and then recorded data from the international websites and literature. I exported the Excel sheet into Stata 14.0 (Stata Corp, College Station, Texas) where I performed all of the analyses. First, I cleaned and validated the data by checking for any inconsistencies using the 'describe' and 'summarize' Stata command for each variable, correcting any data errors and renaming and (re)coding variables into dummy variables as appropriate. Where data were missing, I imputed the values as described below. There were eight different outcomes and each outcome had its own imputation model and analysis model. Therefore, I separately followed the steps indicated in the sections below for each of the eight outcomes.

The initial statistical plan of analysis for all of the studies involved imputing and analysing the data using repeated measures multi-level analyses in order to account for the hierarchical

structure of the data (outcomes for each year were nested in the geographical areas). I attempted to account for this multi-level structure when imputing the data for the missing observations in the five compositional covariates by using the Stata command 'Realcom-Impute'.[363] However, due to the limited number of fully observed years, multi-level data imputation did not work and imputed values could not be generated. As multi-level imputation was not feasible neither was multi-level analysis.

Alternatively, I applied linear regression analysis to analyse the trends in outcomes. Regression analysis has been discussed in the literature as a tool to predict trends[343, 344] and has been previously utilised in other areas of public health and epidemiology to analyse trends across time.[364-368] Furthermore, as the observations were at the ecological level, the required linear regression assumption that observations must be independent is not as problematic as the observations are not related to the same participants/individuals. For the primary outcome of EOGBS, in addition to the linear regression trend analysis, I modelled a repeated measures multi-level analysis. As the data in the analysis could not be multiple imputed to account for the hierarchical structure, I analysed a multi-level model on fully observed data only, known as the complete case analysis.[369, 370] As there were few fully observed covariates, I only analysed an unadjusted multi-level model (with no compositional covariates).

9.8.1 Multiple imputations for compositional covariates

There were five annually reported covariates that had data available for some years but not for others. These were the percentages of preterm births, low birthweights, caesarean sections, skilled deliveries and the human development index. To avoid losing data and increase statistical power in each model, I used multiple imputation so that all observations (or years) of outcomes could be included. First, I calculated the amount of missingness in each of the covariates using the 'misstable summarize' Stata command, which calculates the number of observed and missing cases for each covariate.[371] I then assessed the missingness mechanism. As the reason for missing data among these variables was that the sources only reported data for certain years and not for others, I concluded that data were missing at random. Nevertheless, I investigated the specific missingness mechanism using the 'misstable patterns' Stata command, which shows the patterns of the observed and unobserved observations for covariates with missing data across the dataset.[371] Finally, I created a variable to identify whether a covariate was missing or observed using the 'misstable sum, gen(_)' Stata command. I then performed logistic regressions on each variable investigating whether the probability of each covariate with missing data was associated with the outcomes, predictors, and all compositional variables from the analysis model.[372] This indicated whether the missing

values were missing completely at random (and unrelated to the other variable data) or missing at random (and dependent on other variables).[369]

To impute the data for the covariates in each model, I used multiple imputation using chained equations (MICE). MICE fills in missing observations iteratively using a sequence of univariate imputation methods with fully conditional specification (FCS) of prediction equations.[373, 374] MICE is a flexible approach that accommodates missingness patterns that are arbitrary.[370, 375, 374] The specific MICE model was predictive mean matching (PMM); a semi-parametric method that combines standard linear regression and nearest-neighbour imputation approaches, allowing the missing values to be imputed using the observed value with the closest predicted mean.[370, 376] PMM is more robust than a fully parametric regression MICE model as it uses linear regression to obtain linear predictions and then randomly draws an imputed value from a set of nearest neighbouring donors with complete values. By drawing from the observed values, the advantage of PMM is that it preserves the distribution of the observed values.[377] PMM was appropriate for the aims because the covariates with missing data were percentages whose parameters were bounded from 0-100% or 0.00-1.00. By using PMM, missing data were never outside these bounds and were, therefore, plausible. Furthermore, from an initial examination of the data, the covariates had particular skewed distributions that needed to be accounted for by the missing data. When using PMM, I set the number of nearest neighbours (to consider as donors) at 10 or 'knn(10)' based on the recommendation by Morris, White and Royston (2014).[376]

For the multiple imputation models, the outcomes were the variables with missing data (preterm births, low birthweights, caesarean section, skilled delivery and the human development index) and the covariates that were regressed onto the outcome to obtain the missing values were: the relevant outcome of the analysis model (e.g. EOGBS, LOGBS, early-onset *E. coli* or sepsis, etc.), the predictors of the analysis model (prevention strategy, year and the interactions between year and prevention strategy [see Section 9.8]) and every compositional covariate of the analysis model. It is recommended that the larger the missing data, the bigger the number of imputations.[370] I set the number of imputations to 100 due to the small sample sizes of the fully observed data where only approximately 60 observation years had complete data for all the years for each of the covariates. I evaluated the sufficiency of using 100 imputations by assessing the convergence of imputations on post-imputation diagnostic plots for MICE (see paragraph below) and by assessing the largest fraction of missing information ($_{FMI}$) in the analysis model. The rule of thumb is that *M (number of imputations)* $\geq 100 \times _{FMI}$ provides an adequate number of imputations for analysis.[370]

After running the imputation models, I used multiple imputation diagnostics to assess the reliability of the imputation. I used trace plots to ensure that convergence of the data was achieved, meaning that the algorithm used for data augmentation reached an appropriate stationary state.[375] Trace plots show the estimated parameters against the iteration numbers for each of the five imputed variables. They allow judgement on whether the predicted values remain constant and do not show a trend, as well as the number of iterations it took to achieve a stationary phase.[378, 375] To do this, I saved a trace file containing the mean and standard deviation of each variable in each iteration. I reshaped the data into time series and used the Stata command 'tsline' to plot the iteration on the x-axis and the predicted values on the y-axis. If the data showed signs of not converging, I increased the number of iterations in the imputation model. Finally, I also examined the descriptive summary statistics for the imputed datasets and compared them with the original dataset.

There has been an ongoing debate in the literature about whether data should be transformed and then imputed or vice versa to account for non-linear effects in the data.[370, 379] For the data available to address objectives 4 to 6, I considered many methods and chose PMM which is one approach to alleviate effects of model misspecification.[379] I considered the recommended, substantive model compatible FCS, which involves specifying the analysis model of interest and imputing each variable compatibly with the analysis model.[379] However, with only approximately 60 observations being fully observed, the substantive model compatible FCS model did not work and valid imputed values could not be generated. Likewise, I also considered the 'Just Another Variable' approach,[380] where data are first transformed and then imputed as just another variable. Again, because of the few initial observations, the relationships between the covariate with missing data and the outcomes were difficult to assess and transform because of issues such as collinearity. Furthermore, the relationships between the variables changed after imputation when more data were used for analyses. Therefore, PMM approach as the most appropriate for this context and I transformed the data after imputation. Indeed, PMM can provide results comparable with the results from the 'Just Another Variable' approach.[370] Importantly, there was no missing data in the predictor variable of interest (interaction of year with prevention strategy) or the outcomes, and so the compositional covariates were transformed using the best available solution. The missingness data mechanism in these variables was also missing at random due to data collection and estimation in some years and not others, minimising the bias from imputation.

9.8.2 Descriptive analyses

The descriptive statistics entailed exploratory analyses of the outcomes, predictors, and compositional covariate variables. I reported means and standard deviations for the continuous variables in tables and text (livebirths; EOGBS, early-onset *E. coli*, early-onset sepsis, and LOGBS per 1000 livebirths; percentage EOGBS and neonatal GBS resistant to clindamycin and erythromycin; percentage skilled delivery, preterm births, low birthweights, caesarean section, multiple births, maternal GBS colonisation, prolonged rupture of membranes, intrapartum fever; fertility rate, maternal age, *per capita* government expenditure on health, the human development index). I also described frequencies and percentages for the categorical variables in tables and text (GBS prevention strategy, region, most prevalent GBS serotype, type of surveillance, geographical coverage, age of onset definition). Finally, I tabulated the averages (mean and standard deviation [SD]) of the outcomes for each geographical area, year, world region and each GBS prevention strategy along with a scatterplot.

9.8.3 Unadjusted linear regression analyses

After analysing the descriptive statistics, I analysed the unadjusted relationship between each outcome variable and the predictor, most recently reported GBS prevention strategy, in separate linear regression models for multiple imputed data, using the Stata command 'mi estimate: regress'. This command runs a linear regression on the multiple imputed data and estimates model parameters by adjusting coefficients and standard errors for the variability between imputations according to Rubin's rules.[381, 382, 373, 370] To investigate the difference in the average annual change in each outcome between areas that most recently reported screening with areas that most recently reported other GBS prevention strategies, I interacted the prevention strategy and the year and regressed this to the outcome per 1,000 livebirths or percentage. While the main effect of the year and prevention strategy were included in the model, the most important term was the interaction between year and GBS prevention strategy as this revealed the difference in the average annual change in the outcomes between screening compared with another GBS prevention strategy. I presented the analyses using the coefficients for each of the most recently reported GBS prevention strategies and their 95% CI and p-values. The coefficients show the difference in the average annual change in the outcome, between each GBS prevention strategy and the baseline prevention strategy. Most often, the baseline prevention strategy was universal GBS screening, however, for antibiotic resistance outcomes, the baseline was no prevention strategy, as I was interested in resistance rates in risk-based compared with no prevention.

To present the outcome trends across time graphically and aid the interpretation of the trend analysis for each of the most recently reported GBS prevention strategies calculated by the models, I used the STATA command 'mimgrns'. 'Mimgrns' is the multiple imputation equivalent of the 'margins' post-estimation command for linear regression.[383] Margins provide estimates of the outcomes that are calculated from predictions of a model for specified values of covariates.[384] Once the margin estimates are generated, they can be plotted using 'marginsplot'. Using these methods, I obtained estimates for each outcome for each year under different GBS prevention strategies and was able to plot these trends graphically.

For the compositional covariates, that were later used for adjustment purposes, I investigated the relationship between the covariate and the outcome per 1,000 livebirths or percentage itself, but not the trend. I analysed the unadjusted relationship between each outcome variable and each compositional covariate in separate linear regression models for multiple imputed data, using the same Stata command 'mi estimate: regress'. I also presented these analyses using the coefficients and their 95% CI, as well as the p-values. The coefficients here show the differences in the mean outcome and not the trend.

9.8.4 Adjusted linear regression analyses

After the unadjusted analyses, I performed an adjusted linear regression for multiple imputed data to identify the independent association between the most recently reported GBS prevention strategies and the average annual change for each outcome, after statistically controlling for the compositional covariates. Using 'mi estimate: regress', I regressed the main effect of, and interaction between, year and GBS prevention strategy (to account for the trend in each prevention strategy) as well as the compositional covariates onto each outcome. I only included compositional covariates that were statistically significant at a probability level of $p<0.20$ in 2-sided t-tests in the unadjusted analyses. I chose a probability level higher than $p<0.05$, because adding covariates together in regression models can influence a covariate that was not associated with the outcome on its own, to become associated.

To generate a minimal adjusted model, I used a backwards elimination process, whereby I removed covariates that had low explanatory power (in combination with the others) one at a time, only including variables that were statistically significant at $p<0.05$ in 2-sided t-tests. The exception was a list of covariates that I decided, *a priori,* were important adjustments that would be included in the final model even if they were not statistically significant at $p<0.20$ in the unadjusted models or $p<0.05$ in the adjusted model. The *a priori* list for EOGBS and LOGBS consisted of: preterm births, low birthweights, maternal GBS colonisation, prolonged

rupture of membranes and intrapartum fever (as they are known strong risk factors of EOGBS) and the human development index, world region, surveillance type, geographical coverage, and outcome definition (to account for crucial limitations in the data collection methods). The *a priori* list for the analyses of the remaining outcomes was shorter as the sample sizes were considerably smaller, therefore, the model needed to be simplified. The list included: preterm births and low birthweights (as they are important risk factors), and the human development index, geographic region, surveillance type, geographical coverage and outcome definition (to account for crucial limitations in the data collection methods).

Currently, the recommended methods for running regression diagnostics on multiple imputed data are to run the diagnostics separately for a few imputed datasets.[370, 385] Therefore, I ran regression diagnostics for the minimal adjusted models separately for five imputed datasets to check that the residuals met the assumptions of linear regression. I used five datasets as this was sufficient to get consistent results. The diagnostic plots I created to check the assumptions were the residual-*versus*-fitted plots, kernel density plots, standardised normality plots (pnorm and qnorm) and augmented component-plus-residual plots.[386] I tested these assumptions using Cameron & Trivedi's Information Matrix-test[387] and Breusch-Pagan/Cook-Weisberg test[388, 389] for heteroscedasticity, the linktest[390] and ovtest[391] for regression model specification and the variance inflation factor test (VIF test) for the collinearity of continuous covariates.[386, 392] When assumptions were not met, particularly for linearity, I transformed the covariates using the most appropriate transformation according to the relationship, the normality and the natural measurement of the covariate. Once linearity was achieved and all the assumptions were reasonable for the models, I re-ran the multiple imputed regression models with the transformed covariates. If the assumptions of heteroscedasticity or normality were not strongly supported in the diagnostic analyses, I calculated robust standard errors.[393] If any compositional covariates were no longer significant at $p<0.05$ in the model with transformed data, I removed them (except those on the *a priori* inclusion list) and re-ran the regression diagnostics. This process continued until the regression assumptions were sufficiently met and minimal models were achieved.

These adjusted minimal regression models on multiple imputed and then transformed data were considered the final models. I calculated the adjusted R^2 for the final models, which informs the amount of variance accounted for in models, by using the Stata command 'mi beta' designed for models on multiple imputed data.[394] Finally, I used the command 'mi test' to compare GBS prevention strategies with strategies other than the baseline.[395] The regression equation for the models was:

$$Y_{\text{Outcome}} = \alpha_{\text{Intercept}} + \beta_1 \times \text{Years} + \beta_2 \times \text{Prevention strategy} + \beta_3 \times \text{Years*Prevention Strategy} + \beta_K \times \text{Compositional Covariate}_{K\ldots} + \varepsilon_{\text{Error}}$$

Similar to the unadjusted analysis, the final adjusted models compared the average annual change of outcomes in each of the most recently reported GBS prevention strategies with the baseline prevention strategy (most often universal GBS screening, but no prevention strategy for antibiotic resistance outcomes, as stated above). I presented the adjusted differences in the average annual change of the outcome, by different GBS prevention strategies (adjusting for the compositional variables), using the coefficients for each GBS prevention strategy (compared with the baseline prevention strategy), along with their 95% CI and p-values. In addition, I also reported the adjusted R^2 of the model. To present, graphically, the outcome trends for each GBS prevention strategy (adjusted for compositional covariates), I used the Stata command 'mimgrns' and 'marginsplot' (as described above).

9.8.5 Sensitivity analyses

When the average annual change of the outcome was statistically different ($p<0.05$) by most recently reported GBS prevention strategy in the final model, I tested the stability of the results in sensitivity analyses. This was especially necessary due to the anticipated differences in the data collected across geographical areas. I re-ran the final model with the following sensitivity changes:

a. Restricting the analysis to outcome data that were from two or more centres (i.e. excluding data from one centre only). Data that are mandatory and large scale are more accurate than small centre-based studies, especially as the outcomes were relatively infrequent (e.g. EOGBS incidence is only around 0.5 per 1000 livebirths). Therefore, the larger the sample size coverage the more stable and accurate the incidence rates. Ideally, I would have restricted the data to the mandatory national or regional level only, however, this was not possible due to the limited data available at these levels. As an alternative, I excluded data from only one centre although this meant that voluntary data and data from a few centres only were included.

b. Restricting models to data with the most appropriate definition of the outcome (e.g. 5/6/7 days or less for early-onset and five days onwards for late-onset). As described in Chapter 2, less than seven days is considered early-onset disease and attributed to maternal transmission, while seven to 98 days is considered late-onset and can be transmitted from

other sources.[3] As the definitions of early-onset varied across geographical areas, I selected the closest definition to seven days and ran the final model with only the outcome data defined as such.

c. Restricting models to geographical areas that provided four or more years of data. The number of years provided by institutions varied, with some that only provided data for one year. Only including areas with four or more years of data may be more accurate as the trends would be more stable. Therefore, a sensitivity analysis tested this.

d. Restricting models to outcome data that were within the outer fences of a box plot (i.e. removing extreme box plot outliers). The box plot divides data into four boundaries based on the interquartile ranges (IQR): two are the inner fences and two are the outer fences. Outer fences are "Q1-3 IQR and Q3+3 IQR", and any data points beyond these boundaries are considered extreme outliers.[396 p2] Outer fence outlier data are usually the furthest away, even past the whiskers (inner fences) of a box plot.[396] For this sensitivity analysis, I calculated the outer fences and removed those data lying beyond the outer fences on the right side of the distribution. The outer fence on the left side was less than zero even though the most extreme value on the left side could only be zero, therefore, there were no data to remove. Box plot outliers are only explanatory, thus, I only used them for the purposes of exploring the sensitivity of the models. I analysed the outer fence outliers as they are generally more accurate at identifying unusual distributions compared with inner fence outliers.[396]

e. Restricting models to compositional variables where only less than 10% of the data were imputed from another country. Data from the same country are more accurate than those imputed from neighbouring countries, thus, I tested whether the results changed if only these variables were included. This meant including only the following compositional covariates: region, preterm births, low birthweights, surveillance type, geographical coverage and outcome definitions.

f. Using only survey data for the compositional covariates (i.e. removing data from international websites, except naturally occurring wider covariates, such as the human development index that are only available from international websites). The compositional data from the survey questionnaires match the geographical area from where the outcome data were provided. On the other hand, data from the international websites may be averaged from many different regions across the entire country and may not reflect the context of the particular area the outcome data were from. For example,

data were provided for Emilia-Romagna, a region in the north of Italy that is known for having better economic and health indicators than the south of Italy.[397, 398] Compositional data, averaged for Italy as a whole, may differ from the specific context of Emilia-Romagna. In this analysis, I checked the impact of this difference on the results. I first re-ran multiple imputation on the survey data alone in order to impute missing observations. This included data that were previously missing and data that were now missing due to the removal of international website data. I then re-ran the final model on this new multiple imputed dataset.

g. Using a different 'most prevalent' GBS serotype for geographical areas where more than one was reported from the data source. For the main model, I selected the serotype using the most commonly reported serotype across the years with provided data or the study with the largest sample size where more than one study was available in the literature. In this analysis, I selected the alternative serotype and re-ran the final model.

h. Using alternative maternal GBS colonisation rates for geographical areas where more than one rate was available. Similar to the analysis for serotype, for the main model I selected the colonisation rate using the most commonly reported rate across the years with provided data or the study with the largest sample size where more than one study was available in the literature. For this sensitivity analysis, I selected the alternative lower and/or higher rates separately and re-ran the final model.

i. Using alternative data sources for geographical areas where more than one institution provided data for overlapping areas. Similar to using survey data only, I first re-ran the multiple imputation on the alternative sources of data in order to impute missing observations. I then re-ran the final model on this new multiple imputed dataset to assure confidence in the results.

j. Replacing the GBS prevention strategy that was the most recently reported (predictor variable) with the GBS prevention strategy that was the most frequently reported across the years with outcome data. As discussed in Section 9.3, I created a variable for the most frequently reported GBS prevention strategy for each geographical area using the dates reported by some institutions as well as the dates reported on GBS prevention guidelines. As mentioned in Section 9.3, although this was not the most accurate predictor, it was important to have a comparison between the use of the most recently reported GBS prevention strategy as the predictor variable with the most frequently reported one across the years.

9.8.6 Repeated measures multi-level analyses for EOGBS trends

As mentioned above, I only performed the repeated measures multi-level analysis on the EOGBS data as it this was the primary outcome and was the study with statistically significant results. I ran a two-level linear growth curve model on the fully observed data in the EOGBS dataset using the STATA command 'mixed'.[399, 400] A growth curve model is a type of random slopes model with time as the x-axis and time observations clustered within individuals (or within geographical areas for the purposes of this study). Like other multi-level models, growth curve models allow the estimation of within and between differences, i.e. between geographical area differences and within geographical area differences.[401, 402] The within-geographical patterns of change are the time trends or growth curves.[401] The EOGBS observation years (level 1) were nested within the geographical area from which the data originated (level 2). The outcome variable was the average change in annual EOGBS incidence per 1,000 livebirths and the predictor variable was the GBS prevention strategy.

The growth curve model consists of fixed and random effects that display the trends of EOGBS incidence. The fixed effect represents the mean intercept of EOGBS incidence and the mean slope (or change) in the EOGBS incidence across the entire sample of geographical areas. On the other hand, the random effect represents the between-geographical area variability in the geographical area intercepts and slopes for EOGBS incidence.[401] The model was a growth curve model with an interaction between the year and the most recently reported GBS prevention strategy as a fixed effect, year centred at 2005 (this was around the mean year) random at the level of the geographical area and no compositional variables in the model. As this was a complete case analysis (because multi-level multiple imputation did not work) and there were few fully observed covariates, I was unable to adjust for the compositional covariates.

As per the linear regression analyses, I reported the differences in the trends of EOGBS incidence for each GBS prevention strategy compared with universal GBS screening (fixed effect) using their coefficients with their 95% CI. I also calculated margins for the models and plotted the trends graphically using margin plots as described above. In addition, I reported the between geographical area variance in the intercept at year 2005 (variability in the EOGBS incidence at year 2005 by geographical area) the slope (variability in the pattern of EOGBS incidence by geographical area), and the intercept-slope (the joint variability between the geographical area intercept and slopes). I also reported the within geographical area variance, which is the variability across the years within geographical areas.

9.9 Ethical approval

This study was ethically approved by the University of Warwick Biomedical and Scientific Research Ethics Sub-Committee, BSREC reference: REGO-2014-777 (see Appendix 14). As the data provided were aggregated at the level of the centre, region, or country, they were anonymous and formal consent was not required. I clearly informed institutions of this on the study information sheet. I also told them to make a free decision, reassured them that that there would be no consequences if they chose not to provide data, and that they were free to withdraw their data at any time without giving any reason. I stored the aggregate data in a password protected laptop during the studies, and will store and archive the data as per University policy once the studies are completed.

10. GENERAL DESCRIPTION OF DATA COLLECTED FROM SURVEYS FOR OBJECTIVE 4 TO 6

As discussed in Chapter 9, the data on the outcomes, the predictor and some of the compositional covariates for the three ecological trend analysis studies were collected by sending one survey across institutions worldwide. The remaining compositional covariates were collected from the same international sources (see Chapter 9 for more details on the methodology). As the data were collected using the same surveys and sources, in this chapter, I summarise an overview of the collected outcomes, predictors and covariates across the geographical areas. In some cases, the provided data covered geographical areas that overlap. In the chapters that follow, I summarise the outcomes, predictors and covariates only for the selected geographical areas with the best available outcome data (widest coverage or best surveillance type) for areas where data overlap. I then only use the areas excluded from the main analysis, in the sensitivity analysis. In the sections below, Table 16 and Table 17 I summarise the overall outcome, predictor and compositional data collected, the geographical areas that these data are from, the surveillance and geographical coverage and the sources for the compositional data. I also provide the average rates of the outcomes and the compositional variables.

10.1 Data collected

I advertised the study to 520 institutions across 167 countries. There were five identified institutions whose contact details I could not find (one covering Asia, one in India, one in Argentina and two in Slovakia) and two whose contact details did not work (one in Saudi Arabia and one in Brazil). Of the 520 institutions contacted, some of the institutions were international and covered more than one country. Altogether, 467 institutions did not provide data: 257 did not respond, 158 did not have available data, 29 did not have the availability, resources or had management issues, for example, were unable to obtain ethical approval from their institution altogether or by the time data collection ended, eight did not send data after initially agreeing to participate, seven either did not have their data ready yet or had yet to publish them so could not share, seven did not want to participate or did not want to share data and one required payment which was not possible. Six of the institutions that provided outcome data referred me to other institutions that covered the same geographical area to provide data on the compositional covariates. Of the six institutions suggested, three provided

data or referred me to the website to collect data myself, while one did not respond and two did not have the data. One institute covering Ontario and one covering Norway provided compositional covariate data and one online database covering England was suggested, from which I extracted data.

Altogether, 53 institutions provided outcome data across 60 geographical areas. However, I excluded data from one institution in Hebei, China, as the denominator was the number of neonates in an NICU, instead of livebirths. This may over-represent the number of diseases in a population and not be comparable with data from other countries. This left 52 institutions across 59 geographical areas. One institution provided outcome data on three different geographical areas in South Africa (two different hospitals in Johannesburg and the township of Soweto) and one institution provided outcome data on six different geographical areas within the British Isles (England, Northern Ireland, Republic of Ireland, Scotland, Wales and the United Kingdom and Republic of Ireland combined). Table 16 shows the geographical areas for which I collected data for each outcome. There were EOGBS data from 55 geographical areas, early-onset neonatal sepsis and early-onset *E. coli* data from 28 areas, LOGBS data from 47 areas, EOGBS clindamycin resistance from 23 areas, EOGBS erythromycin resistance data from 24, neonatal GBS clindamycin resistance from 17 areas and neonatal GBS erythromycin resistance from 19 areas.

Table 17 shows the data source for each compositional covariate: survey data, international websites or imputed from neighbouring countries. There were five geographical areas or institutions from North America, four from Oceania, eight from Asia, 27 from Europe, eight from Latin America and the Caribbean, four from Sub-Saharan Africa and three from North Africa and the Middle East. Data for the human development index, skilled attendance at delivery, mean government expenditure on health and fertility rate were from international websites, and as such, were at the national level for each geographical area. Data for the average maternal age were from international websites for all areas, except six (Buenos Aires, Cordoba, Guangzhou, Kuwait, Macau and Mauritius) for which I imputed data from the closest neighbouring area. Data for preterm births were from the survey questionnaires for 23 geographical areas and from international websites for 36 areas while data for low birthweights were from survey questionnaires for 27 areas and from international websites for 32 areas. Data for caesarean sections were from survey questionnaires for 21 areas, from international websites for 36 areas and I imputed data from a neighbouring country for Singapore City. Data for multiple births were from international sources, except for 14 areas where the data were from surveys and 10 that I imputed from the nearest neighbouring area. Maternal GBS colonisation rates were from surveys for 25 areas, an international source for

25 areas and I imputed data from the closest neighbouring area for nine areas. Data on the most prevalent serotype were provided in surveys for 21 areas, from international sources for eight areas and I data from neighbouring areas for 30 areas. Data for prolonged rupture of membranes were from surveys for 17 areas and I imputed them from neighbouring areas for 42 areas, while data for intrapartum fever were from surveys for 14 areas and I imputed them from neighbouring areas for 45 areas. Evidently, few data were available for prolonged rupture of membranes and intrapartum fever, however, as these compositional covariates were important for EOGBS, I imputed them from the other countries so that the models had some, rather than no, adjustment.

10.2 General characteristics of the data

There were 59 geographical areas across 42 countries. Data from 26 areas had national coverage, three had regional coverage, two had city or town wide coverage and the remaining were from one centre in a city or town. Data for 11 areas were from mandatory or enhanced population surveillance, nine from voluntary population surveillance, 11 from multi-centre surveillance and the remaining were surveillance from one centre.

Table 16 shows the mean outcome rates for each geographical area (across the years) and a total mean rate across all geographical areas and years is provided below. Note that the total mean outcome rate provides an overview of the data collected, however, as some of the areas overlap, there may be double counting. Therefore, the overall mean outcome rates provided in the separate chapters are more accurate. The years ranged from 1989 to 2015 and the livebirths ranged from 414 to 4,316,233. The mean EOGBS incidence was 0.55 per 1,000 livebirths (SD 1.48) and it ranged from 0.00 to 28.99 per 1,000 livebirths. Mean early-onset sepsis was 4.60 per 1,000 livebirths (SD 8.79), and it ranged from 0.00 to 72.46 per 1,000 livebirths. Mean early-onset *E. coli* was 0.42 per 1,000 livebirths (SD 1.99), and it ranged from 0.00 to 28.99 per 1,000 livebirths. The mean LOGBS was 0.37 per 1,000 livebirths (SD 1.37), ranging from 0.00 to 24.15 per 1,000 livebirths. The disease incidence rates of 0.00 per 1,000 livebirths might be a result of diagnostic related issues, voluntary surveillance or too small a sample size to detect cases. The highest incidence rates of all of the disease outcomes across the board were in Mansoura city, Egypt. The mean percentage of EOGBS cases resistant to clindamycin was 15.57% (SD 29.22) and resistance to erythromycin was 19.22% (SD 30.08), both ranging from 0.00 to 100.00%. The mean percentage of neonatal GBS

resistant to clindamycin was 14.73% (SD 25.01) and the mean percentage of neonatal GBS cases resistant to erythromycin was 15.33 (SD 21.75), both ranging from 0.00 to 100.00%.

Table 17 shows the averages for the predictor variable and the compositional covariates across geographical areas. Twenty-six centres, regions, or countries most recently reported a universal GBS screening programme, 22 reported a risk-based prevention strategy, three reported either a screening or risk-based strategy (two of which were Australia) and eight reported no prevention strategy. The mean human development index was 0.83 (SD 0.09), the mean percentage of skilled deliveries was 97.80% (SD 4.98) and the mean government expenditure on health was $3,502.46 (SD 2,268.15). The mean percentage of preterm births was 8.96% (SD 3.70), the mean percentage of low birthweights was 8.88% (SD 6.73) and the mean percentage of caesarean sections was 25.84% (SD 10.09). The mean fertility rate was 1.83 per woman (SD 0.45), the mean maternal age was 26.39 (SD 3.21) and the mean multiple or twin birth rate was 17.29 per 1,000 livebirths (SD 12.44). The mean maternal GBS colonisation was 20.85% (SD 10.05, range 0.14 to 44%) and the most prevalent GBS serotype was serotype III. Finally, the mean percentage of prolonged rupture of membranes was 7.08% (SD 6.24) and the mean percentage of intrapartum fever was 1.51% (SD 2.30).

In the following three chapters, I will focus separately on the results on each outcome.

Table 16. Outcomes collected for objectives 4 to 6

No	Area	Country	Surveillance type	Geographical coverage	Overall Years	Mean Livebirths (SD)	Mean EOGBS (SD)	Mean early-onset sepsis (SD)	Mean early-onset *E.coli* (SD)	Mean LOGBS (SD)	Mean EOGBS clindamycin resistance (SD)	Mean EOGBS erythromycin resistance (SD)	Mean neonatal GBS clindamycin resistance (SD)	Mean neonatal GBS erythromycin resistance (SD)
1	Alberta	Canada	Mandatory/ Enhanced population	Regional	2003-2013	47,896 (4854.45)	0.25 (0.10)	0.21 (0.08)	-	0.24 (0.09)	20.85 (11.05)	36.17 (18.41)	19.51 (10.12)	32.94 (14.80)
2	Australia	Australia	Multi-centre	National	2008	847,783	0.38	-	-	-	-	-	-	-
3	Australia	Australia	Multi-centre	National	2002-2012	29,199.82 (4802.03)	0.38 (0.16)	2.24 (0.51)	0.34 (0.12)	0.20 (0.12)	-	-	-	-
4	Bangalore	India	Multi-centre	City/town-wide	2013-2015	4,044.33 (1605.77)	0.15 (0.26)	5.68 (0.72)	0.00 (0.00)	0.07 (0.11)	-	-		-
5	Barcelona	Spain	One centre	One centre in a city/town	1996-2013	1,776.89 (247.95)	0.33 (0.52)	0.98 (0.81)	0.25 (0.33)	0.10 (0.23)	0.00 (0.00)	0.00 (0.00)	0.00	0.00 (0.00)
6	Brno	Czech Republic	One centre	One centre in a city/town	2010	6,415	0.31	-	-	0.00	0.00	0.00	0.00	0.00
7	Buenos Aires	Argentina	One centre	One centre in a city/town	1996-2013	6,473.22 (923.54)	0.14 (0.20)	0.68 (0.32)	0.13 (0.12)	-	-	-	-	-
8	Canada	Canada	Voluntary population	National	2000-2013	-	-	-	-	-	22.95 (5.50)	56.60 (17.74)	22.32 (7.65)	56.01 (13.37)
9	Canada	Canada	Voluntary population	National	2011-2012	379,037.50 (1,982.02)	0.07 (0.03)	0.17 (0.01)	0.06 (0.01)	-	9.98 (6.68)	8.51 (4.60)	-	-
10	Cordoba	Argentina	One centre	One centre in a city/town	2013-2014	1,573 (152.74)	0 .00 (0.00)	6.12 (4.80)	0.00 (0.00)	0.00 (0.00)	-	-	-	-
11	Denmark	Denmark	Voluntary population	National	1999-2013	63,296.47 (3182.94)	0.17 (0.05)	-	-	0.13 (0.05)	27.10 (28.39)	26.80 (27.26)	23.48 (29.13)	24.79 (28.82)
12	Emilia-Romagna	Italy	Mandatory/ Enhanced population	Regional	2003-2013	38,686.09 (2,477.04)	0.26 (0.07)	0.61	0.13	0.30 (0.10)	23.81	16.67	-	-
13	England	United Kingdom	Mandatory/ Enhanced population	National	2000, 2014	668,696 (68,071.76)	0.54 (0.06)	-	-	0.31 (0.08)	-	-	-	-
14	England	United Kingdom	Voluntary population	National	1998-2013	629,548.60 (46,740.74)	0.35 (0.03)	-	0.14 (0.04)	0.20 (0.05)	5.91 (5.07)	9.46 (7.04)	6.44 (4.88)	8.79 (6.48)
15	Finland	Finland	Mandatory/ Enhanced population	National	1995-2013	58,576.38 (1,752.01)	0.55 (0.14)	-	-	0.25 (0.09)	-	-	-	-
16	Flanders	Belgium	Multi-centre	City/town-wide	2004-2009	-	0.21 (0.08)	-	-	-	-	-	-	-
17	France	France	Voluntary population	National	1996-2013	570,199.50 (57,015.64)	0.36 (0.19)	-	-	0.22 (0.04)	-	-	-	-
18	France	France	Voluntary population	National	2007-2013	-	-	-	-	-	14.90 (8.42)	23.27 (9.16)	10.35 (5.09)	16.58 (3.04)
19	Guangzhou	China	One centre	One centre in a city/town	1997-2014	1,820.89 (488.22)	0.61 (0.29)	7.24 (3.00)	2.30 (1.66)	0.97 (0.45)	100.00 (81.65)	100.00 (81.65)	43.75 (33.69)	43.75 (33.69)

No	Area	Country	Surveillance type	Geographical coverage	Overall Years	Mean Livebirths (SD)	Mean EOGBS (SD)	Mean early-onset sepsis (SD)	Mean early-onset *E.coli* (SD)	Mean LOGBS (SD)	Mean EOGBS clindamycin resistance (SD)	Mean EOGBS erythromycin resistance (SD)	Mean neonatal GBS clindamycin resistance (SD)	Mean neonatal GBS erythromycin resistance (SD)
20	Ho Chi Minh	Vietnam	One centre	One centre in a city/town	2011-2013	45,589.33 (5,934.90)	-	-	-	0.04 (0.03)	-	-	-	-
21	Johannesburg	South Africa	One centre	One centre in a city/town	2012	9,028	1.22	-	-	1.22	-	-	-	-
22	Johannesburg	South Africa	One centre	One centre in a city/town	2012	11,894	0.67	-	-	0.84	-	-	-	-
23	Kaunas	Lithuania	One centre	One centre in a city/town	2007-2013	3,524.86 (194.83)	1.13 (0.50)	5.18 (2.15)	0.81 (0.30)	0.79 (0.90)	-	-	-	-
24	Kingston	Jamaica	One centre	One centre in a city/town	1991-2010	2,744.69 (360.63)	0.69 (0.40)	2.59 (1.03)	0.18 (0.40)	0.33 (0.31)	-	-	-	-
25	Kuala Terengganu	Malaysia	One centre	One centre in a city/town	2006-2014	12,578.56 (1141.11)	0.26 (0.25)	0.93 (0.42)	0.02 (0.03)	0.03 (0.04)	3.17 (8.40)	19.05 (26.23)	3.17 (8.40)	19.73 (27.69)
26	Kuwait	Kuwait	One centre	One centre in a city/town	2005-2014	11,074.1 (486.46)	1.14 (0.76)	3.52 (1.14)	0.43 (0.28)	-	16.31 (12.20)	15.44 (10.60)	-	-
27	Macau	China	One centre	One centre in a city/town	2005-2014	2,667.70 (697.83)	0.64 (0.70)	0.84 (0.77)	0.08 (0.17)	0.68 (0.66)	66.67 42.49	33.33 (57.74)	79.17 (21.08)	17.78 (16.78)
28	Manila	Philippines	One centre	One centre in a city/town	2014	6,682	0.15	10.18	-	0.00	-	-	-	-
29	Mansoura city- Dakahlia Governorate	Egypt	One centre	One centre in a city/town	2014	414	28.99	72.46	28.99	24.15	16.67	25.00	18.18	27.27
30	Mauritius	Mauritius	Mandatory/ Enhanced surveillance	National	2013	12,986	1.46	-	-	0.39	-	0.00	-	0.00
31	Mexico City	Mexico	One centre	One centre in a city/town	1995-2013	5,055.11 (594.00)	0.27 (0.22)	24.52 (8.20)	0.96 (0.84)	0.99 (1.40)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
32	Netherlands	Netherlands	Voluntary population	National	1995-2015	190,186.70 (9,833.44)	0.17 (0.04)	-	0.03 (0.02)	0.10 (0.04)	-	-	-	-
33	New Zealand	New Zealand	Multi-centre	National	2002-2012	5,929.55 (1891.91)	0.96 (0.44)	3.00 (0.82)	0.54 0.33	0.68 (0.51)	-	-	-	-
34	New Zealand	New Zealand	Multi-centre	National	1998, 2010	119,768 (10,417.10)	0.36 (0.19)	0.49	0.09	0.02	3.57	1.79	-	-
35	Northern Ireland	United Kingdom	Mandatory/ Enhanced population	National	2000, 2004	24,866 (2,207.59)	0.69 (0.06)	-	-	0.26 (0.11)	-	-	-	-
36	Norway	Norway	Mandatory/ Enhanced population	National	1996-2013	59,343.44 (1,936.13)	0.44 (0.12)	-	-	0.22 (0.08)	-	-	-	-
37	Ontario	Canada	Voluntary population	Regional	1997-2013	135,098.60 (4,563.32)	0.27 (0.06)	-	0.003 (0.003)	0.08 (0.03)	-	-	-	-
38	Panama City	Panama	One centre	One centre in a city/town	1992, 2011	131,288 (163,749)	0.40 (0.53)	1.09	0.22	0.30 0.40	-	-	-	-

No	Area	Country	Surveillance type	Geographical coverage	Overall Years	Mean Livebirths (SD)	Mean EOGBS (SD)	Mean early-onset sepsis (SD)	Mean early-onset *E.coli* (SD)	Mean LOGBS (SD)	Mean EOGBS clindamycin resistance (SD)	Mean EOGBS erythromycin resistance (SD)	Mean neonatal GBS clindamycin resistance (SD)	Mean neonatal GBS erythromycin resistance (SD)
39	Podgorica	Montenegro	One centre	One centre in a city/town	2012-2014	4,630.333 (2,449.98)	0.00 (0.00)	-	-	0.00 (0.00)	-	-	-	-
40	Portugal	Portugal	Multi-centre	National	2001-2007	109,472.90 (4,266.57)	0.24 (0.13)	-	-	0.13 (0.08)	-	-	-	-
41	Portugal	Portugal	Multi-centre	National	2008-2013	95,824.83 (8,100.16)	-	5.13 (0.20)	0.21 (0.03)	-	-	-	-	30.19
42	Republic of Ireland	Republic of Ireland	Mandatory/ Enhanced population	National	2000, 2014	65,921.50 (10,129.30)	0.40 (0.08)	-	-	0.27 (0.02)	-	-	-	-
43	Riga	Latvia	One centre	One centre in a city/town	2014	2,060	0.49	9.71	0.00 (0.00)	0.00	0.00	0.00	0.00	0.00
44	Santo Domingo	Dominican Republic	One centre	One centre in a city/town	2011	18,000	2.33	-	-	0.83	4.76	83.33	-	-
45	Sao Paolo	Brazil	One centre	One centre in a city/town One centre in a city/town	1991-2001, 2007-2011	8,671.19 (4,906.72)	0.44 (0.24)	-	-	0.15	-	-	-	-
46	Scotland	United Kingdom	Mandatory/ Enhanced population	National	2000, 2014	59,475.5 (2,795.19)	0.35 (0.20)	-	-	0.32 (0.15)	-	-	-	-
47	Singapore City	Singapore	One centre	One centre in a city/town	2001-2014	2,658.64 (399.24)	0.26 (0.40)	-	-	-	-	-	-	-
48	Slovenia	Slovenia	Voluntary population	National	2009-2013	21,839 (448.82)	0.00 (0.00)	-	-	0.00 (0.00)	-	-	-	-
49	Sofia	Bulgaria	One centre	One centre in a city/town	2009-2014	3,894.17 (229.22)	0.78 (0.25)	-	-	-	1.00 (2.45)	-	-	-
50	Soweto	South Africa	One centre	One centre in a city/town	2004-2012	29,064.50 (2,561.84)	1.50 (0.15)	-	-	1.20 (0.13)	0.39 (0.88)	5.74 (4.32)	0.23 (0.51)	3.41 (2.66)
51	Spain	Spain	Multi-centre	National	1996-2012	96,036.63 (13,710)	0.50 (0.31)	1.35 (0.51)	-	-	-	-	-	-
52	St Augustine	Trinidad and Tobago	One centre	One centre in a city/town	1989, 1990, 1994, 1996, 1997, 2000, 2002	5,145 (375.28)	3.74 (1.14)	10.18	-	-	-	-	-	-
53	Switzerland	Switzerland	Multi-centre	National	2011-2013	81,901 (988.11)	0.06 (0.05)	0.30 (0.18)	0.04 (0.05)	0.13 (0.10)	-	-	-	-
54	Tokyo	Japan	One centre	One centre in a city/town	2003-2013	1,627.73 (192.70)	0.39 (0.63)	1.05 (0.92)	0.12 (0.26)	0.12 (0.26)	25.00 (50.00)	0.00 (0.00)	16.67 (40.82)	0.00 (0.00)

No	Area	Country	Surveillance type	Geographical coverage	Overall Years	Mean Livebirths (SD)	Mean EOGBS (SD)	Mean early-onset sepsis (SD)	Mean early-onset *E.coli* (SD)	Mean LOGBS (SD)	Mean EOGBS clindamycin resistance (SD)	Mean EOGBS erythromycin resistance (SD)	Mean neonatal GBS clindamycin resistance (SD)	Mean neonatal GBS erythromycin resistance (SD)
55	Tunis	Tunisia	One centre	One centre in a city/town	2011-2013	3,818 (115.17)	1.14 (0.32)	-	0.18 (0.31)	0.00 (0.00)	100.00	60.00 (56.57)	-	-
56	United Kingdom and the Republic of Ireland	United Kingdom and Republic of Ireland	Mandatory/ Enhanced population	National	2000, 2004	854,084.5 (84,919.99)	0.52 (0.06)	-	-	0.31 (0.09)	-	-	-	-
57	United States of America	United States of America	Multi-centre	National	1990, 1993, 1995, 1997-2014	384,732.30 (95,631.70)	0.53 (0.43)	0.56 (0.22)	0.19	0.33 (0.03)	26.09	47.83	21.64	49.71
58	Wales	United Kingdom	Mandatory/ Enhanced population	National	2000, 2014	35,126 (1,715.44)	0.41 (0.08)	-	-	0.35 (0.16)	-	-	-	-
59	Zagreb	Croatia	One centre	One centre in a city/town	2008-2013	4,254.50 (102.26)	0.35 (0.13)	3.23 (2.52)	0.27 (0.23)	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

E. coli Escherichia coli, EOGBS early-onset GBS, GBS group B *Streptococcus*, LOGBS late-onset GBS, SD standard deviation

Notes:

- The range of years covers the entire period given for the data overall, however, not all outcomes are available for all years
- All outcomes per 1,000 livebirths, except resistance rates which are percentages

Table 17. Average population compositional variables for objective 4 to 6

No	Area	Region	GBS prevention strategy	Mean HDI (SD)	Mean skilled delivery (SD)	Mean government expenditure on health (SD)	Mean preterm births (SD)	Mean low birthweights (SD)	Mean caesarean section (SD)	Mean fertility rate (SD)	Mean maternal age (SD)	Mean multiple or twin births (SD)	Mean maternal GBS (SD)	Most prevalent GBS strain	Mean PROMs (SD)	Mean intrapartum fever (SD)
1	Alberta	North America	Screening	0.91 (0.00)	98.55 (0.82)	4,759	7.8 (0.00)	6.00	27.05 (0.54)	1.61 (0.05)	27.60	*18.26*	19.50	III	*3.84*	*1.15*
2	Australia	Oceania	Either screening and risk-based	-	99.00	4,191	7.6 (0.00)	6.00	32.00	1.95	30.50	16.20	24.50	**III**	*16.15*	*7.60*
3	Australia	Oceania	Either screening and risk-based	0.93 (0.00)	99.00 (0.00)	4,191	7.6 (0.00)	6.00	31.75 (0.42)	1.89 (0.08)	30.50	16.20	24.50	*III*	*16.15*	*7.60*
4	Bangalore	Asia	None	0.62 (0.00)	67.00 (0.00)	215	**2.05 (0.76)**	**6.62 (2.10)**	**63.93 (1.35)**	2.48 (0.00)	19.90	**10.93**	**0.13**	*III, Ia*	**7.06**	*1.36*
5	Barcelona	Europe	Screening	0.86 (0.02)	*97.86 (0.95)*	2,846	**8.96 (0.99)**	**3.78 (1.49)**	**24.91 (3.11)**	1.30 (0.07)	29.30	17.20	**14.60**	III	**4.23**	**1.57**
6	Brno	Europe	Screening	0.86	100.00	1,982	7.30	8.00	52.00	1.45	27.40	19.20	**17.40**	*Ib*	*6.22*	*0.85*
7	Buenos Aires	Latin America and the Caribbean	Screening	0.81 (0.03)	98.94 (1.16)	1,725	7.65 (0.49)	7.00 (0.00)	25.89 (3.23)	2.47 (0.11)	*22.10*	*15.02*	9.40	*Ib*	*0.03*	*0.08*
8	Canada	North America	Screening	0.90 (0.02)	98.86 (0.95)	4,759	7.67 (0.23)	6.00 (0.00)	26.89 (0.57)	1.59 (0.05)	27.60	*18.26*	19.50	**III**	*3.84*	*1.15*
9	Canada	North America	Screening	0.91 (0.00)	98.00 (0.00)	4,759	**7.58**	**6.11**	27.00 (0.00)	1.61 (0.00)	27.60	*18.26*	19.50	**III**	*3.84*	*1.15*
10	Cordoba	Latin America and the Caribbean	Screening	0.83 (0.00)	100.00 (0.00)	1,725	**8.83 (1.13)**	7.00 (0.00)	**47.96 (4.01)**	2.35 (0.00)	*22.10*	**15.02**	**16.03**	*Ib*	*0.03*	*0.08*
11	Denmark	Europe	Risk-based	0.91 (0.03)	97.43 (1.16)	4,552	6.47 (0.25)	5.00 (0.00)	20.86 (0.20)	1.78 (0.05)	28.40	**21.15**	37.90	**III**	*15.86*	*0.30*
12	Emilia-Romagna	Europe	Screening	0.87 (0.00)	99,82 (0.40)	3,126	**7.44 (0.19)**	**2.00 (1.88)**	36.08 (4.73)	1.40 (0.05)	29.90	13.00	**21.40**	**III**	**8.50**	**0.50**
13	England	Europe	Risk-based	0.87 (0.00)	99.00 (0.00)	3,311	**7.40**	**4.00**	**21.50**	1.79 (0.18)	29.90	**14.77**	**21.00**	**III**	**0.25**	**0.13**
14	England	Europe	Risk-based	0.90 (0.02)	99.00	3,311	**7.63 (0.61)**	**6.63 (0.18)**	**22.93 (2.19)**	1.80 (0.11)	29.90	**14.77**	**21.00**	**III**	**0.25**	**0.13**
15	Finland	Europe	Either screening and risk-based	0.88 (0.01)	99.89 (0.31)	3,604	5.61 (0.13)	4.00 (0.00)	15.96 (0.05)	1.77 (0.04)	28.20	15.00	*37.90*	*III*	*15.86*	*0.30*
16	Flanders	Europe	Screening	0.88 (0.01)	99.00 (0.00)	4,526	7.90	7.00	22.68 (6.57)	1.80 (0.06)	27.70	17.40	22.00	*III*	*0.25*	*0.13*
17	France	Europe	Screening	0.88 (0.02)	97.86 (0.95)	4,334	6.40 (0.30)	7.00 (0.00)	20.71 (0.40)	1.91 (0.09)	28.60	16.30	15.40	*III*	*0.25*	*0.13*
18	France	Europe	Screening	0.88 (0.00)	97.00 (0.00)	4,334	6.70	-	21.00 (0.00)	1.99 (0.02)	28.60	16.30	15.40	*III*	*0.25*	*0.13*

No	Area	Region	GBS prevention strategy	Mean HDI (SD)	Mean skilled delivery (SD)	Mean government expenditure on health (SD)	Mean preterm births (SD)	Mean low birthweights (SD)	Mean caesarean section (SD)	Mean fertility rate (SD)	Mean maternal age (SD)	Mean multiple or twin births (SD)	Mean maternal GBS (SD)	Most prevalent GBS strain	Mean PROMs (SD)	Mean intrapartum fever (SD)
19	Guangzhou	Asia	Screening	0.69 (0.05)	96.61 (3.97)	646	**10.37 (6.39)**	**5.66 (5.77)**	**15.59 (16.00)**	1.52 (0.03)	*22.60*	7.90	7.10	**III, Ib**	**17.87**	**1.36**
20	Ho Chi Minh	Asia	None	0.66 (0.00)	94.00 (0.00)	308	9.4 (0.00)	5.00	**43.59 (0.59)**	1.96 (0.00)	22.60	6.20	*14.10*	**Ia**	*2.47*	*1.36*
21	Johannesburg	Sub-Saharan Africa	Risk-based	0.66	94.00	1,121	8.00	15.00	20.60	2.40	22.50	12.60	28.40	**Ia**	*9.66*	*2.42*
22	Johannesburg	Sub-Saharan Africa	Risk-based	0.66	94.00	1,121	8.00	15.00	20.60	2.40	22.50	12.60	28.40	**III**	*9.66*	*2.42*
23	Kaunas	Europe	Screening	0.83 (0.00)	100.00	1,579	**16.73 (0.78)**	**12.15 (0.62)**	**28.96 (0.89)**	1.51 (0.08)	26.10	**37.56**	**15.30**	**III**	**2.61**	**1.03**
24	Kingston	Latin America and the Caribbean	Risk-based	0.71 (0.02)	98.57 (0.53)	512	9.28 (0.81)	10.00 (1.41)	18.43 (3.21)	2.55 (0.26)	19.20	*11.20*	*44.00*	*III*	*12.00*	*0.08*
25	Kuala Terengganu	Asia	Risk-based	0.77 (0.00)	99.00 (0.00)	1,579	12.30	**12.18 (1.68)**	16.00 (0.00)	2.01 (0.05)	22.30	*11.00*	*14.10*	*Ia, III*	*2.47*	*1.36*
26	Kuwait	North Africa and the Middle East	Risk-based	0.81 (0.00)	99.00 (0.00)	2,375	10.60	8.00	**30.32 (2.63)**	2.35 (0.21)	*22.90*	**18.25**	**12.94**	*Ib*	*9.66*	*2.42*
27	Macau	Asia	None	0.71 (0.01)	99.20 (1.69)	646	7.10	5.00 (0.00)	27.00 (0.00)	1.07 (0.13)	*22.60*	7.90	7.10	III	*17.87*	*1.36*
28	Manila	Asia	Risk-based	0.67	73.00	287	**18.89**	**28.76**	28.00	3.04	23.10	**9.28**	*14.10*	*Ia*	**2.47**	*1.36*
29	Mansoura city-Dakahlia Governorate	North Africa and the Middle East	None	0.69	92.00	539	**12.08**	**14.49**	**24.15**	3.38	22.90	17.70	30.00	V	**9.66**	**2.42**
30	Mauritius	Sub-Saharan Africa	Risk-based	0.78	100.00	864	**12.60**	**18.69**	**42.40**	1.50	*22.50*	*12.60*	*32.30*	*III, Ia*	*9.66*	***2.42***
31	Mexico City	Latin America and the Caribbean	None	0.74 (0.02)	91.05 (10.77)	1,061	7.50 (0.24)	**24.22 (3.03)**	41.75 (4.47)	2.57 (0.23)	20.80	10.50	**3.67**	*III*	**0.03**	**0.08**
32	Netherlands	Europe	Risk-based	0.91 (0.01)	100.00	5,601	7.63 (0.35)	6.00	15.64 (0.39)	1.71 (0.07)	29.20	18.60	22.90	**III**	*0.25*	*0.13*
33	New Zealand	Oceania	Risk-based	0.91 (0.00)	96.55 0.52	3,405	7.45 (0.21)	6.00	24.05 (0.09)	2.06 (0.08)	27.70	15.50	21.70	**III**	*16.15*	**11.55**
34	New Zealand	Oceania	Risk-based	0.91 (0.00)	95.00 (2.83)	3,405	7.40	6.00	23.60	1.83 (0.32)	27.70	15.50	21.70	**III**	*16.15*	**11.55**
35	Northern Ireland	Europe	Risk-based	0.87	99.00	3,311	7.00	8.00	27.60	1.79 (0.18	29.90	14.90	**21.00**	*III*	*0.25*	*0.13*
36	Norway	Europe	Risk-based	0.94 (0.01)	99.00 (0.00)	6,308	**6.86 (0.43)**	**5.15 (0.16)**	**14.86 (1.57)**	1.85 (0.05)	28.10	18.30	*37.90*	*III*	**15.86**	**0.30**

No	Area	Region	GBS prevention strategy	Mean HDI (SD)	Mean skilled delivery (SD)	Mean government expenditure on health (SD)	Mean preterm births (SD)	Mean low birthweights (SD)	Mean caesarean section (SD)	Mean fertility rate (SD)	Mean maternal age (SD)	Mean multiple or twin births (SD)	Mean maternal GBS (SD)	Most prevalent GBS strain	Mean PROMs (SD)	Mean intrapartum fever (SD)
37	Ontario	North America	Screening	0.90 (0.02)	98.71 (0.92)	4,759	**7.50 (0.22)**	**6.97 (0.12)**	**27.61 (0.49)**	1.58 (0.05)	27.60	**18.26**	**21.69**	*III*	**3.84**	**1.15**
38	Panama City	Latin America and the Caribbean	None	0.71 (0.07)	88.50 (3.54)	796	8.30	**11.00**	**20.40**	2.86 (0.54)	21.10	*11.20*	**13.00**	**III**	**12.00**	*0.08*
39	Podgorica	Europe	Screening	0.80 (0.00)	100.00 (0.00)	926	**5.65 (1.77)**	**6.40 (0.60)**	**41.72 (23.38)**	1.71 (0.00)	25.50	**25.90**	**15.46**	*Ib*	*6.22*	*0.85*
40	Portugal	Europe	Screening	-	100.00 (0.00)	2,508	**7.02 (1.12)**	**7.48 (0.21)**	**33.00 (2.22)**	1.42 (0.04)	27.90	12.90	**22.50**	**III**	*4.23*	*1.57*
41	Portugal	Europe	Screening	0.82 (0.00)	100.00 (0.00)	2,508	-	-	-	1.31 (0.05)	27.90	12.90	**22.50**	**III**	*4.23*	*1.57*
42	Republic of Ireland	Europe	Risk-based	0.86	100.00	3311	5.6	6.00	26.00	1.90 (0.02)	28.90	15.50	25.60	III	*0.25*	*0.13*
43	Riga	Europe	Screening	0.82	99.00	1,310	**2.57**	**2.57**	23.00	1.48	25.60	*10.40*	*15.30*	*III*	*2.61*	*1.03*
44	Santo Domingo	Latin America and the Caribbean	None	0.70	98.00	1,454	16.00	**18.00**	56.00	2.53	20.30	11.20	**44.00**	*III*	*12.00*	*0.08*
45	Sao Paolo	Latin America and the Caribbean	Screening	0.72 (0.03)	97.73 (0.96)	1,454	8.63 (0.49)	9.50 (0.71)	53.97 (4.98)	2.28 (0.32)	22.10	8.80	**21.80**	Ib	*0.60*	*0.08*
46	Scotland	Europe	Risk-based	0.87	99.00	3,311	7.00	8.00	24.9	1.79 (0.18)	29.90	14.90	**21.00**	*III*	*0.25*	*0.13*
47	Singapore City	Asia	Screening	0.91 (0.00)	100.00 (0.00)	3,578	11.50	**1.73 (0.31)**	***16.00 (0.00)***	1.28 (0.05)	29.80	11.00	**14.10**	*III*	*2.47*	*1.36*
48	Slovenia	Europe	Screening	0.88 (0.00)	100.00 (0.00)	2,595	7.50	6.00	19.00 (0.00)	1.54 (0.09)	28.90	*13.70*	*18.50*	*Ib*	*6.22*	*0.85*
49	Sofia	Europe	Risk-based	0.78 (0.00)	100.00 (0.00)	1,213	7.50	**18.44 (1.10)**	33.00 (0.00)	1.52 (0.01)	26.60	9.80	**1.70**	*Ib, III*	*6.22*	*0.85*
50	Soweto	Sub-Saharan Africa	Risk-based	0.66	92.50 (1.64)	1,121	**18.00 (0.00)**	**17.53 (1.14)**	20.60 (0.00)	2.57 (0.13)	22.50	12.60	28.40	**III, Ia**	*9.66*	*2.42*
51	Spain	Europe	Screening	0.86 (0.02)	*97.92 (0..95)*	2,846	6.97 (0.45)	7.00 (1.41)	26.04 (0.93)	1.31 (0.07)	29.30	17.20	15.90	III	*4.23*	*1.57*
52	St Augustine	Latin America and the Caribbean	None	0.70 (0.03)	98.43 (0.79)	1,663	**10.21 (0.85)**	**13.84 (0.33)**	**9.16 (1.06)**	2.04 (0.37)	22.20	**107.46**	32.80	*III*	**0.60**	*0.08*
53	Switzerland	Europe	Screening	0.93 (0.00)	100 (0.00)	6,187	**7.26 (0.11)**	**2.28 (0.02)**	**32.95 (0.10)**	1.52 (0.00)	30.00	**18.17**	**18.70**	**III**	*8.50*	*0.50*
54	Tokyo	Asia	Screening	0.89 (0.00)	100.00 (0.00)	3,741	**15.33 (2.29)**	**20.32 (3.15)**	**33.69 (2.01)**	1.35 (0.04)	29.90	**49.88**	22.40	*III*	**16.15**	*7.60*
55	Tunis	North Africa and the Middle East	Risk-based	0.72 (0.00)	74.00 (0.00)	1,663	**9.25**	**8.29 (0.95)**	35.28 (3.06)	1.80 (0.00)	24.50	14.30	13.00	*V*	*9.66*	*2.42*

No	Area	Region	GBS prevention strategy	Mean HDI (SD)	Mean skilled delivery (SD)	Mean government expenditure on health (SD)	Mean preterm births (SD)	Mean low birthweights (SD)	Mean caesarean section (SD)	Mean fertility rate (SD)	Mean maternal age (SD)	Mean multiple or twin births (SD)	Mean maternal GBS (SD)	Most prevalent GBS strain	Mean PROMs (SD)	Mean intrapartum fever (SD)
56	United Kingdom and the Republic of Ireland	Europe	Risk-based	0.87	99.00	3,311	7.00	8.00	25.84	1.79 (0.18)	29.90	14.90	**21.00**	*III*	*0.25*	*0.13*
57	United States of America	North America	Screening	0.90 (0.02)	98.76 (0.44)	9,146	11.75 (0.77)	8.00 (0.00)	32.68 (0.54)	2.01 (0.06)	25.00	16.40	18.80	Ia, II	**7.20**	**3.30**
58	Wales	Europe	Risk-based	0.87	99.00	3,311	7.00	8.00	**21.50**	1.78 (0.18)	29.90	14.90	**21.00**	*III*	*0.25*	*0.13*
59	Zagreb	Europe	Screening	0.81 (0.00)	100.00 (0.00)	1,517	**7.38 (0.93)**	**3.10 (0.53)**	**23.97**	1.52 (0.00)	27.10	**29.49**	**18.50**	**Ia**	**6.22**	**0.85**

E. coli Escherichia coli, GBS group B *Streptococcus*, HDI human development index, PROMs prolonged rupture of membranes, SD standard deviation

Notes:

- All outcomes are percentages, except multiple or twin births, which are per 1,000 livebirths, and government expenditure on health, which is in PPP int $.
- Number is bold are from surveys while numbers not in bold are from international websites.
- Numbers in italics are imputed from the closest neighbouring geographical area.
- GBS prevention strategy refers to the most recently reported strategy during the period that outcome data were reported.

11. THE IMPACT OF UNIVERSAL GBS SCREENING ON THE TRENDS OF ANNUAL EOGBS INCIDENCE

This chapter presents the results for the ecological trend analysis study that combines international data to explore the impact of universal GBS screening on the trends of annual EOGBS incidence, compared with other GBS prevention strategies. While I discussed the methodology used to address this study in Chapter 9, here I will first present the study specific aim and objectives followed by some study specific methodological procedures. I will then present the detailed results from the statistical analyses: first the MICE imputation results, then the descriptive statistics, followed by the unadjusted analyses, the main adjusted model, the sensitivity analyses and the multi-level analysis model. Finally, I will summarise the principal findings and how they relate to previous literature.

11.1 Aims and objectives

The aim of this chapter is to measure the effect of universal GBS screening on the trend of annual EOGBS incidence across time, compared with other GBS prevention strategies, in a statistical model that combines data from geographical areas with different prevention strategies, and adjusts for compositional differences between the areas.

The research objectives are to:

a) Describe the frequency of the GBS prevention strategy as well as the mean or frequency of the compositional covariates in general (irrespective of EOGBS incidence);
b) Describe the mean EOGBS incidence across time, geographical areas, world regions and GBS prevention strategies;
c) Investigate the unadjusted relationship between universal GBS screening and the trend of annual EOGBS incidence across time compared with other prevention strategies, using linear regression;
d) Investigate the unadjusted relationship between each compositional covariate and the mean EOGBS incidence across time, using linear regression;

e) Investigate the relationship between universal GBS screening and the trend of annual EOGBS incidence across time compared with other prevention strategies, using linear regression and adjusting for the compositional covariates;
f) Examine the stability of the adjusted relationship between universal GBS screening and the trend of annual EOGBS incidence across time compared with other prevention strategies in a range of sensitivity analyses, if the relationship was statistically significant; and
g) Investigate the multi-level unadjusted relationship between universal GBS screening and the trend of annual EOGBS incidence across time compared with other prevention strategies, using a multi-level growth curve model to account for the structure of the data. (Note that this was an unadjusted analysis as multi-level multiple imputation did not work and there were few fully observed covariates).

11.2 Methods

I have detailed the methodology used for this study in Chapter 9. Here, I describe the data selected to perform the analysis.

11.2.1 Geographical data included

As discussed in Chapter 10, there were annual EOGBS data from 55 geographical areas. However, some of them overlapped with one another in terms of coverage, giving rise to the potential of double-counting. These were: Alberta and Ontario that overlapped with multi-centre data across Canada; data from Barcelona *versus* multiple centres across Spain; voluntary *versus* enhanced surveillance from England; two hospitals from Johannesburg *versus* data from the township of Soweto; data from multiple centres *versus* enhanced surveillance in New Zealand; two different multi-centre sources for Australia; and enhanced surveillance from the United Kingdom and Republic of Ireland *versus* data from each of the five countries in the British Isles. I included the following data sources (and excluded the others): Alberta and Ontario as both had population-based surveillance, and Alberta in particular had mandatory surveillance; multi-centres from Spain to have broader national coverage; the enhanced surveillance for England and individual UK countries instead of the United Kingdom and Republic of Ireland overall as the EOGBS incidence varies across the countries; Soweto over the two hospitals in Johannesburg for population and wider coverage; the enhanced surveillance for New Zealand; and the multi-centre data that had a larger number

of years for Australia. This left 47 geographical areas. However, after analysing the EOGBS incidence across the years and countries, I excluded Mansoura City in Egypt where the EOGBS incidence for 2014 was 28.99 per 1,000 livebirths. This was an extreme outlier and was vastly different from incidence rates in the UK. This left data from a total of 46 geographical areas in the analyses that follow.

Further descriptive analyses also revealed that St. Augustine had a strong influence on the descriptive trends of EOGBS incidence over time. However, as the incidence rates themselves were not extreme outliers, there is a separate unadjusted and adjusted linear regression, multi-level regression and sensitivity analyses with and without St. Augustine.

11.3 Results

11.3.1 Multiple imputation for compositional covariates

There were 60 (16%) observations that had complete compositional covariate data for every year. The proportion of missing data is shown in Table 18. The covariate with the largest number of missing years was the human development index, which had 63% of years missing. The covariate with the least amount of missing data was skilled attendance at delivery, which had 7% of years missing.

Table 18. Proportion of missing data in compositional covariates for the EOGBS dataset

Variable	Observed (%)	Missing (%)
Preterm births	153 (40)	231 (60)
Low birthweights	175 (46)	209 (54)
Caesarean section	302 (79)	82 (21)
Skilled attendance at delivery	347 (93)	37 (7)
Human development index	142 (37)	242 (63)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*

There were multiple patterns of missing data. The most common pattern was for skilled attendance at delivery and caesarean section to be observed, with the remaining three variables not observed. This was followed by all covariates observed, all but the human development index observed, only skilled delivery observed, and less frequent patterns. The logistic regression for whether the missingness of a variable was related to the other variables, revealed that missingness of preterm births was only related to multiple births. Missingness of low birthweights was related to year, fertility rate, EOGBS definition, maternal GBS

colonisation, GBS serotype, prolonged rupture of membranes, most recently reported GBS prevention strategy, geographical coverage and geographical region. Missingness of caesarean section was related to year, fertility rate, multiple births, prolonged rupture of membranes, intrapartum fever, geographical coverage and geographical region. Missingness for the human development index was related to year and EOGBS incidence. Finally, missingness of skilled attendance at delivery was related to year, EOGBS incidence, multiple births, maternal GBS colonisation and most recently reported GBS prevention strategy. As data were missing because they were available from some years but not others and their missingness was related to other data, the mechanism for the missing data was 'missing at random'.

As mentioned in Chapter 9, there were 100 imputations because of the large amount of missing data and the results showed that convergence was reached within these imputations. Additionally, the largest fraction of missing information ($_{FMI}$) from the analysis models was below 67%, confirming that the number of imputations was sufficient. Below, I present the descriptive statistics for some of the imputed datasets and the original dataset for comparison.

11.3.2 Descriptive analysis (objectives a & b)

This section presents the mean EOGBS incidence across time, geographical areas, world regions and GBS prevention strategies (objective b) as well as the frequency of the GBS prevention strategy and the mean or frequency of the compositional covariates in general (irrespective of EOGBS incidence) (objective a).

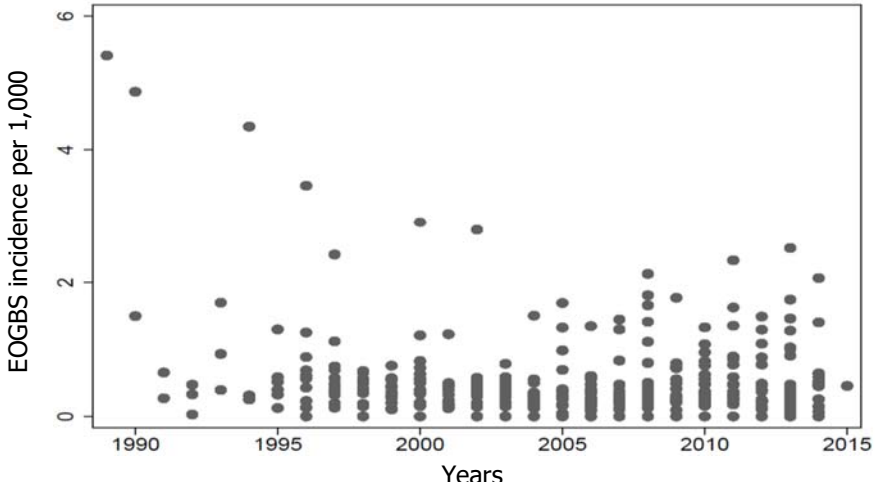
EOGBS incidence

The mean EOGBS incidence across 384 observations (46 geographical areas, 27 years between 1989 and 2015) was 0.49 per 1,000 livebirths (SD 0.62, range: 0.00 to 5.40). Across the years, EOGBS incidence varied between 0.28 and 5.04 per 1,000 livebirths. The incidence varied between the years, but there was no overall trend (see Table 19). Figure 11 shows the scatterplot of all EOGBS incidence observations by year, demonstrating that the range of EOGBS incidence was wider and higher in the earlier years. Seven observations before 2002 were above 2.00 to 5.00 per 1,000 livebirths, while the majority were below 2.0 per 1,000 livebirths. As the years progressed, these rates reduced and stabilised at a lower rate. As evident from Table 19, the geographical areas that contributed to each year varied, therefore, the patterns may be reflective of those over time or a result of different areas reporting during different time periods.

Table 19. EOGBS incidence per 1,000 livebirths across the years

Year	Number of geographical areas	Mean livebirths (Standard deviation)	Mean EOGBS incidence (Standard deviation)
1989	1	5,731.00	5.40
1990	2	5,547.00	3.18 (2.38)
1991	2	5,214.00 (3063.19)	0.46 (0.27)
1992	3	86,181.00 (139,365.30)	0.28 (0.23)
1993	3	6,669.50 (4,891.06)	1.01 (0.66)
1994	3	6,707.33 (4,570.78)	1.64 (2.35)
1995	6	55,007.40 (79,601.10)	0.54 (0.40)
1996	10	85,310.77 (136,912.90)	0.83 (1.00)
1997	12	110,751.70 (143,861.70)	0.64 (0.63)
1998	11	125,676.70 (152,557.00)	0.41 (0.23)
1999	12	126,738.90 (165,442.10)	0.37 (0.19)
2000	18	128,990.20 (187,815.40)	0.63 (0.64)
2001	12	144,928.40 (178573.20)	0.39 (0.30)
2002	14	125,956.50 (167,647.50)	0.48 (0.70)
2003	16	115,542.60 (165,273.50)	0.37 (0.19)
2004	18	111,318.20 (167,775.50)	0.32 (0.33)
2005	21	96,630.87 (15,9638.70)	0.39 (0.44)
2006	22	95,396.27 (160,016.10)	0.30 (0.29)
2007	24	87,147.15 (153,155.20)	0.35 (0.37)
2008	24	83,339.56 (155,873.10)	0.60 (0.59)
2009	25	78,781.24 (151,121.20)	0.36 (0.38)
2010	26	77,229.19 (143,812.00)	0.42 (0.34)
2011	28	70,159.65 (135,949.40)	0.48 (0.53)
2012	26	75,572.28 (140,237.60)	0.40 (0.40)
2013	27	66,003.58 (135,981.50)	0.51 (0.63)
2014	17	82,560.06 (193,064.80)	0.48 (0.54)
2015	1	2,195.00	0.46
Total	384 observations (46 areas, 27 years)	90,967.03 (151,485.80)	0.49 (0.62)

EOGBS early-onset group B *Streptococcus* disease



EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Each dot represents the EOGBS incidence for one year for one geographical area

Figure 11. Scatterplot of EOGBS incidence by year

When plotting the EOGBS incidence by year for every geographical area, we see that the early trend of high EOGBS incidence that dramatically decreased in the seven observations above 2.0 per 1,000 livebirths is from one geographical area of St. Augustine in Trinidad and Tobago (see Appendix 15-A). Indeed, besides Mansoura City which was excluded, the highest mean EOGBS incidence per 1,000 livebirths across years was found in St. Augustine (3.74 [SD 1.14]), followed by Sofia in Bulgaria (0.78 [SD 0.25]). Three areas reported zero cases of EOGBS across years: Cordoba in Argentina, Podgorica in Montenegro and Slovenia (this might be a result of diagnostic related issues, voluntary surveillance or too small a sample size to detect cases). Otherwise, the lowest mean EOGBS incidences per 1,000 livebirths across years were found in Bangalore (0.15 [SD 0.26]), Buenos Aires (0.14 [SD 0.20]), and Manila (0.15) (see Table 20). These are the averages across the years for each geographical area but, as can be seen in Appendix 15-A, for many areas the rates of EOGBS fluctuated from the first year to the last.

Table 20. EOGBS incidence per 1,000 livebirths by geographical area

Geographical area	Number of years	Mean number of livebirths (Standard deviation)	Mean EOGBS incidence (Standard deviation)
Alberta	11	47,896.00 (4,854.45)	0.25 (0.10)
Australia	11	29,199.82 (4,802.03)	0.38 (0.16)
Bangalore	3	4,044.33 (1,605.77)	0.15 (0.26)
Brno	1	6,415.00	0.31
Buenos Aires	18	6,473.22 (923.54)	0.14 (0.20)
Cordoba	2	1,573.00 (152.74)	0.00 (0.00)
Denmark	15	63,296.47 (3,182.94)	0.17 (0.05)
Emilia-Romagna	11	38,686.09 (2,477.04)	0.26 (0.07)
England	2	668,696.00 (68,071.76)	0.54 (0.06)
Finland	19	58,658.79 (1,898.58)	0.55 (0.14)
Flanders	6	-	0.21 (0.08)
France	18	570,199.50 (57,015.64)	0.36 (0.17)
Guangzhou	4	2,097.50 (265.54)	0.61 (0.29)
Kaunas	7	3,524.86 (194.83)	1.13 (0.50)
Kingston	16	2,744.69 (360.63)	0.69 (0.40)
Kuala Terengganu	9	12,578.56 (1.141.11)	0.26 (0.25)
Kuwait	10	11,074.1 (486.46)	1.14 (0.76)
Macau	10	2,667.7 (697.83)	0.64 (0.70)
Manila	1	6,682.00	0.15
Mauritius	1	12,986.00	1.46
Mexico City	19	5,055.11 (594.00)	0.27 (0.22)
Netherlands	19	190,186.70 (9,833.44)	0.17 (0.04)
New Zealand	2	119,768.00 (10,417.10)	0.36 (0.19)
Northern Ireland	2	24,866.00 2,207.59	0.69 (0.06)
Norway	18	59,343.44 (1,936.13)	0.44 (0.12)
Ontario	17	135,098.60 (4,563.32)	0.27 (0.06)
Panama City	2	131,288.00 (163,749.00)	0.40 (0.53)
Podgorica	2	3,216 52. (3,259.00)	0.00 (0.00)
Portugal	7	109,472.90 (4,266.57)	0.24 (0.13)
Republic of Ireland	2	65,921.50 (10,129.30)	0.40 (0.08)
Riga	1	2,060.00	0.49
Santo Domingo	1	18,000.00	2.33
Sao Paolo	15	8,332.67 (4,881.71)	0.44 (0.25)
Scotland	2	59,475.50 (2,795.19)	0.35 (0.20)
Singapore city	14	2,658.64 (399.24)	0.26 (0.40)
Slovenia	5	21,839.00 (448.82)	0.00 (0.00)
Sofia	6	3,894.17 (229.22)	0.78 (0.25)
Soweto	6	29,064.50 (2,561.84)	1.50 (0.16)
Spain	16	96,036.63 (13,710.00)	0.50 (0.32)
St Augustine	7	5,145.00 (375.28)	3.74 (1.14)
Switzerland	3	81,901.00 (988.11)	0.06 (0.05)
Tokyo	11	1,627.73 (192.70)	0.39 (0.63)
Tunis	3	3,818.00 (115.17)	1.14 (0.32)
US	18	422,561.70 (52,171.40)	0.53 (0.43)
Wales	2	35,126.00 (1,715.44)	0.41 (0.08)
Zagreb	6	4,254.50 (102.26)	0.35 (0.13)
Total	384	90,967.03 (151,485.80)	0.49 (0.62)

EOGBS early-onset group B *Streptococcus* disease, US United States of America

Taking a wider global perspective, the highest mean EOGBS incidence was reported in Sub-Saharan Africa (1.50 per 1,000 livebirths [SD 0.15]) whereas the lowest was reported in Asia, Europe, North America and Oceania which all showed a mean rate of 0.38 per 1,000 livebirths (see Table 21). Again, these rates vary across time and, within regions may fluctuate by year as shown in the scatterplots of EOGBS incidence by world region presented in Appendix 15-B.

Table 21. EOGBS incidence per 1,000 livebirths by region

Region	Number of observations/ years (%)	Mean EOGBS incidence (standard deviation)
Asia	52 (13.54)	0.38 (0.50)
Europe	170 (44.27)	0.38 (0.29)
Latin America and the Caribbean	80 (20.83)	0.68 (1.08)
North Africa and the Middle East	13 (3.39)	1.14 (0.67)
North America	49 (12.76)	0.38 (0.31)
Oceania	13 (3.39)	0.38 (0.16)
Sub-Saharan Africa	7 (1.82)	1.50 (0.15)
Total	384 (100.00)	0.49 (0.62)

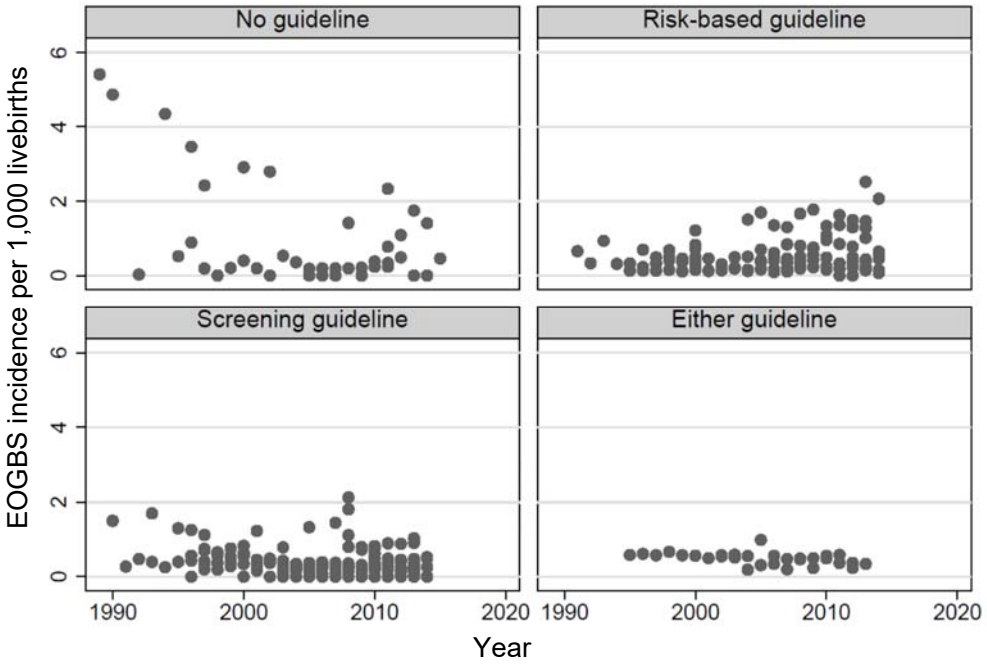
EOGBS early-onset group B *Streptococcus* disease

Finally, the mean EOGBS incidence by most recently reported GBS prevention strategy is presented in Table 22. The highest mean EOGBS incidence per 1,000 livebirths was reported under no prevention (0.99 [SD 1.42]), followed by risk-based prevention (0.54 [SD 0.48]), either risk-based or screening prevention (0.48 [SD 0.16]) and the lowest mean rate was reported under universal screening (0.35 [SD 0.35]). Figure 12 shows the scatterplots for EOGBS incidence by the most recently reported GBS prevention strategy, highlighting a potential increase in EOGBS incidence in areas that reported a risk-based strategy. By contrast, there is a potential decrease in areas that reported no prevention, a smaller decrease in areas that reported universal screening and an even smaller decrease in areas that reported either risk-based or screening prevention. Again, it seems that under no prevention there may be a substantial impact of the drastic decrease in EOGBS incidence in St. Augustine, as the plot shows the same pattern; removing these observations might reveal a different pattern.

Table 22. EOGBS incidence per 1,000 livebirths by recently reported GBS prevention strategy

Most recent GBS prevention strategy	Number of observations/ years (%)	Mean EOGBS incidence (standard deviation)
No prevention	42 (10.94)	0.99 (1.42)
Risk-based prevention	116 (30.21)	0.54 (0.48)
Screening prevention	196 (51.04)	0.35 (0.35)
Either risk-based or screening prevention	30 (7.81)	0.48 (0.16)
Total	384 (100.00)	0.49 (0.62)

EOGBS early-onset group B *Streptococcus* disease, GBS group B *Streptococcus*



EOGBS early-onset group B *Streptococcus* disease, GBS group B *Streptococcus*
Each dot represents the EOGBS incidence for one year for one geographical area

Figure 12. Scatterplot of EOGBS incidence by recently reported GBS prevention strategy

Predictor and compositional covariates (objective a)

Most geographical areas (and observations) recently reported a universal screening strategy (51% of observations and 46% of areas), followed by risk-based prevention (30% of observations and 37% of areas), no prevention (11% of observations and 13% of areas) and either risk-based or screening prevention (8% of observations and 4% of areas) (see Table 23 and Table 17). With respect to world region, the majority of observations were from Europe (44%) and the fewest were from Sub-Saharan Africa (2%) (see Table 21).

Table 23. Frequencies of the recently reported GBS prevention strategy for the EOGBS dataset

Most recent GBS prevention strategy	Frequency (%)
No prevention	6 (13.04)
Risk-based prevention	17 (36.96)
Screening prevention	21 (45.65)
Either risk-based or screening prevention	2 (4.35)
Total	46 (100.00)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*

The mean values for the compositional covariates that were multiple imputed are presented in Table 24. Averages are provided for the data with no imputation, followed by imputed datasets

1 and 100, to provide an indication of how well the multiple imputation performed. The mean percentage of preterm births across the years in the original dataset was 9.40% (SD 4.25) and the imputed means were around 8.70% and, therefore within 0.7% of the original mean. Similarly, the mean percentage of low birthweights was 10.17% (SD 7.25) and the imputed mean values were around 8.5% to 8.6%, thus, within 1.7% of the original mean and not as close to the original data as the other covariates. The mean percentage of caesarean section deliveries was 26.80% (SD 10.42) and the imputed mean values were within 0.6%. The mean skilled attendance at delivery was 97.85% (SD 5.20) and the imputed mean values were within 0.13%. Finally, the mean human development index across the data was 0.83 (SD 0.08) and were identical in the other two sample datasets.

Table 24. Mean values for the multiple imputed covariates in the EOGBS dataset

Covariate	Original dataset Mean (SD)	Imputed dataset 1 Mean (SD)	Imputed dataset 2 Mean (SD)
Preterm births (%)	9.40 (4.25)	8.73 (3.51)	8.70 (3.35)
Low birthweights (%)	10.17 (7.25)	8.50 (5.89)	8.57 (5.74)
Caesarean delivery (%)	26.80 (10.42)	26.42 (10.57)	26.24 (10.70)
Skilled attendance at delivery (%)	97.85 (5.20)	97.96 (4.96)	97.98 (4.96)
Human development index	0.83 (0.08)	0.83 (0.08)	0.83 (0.08)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*, SD standard deviation

The mean values for compositional covariates that only had one value across all of the years and, thus, were not multiple imputed are presented in Table 25. Across the geographical areas, mothers were 26 years old at first child (SD 3.41) and had an average of 1.86 children (SD 0.44). Eighteen percent of births were multiple or twin births (SD 14.34), maternal colonisation of GBS was approximately 21% (SD 10.68), mean percentage of prolonged rupture of membranes was 6.80% (SD 6.20) and intrapartum fever was 1.28% (SD 1.95). Mean government expenditure on health *per capita* was $3,348.76 (SD 2,163.51). Table 25 also presents the frequency and percentage of the observations for each category of the categorical compositional covariates. The most frequent GBS serotype was serotype III (74%), followed by Ib (17%), Ia (8%) and V (1%). Most data covered one centre only and approximately the same amount of data was from mandatory/enhanced (18%) or voluntary (19%) population surveillance or multiple centres (18%). After data from one centre, most data covered a country (43%), a region (10%) or were city or town-wide (2%). Finally, the most common definition of EOGBS was 5/6/7 days or less (67%), followed by 2/3 days (28%), vertical onset (4%) and was not stated in 0.5%.

Table 25. Mean values for the un-imputed covariates for the EOGBS dataset

Covariate	Descriptive statistic
Continuous variables	*Mean (SD)*
Fertility rate	1.86 (0.44)
Average maternal age	26.11(3.41)
Multiple or twin births (per 1,000 livebirths)	18.16 (14.34)
Per capita government expenditure on health (PPP int $)	3,348.76 (2,163.51)
Maternal GBS colonisation	21.02 (10.68)
Prolonged rupture of membranes	6.80 (6.20)
Intrapartum fever	1.28 (1.95)
Categorical variables	*Frequency (%)*
Most prevalent GBS serotype	
Ia	31 (8.07)
Ib	65 (16.93)
III	285 (74.22)
V	3 (0.78)
Surveillance type	
Mandatory or enhanced population surveillance	70 (18.23)
Voluntary population surveillance	74 (19.27)
Multiple centres/counties	69 (17.79)
One centre	171 (44.53)
Geographical coverage	
National	165 (42.97)
Regional	39 (10.16)
City/town wide	9 (2.34)
One centre in a city/town	171 (44.53)
EOGBS definition	
2/3 days or less	108 (28.13)
5/6/7 days or less	258 (67.19)
Vertical onset	16 (4.17)
Not stated	2 (0.52)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*, SD Standard deviation

11.3.3 Unadjusted linear regression analysis (objective c & d)

This section presents the results of the linear regression analysis showing the unadjusted relationship between the most recently reported GBS prevention strategy and the trends of annual EOGBS incidence across time for all data and then for the data excluding St. Augustine (objective c). This is followed by the linear regression analysis showing the unadjusted relationship between each compositional covariate and mean EOGBS incidence (objective d).

Most recently reported GBS prevention strategy: all data

The results of the unadjusted analysis are summarised in see Table 26 and Figure 13 for all data. Contrary to expectation, there was a continuous decrease in annual EOGBS incidence in areas with no prevention. Similarly, there was a decrease in annual EOGBS incidence in screening prevention and 'either prevention' (i.e. risk-based or screening) areas. Compared with screening prevention areas, the EOGBS incidence decreased by 0.082 (95% CI -0.108 to

-0.055) yearly in no prevention areas. However, the incidence of EOGBS increased by 0.037 (95% CI 0.016 to 0.059) yearly in risk-based prevention areas compared with screening areas. There was no statistically significant difference in the trends of annual EOGBS incidence between screening and 'either prevention' areas.

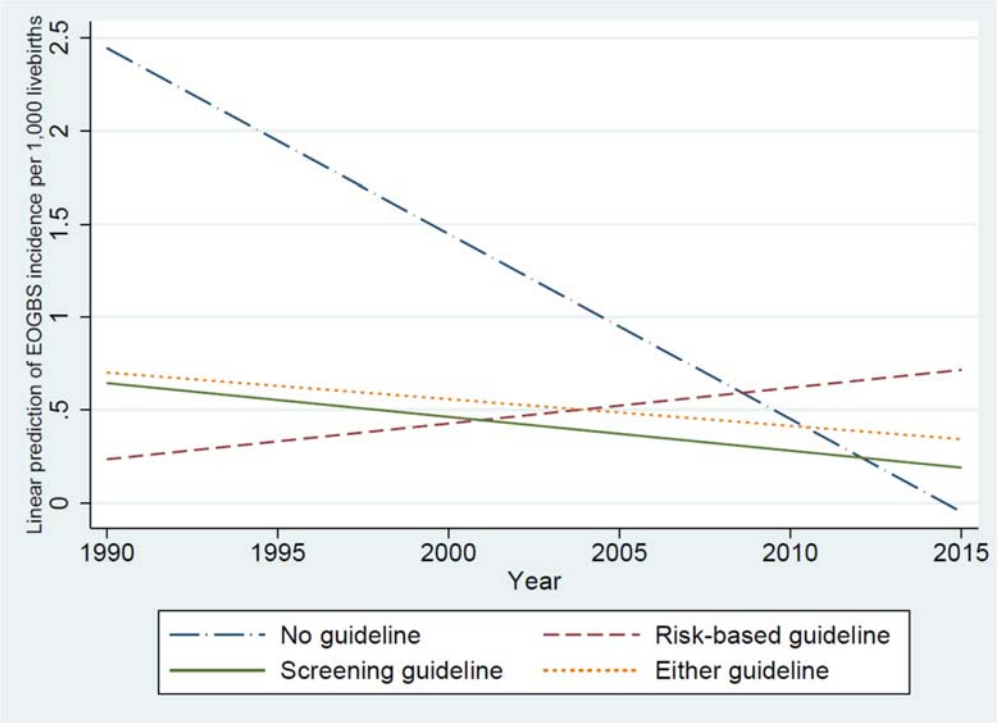
Most recently reported GBS prevention strategy: excluding data from St. Augustine

The results of excluding St. Augustine from the analysis (as it had much higher EOGBS rates and a trend that drastically decreased) are shown in Table 26 and Figure 14. When St Augustine data were excluded, there were decreases in annual EOGBS incidence in both screening and 'either prevention' areas, and increases in annual EOGBS incidence in no prevention and risk-based prevention areas. Compared with screening prevention areas, the EOGBS incidence increased by 0.042 (95% CI 0.018 to 0.066) and 0.037 (95% CI 0.022 to 0.053) yearly for no prevention and risk-based prevention areas respectively. There was no statistically significant difference in the trends of annual EOGBS incidence between screening prevention and 'either prevention' areas.

Table 26. Unadjusted linear regression analyses of the average annual change in EOGBS incidence by recently reported GBS prevention strategy

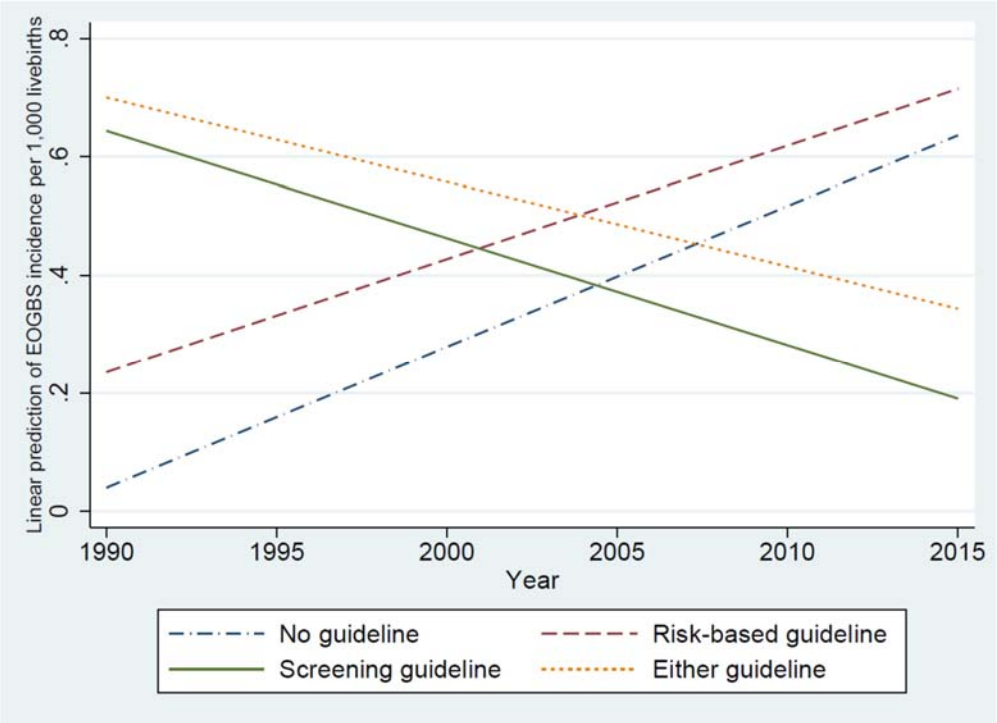
Most recent reported GBS prevention (baseline: screening prevention)	Average annual change in EOGBS incidence (95% confidence interval)	p-value
All data		
Screening prevention	(reference)	
No prevention	-0.082 (-0.108 to -0.055)	0.000
Risk-based prevention	0.037 (0.016 to 0.059)	0.001
Either screening or risk-based prevention	0.004 (-0.037 to 0.05)	0.855
Excluding St. Augustine		
Screening prevention	(reference)	
No prevention	0.042 (0.018 to 0.066)	0.001
Risk-based prevention	0.037 (0.022 to 0.053)	0.000
Either screening or risk-based prevention	0.004 (-0.026 to 0.034)	0.803

EOGBS early-onset GBS disease, GBS group B *Streptococcus*



EOGBS early-onset group B *Streptococcus* disease

Figure 13. Unadjusted trends of annual EOGBS incidence for each recently reported GBS prevention strategy using linear regression analysis



EOGBS early-onset group B *Streptococcus* disease

Figure 14. Unadjusted trends of annual EOGBS incidence for each recently reported GBS prevention strategy using linear regression analysis excluding St. Augustine

Compositional covariates

Compared with North America, the EOGBS incidence per 1,000 livebirths was higher in Latin America and the Caribbean (0.301, 95% CI 0.093 to 0.510), North Africa and the Middle East (0.758, 95% CI 0.398 to 1.116) and Sub-Saharan Africa (1.114, 95% CI 0.649 to 1.579) (see Table 27). Compared with national and mandatory surveillance, EOGBS incidence per 1,000 livebirths was higher in surveillance from one centre (0.280 95% CI 0.151 to 0.410 and 0.230 95% CI 0.062 to 0.397 respectively). As preterm births increased by one percent, EOGBS incidence increased by 0.048 per 1,000 livebirths (95% CI 0.027 to 0.070). As low birthweights increased by one percent, the rate of EOGBS incidence increased by 0.02 per 1,000 livebirths (95% CI 0.009 to 0.031). For a one unit increase in fertility rate, EOGBS incidence increased by 0.148 per 1,000 livebirths (95% CI 0.007 to 0.290) while a one percent increase in multiple births led to an increase of 0.026 per 1,000 livebirths (95% CI 0.023 to 0.030). A one percent increase in the prevalence of maternal GBS colonisation led to an increase in EOGBS incidence of 0.010 per 1,000 livebirths (95% CI 0.004 to 0.015).

By contrast, EOGBS incidence per 1,000 livebirths decreased by 0.008 (95% CI -0.014 to -0.003) with a one percent increase in caesarean sections and by 0.045 (95% CI -0.063 to -0.028) when average maternal age increased by one year (see Table 27). EOGBS incidence per 1,000 livebirths decreased by 0.00005 (95% CI -0.00008 to -0.00002) when government health expenditure increased by one unit and by 2.522 (95% CI -3.314 to -1.731) as the human development index increased by one unit. The percentages of skilled attendance at delivery, prolonged rupture of membranes, EOGBS definition and intrapartum fever were not statistically associated with EOGBS incidence. There was no statistically significant difference in EOGBS incidence between serotype Ia and the other most prevalent GBS serotypes, however, as serotype V was below p=0.2, the most prevalent GBS serotype was taken forward into the adjusted analysis.

Table 27. Unadjusted linear regression analyses of EOGBS incidence per 1,000 livebirths by compositional covariates

Covariate	EOGBS coefficient (95% confidence interval)	p-value
Region		
North America	(reference)	
Asia	-0.004 (-0.233 to 0.225)	0.970
Europe	0.000 (-0.186 to 0.187)	0.999
Latin America and the Caribbean	0.301(0.093 to 0.510)	0.005
North Africa and the Middle East	0.758 (0.398 to 1.116)	0.000
Oceania	-0.003 (-0.362 to 0.356)	0.985
Sub-Saharan Africa	1.114 (0.649 to 1.579)	0.000
Preterm births	0.048 (0.027 to 0.070)	0.000
Low birthweights	0.020 (0.009 to 0.031)	0.001
Caesarean section	-0.008 (-0.014 to -0.003)	0.005
Fertility rate	0.148 (0.007 to 0.290)	0.040
Skilled attendance at delivery	-0.003 (-0.016 to 0.010)	0.631
Average maternal age	-0.045 (-0.063 to -0.028)	0.000
Multiple or twin birth	0.026 (0.023 to 0.030)	0.000
Per capita government expenditure on health	-0.00005 (-0.00008 to -0.00002)	0.001
Human development index	-2.522 (-3.314 to -1.731)	0.000
Maternal GBS colonisation	0.010 (0.004 to 0.015)	0.001
Prolonged rupture of membranes	0.004 (-0.006 to 0.014)	0.459
Intrapartum fever	0.002 (-0.030 to 0.034)	0.911
Most prevalent GBS serotype		
Ia	(reference)	
Ib	-0.019 (-0.286 to 0.247)	0.887
III	0.060 (-0.0171 to 0.290)	0.612
V	0.695 (-0.043 to 1.433)	0.065
Surveillance type		
Mandatory population surveillance	(reference)	
Voluntary population surveillance	-0.201 (-0.398 to 0.004)	0.046
Multiple centres/counties	-0.031 (-0.233 to 0.169)	0.761
One centre	0.230 (0.062 to 0.397)	0.007
Geographical coverage		
National	(reference)	
Regional	-0.116 (-0.327 to 0.096)	0.282
City/town wide	-0.192 (-0.598 to 0.214)	0.354
One centre in a city/town	0.280 (0.151 to 0.410)	0.000
EOGBS definition		
2/3 days or less	(reference)	
5/6/7 days or less	0.113 (-0.027 to 0.253)	0.114
Vertical onset	0.085 (-0.243 to 0.412)	0.612
Not stated	-0.412 (-1.283 to 0.459)	0.353

EOGBS early-onset GBS disease, GBS group B *Streptococcus*

11.3.4 Adjusted linear regression analysis (objective e)

This section presents the results of the linear regression analysis showing the relationship between the most recently reported GBS prevention strategy and the trends of annual EOGBS incidence across time adjusted for the compositional covariates (objective e): first for all data and then for the data excluding St. Augustine.

All data

Given the results of the unadjusted linear regression analyses and the *a priori* list of covariates, I included all of the covariates with the exception of skilled attendance at delivery in the initial adjusted regression analysis. As I removed statistically non-significant covariates ($p>0.05$), this left the minimal and final model shown in Table 28. The results of the adjusted analysis are summarised in Table 28 and Figure 15 for all data. In contrast with the unadjusted analysis, when compositional covariates were accounted for, there was no longer a decrease in annual EOGBS incidence in no prevention areas compared with screening prevention areas, nor a statistically significant difference in the trends of annual EOGBS incidence between no prevention and screening areas. In line with the unadjusted analyses, compared with screening prevention areas, the incidence of EOGBS increased by 0.040 (95% CI 0.026 to 0.055) yearly in risk-based prevention areas. In line with the unadjusted analysis, there was a decrease in annual EOGBS incidence in screening and 'either prevention' areas. However, the decrease of EOGBS incidence in screening prevention areas was larger than compared with ''either prevention'' areas, by 0.017 (95% CI 0.002 to 0.031) yearly. There was also a statistically significant difference in the trends of annual EOGBS incidence between risk-based prevention areas and no prevention areas ($p<0.0001$). The mean adjusted R^2 for this model was 73%.

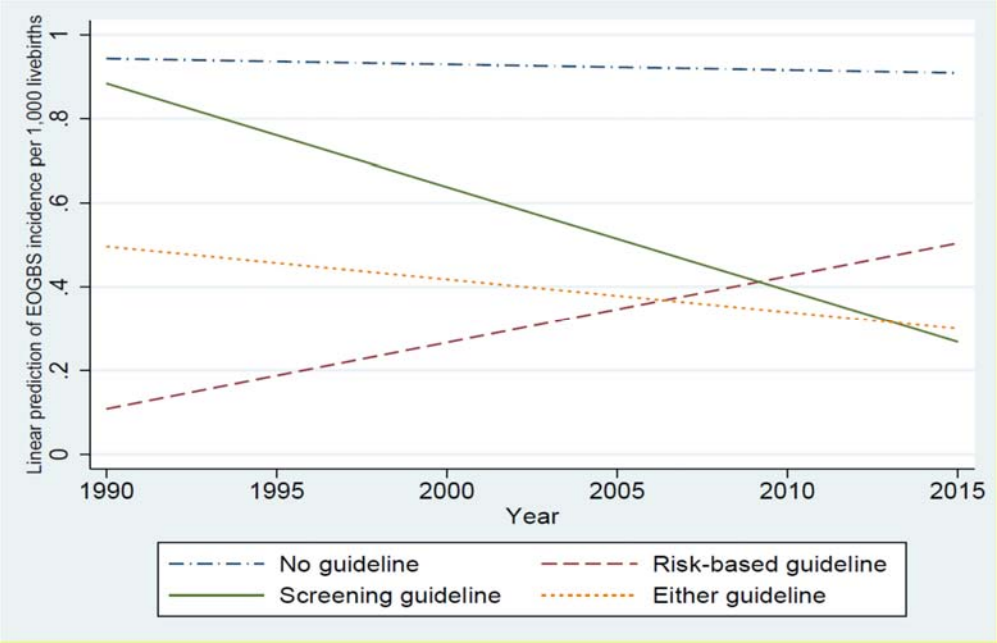
Table 28. Adjusted linear regression analyses of annual EOGBS incidence per 1,000 livebirths by recently reported GBS prevention strategy

Most recent reported GBS prevention	Average annual change in EOGBS incidence (95% confidence interval)	p-value
All data		
Screening prevention	(reference)	
No prevention	0.023 (-0.012 to 0.058)	0.194
Risk-based prevention	0.040 (0.026 to 0.055)	0.000
Either screening or risk-based prevention	0.017 (0.002 to 0.031)	0.026
Excluding St Augustine		
Screening prevention	(reference)	
No prevention	0.050 (0.022 to 0.078)	0.000
Risk-based prevention	0.038 (0.023 to 0.053)	0.000
Either screening or risk-based prevention	0.014 (-0.0002 to 0.029)	0.055

EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Models adjusted for region, preterm births, low birthweights, maternal GBS colonisation, prolonged rupture of membranes, intrapartum fever, human development index, multiple or twin births, geographical coverage, surveillance type, and EOGBS definition

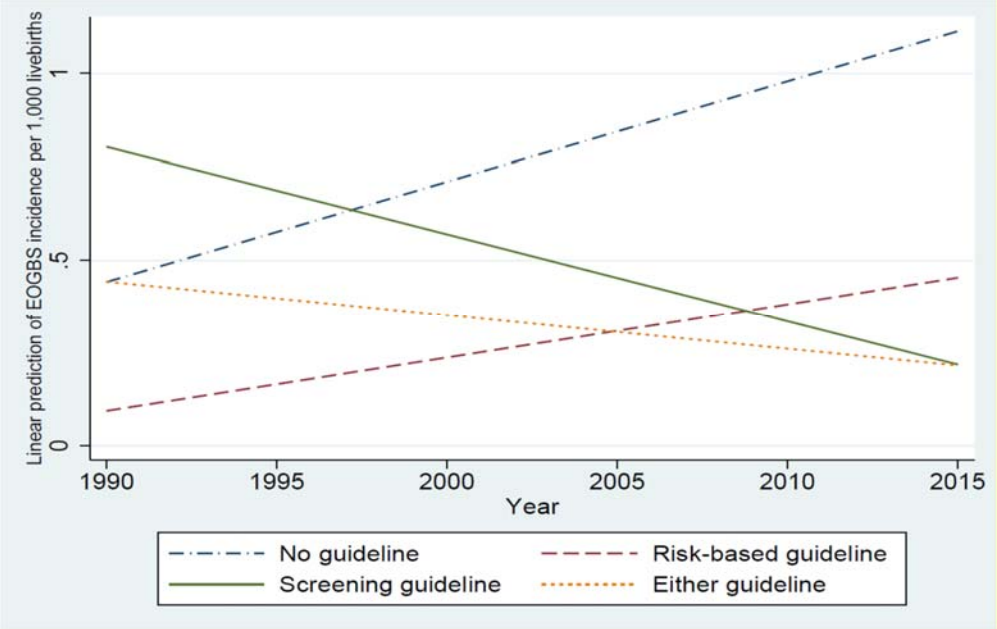
Excluding St. Augustine data

The results of the final model excluding St. Augustine from the analysis are shown in Table 28 and Figure 16. In line with the unadjusted analysis, there was a decrease in annual EOGBS incidence in screening prevention and 'either prevention' areas, and an increase in annual EOGBS incidence in no prevention and risk-based prevention areas. Compared with screening prevention areas, EOGBS incidence increased by 0.038 (95% CI 0.023 to 0.053) yearly in risk-based prevention areas and 0.050 (95% CI 0.022 to 0.078) yearly in no prevention areas. The difference in the trends of annual EOGBS incidence between no prevention areas and risk-based prevention areas was also statistically significant ($p<0.0001$). The difference in the trends of annual EOGBS incidence between screening prevention and 'either prevention' areas was not statistically significant. The mean adjusted R^2 for this second model was 53%.



EOGBS early-onset group B *Streptococcus* disease

Figure 15. Adjusted trends of annual EOGBS incidence for each recently reported GBS prevention strategy using linear regression analysis



EOGBS early-onset group B *Streptococcus* disease

Figure 16. Adjusted trends of annual EOGBS incidence for each recently reported GBS prevention strategy using linear regression analysis excluding St. Augustine

11.3.5 Sensitivity analysis of the adjusted linear regression analysis (objective f)

This section presents the results of the sensitivity analyses for the model with all of the data and then for the model without St. Augustine data, as the adjusted relationship between universal GBS screening and the trend of annual EOGBS incidence across time was statistically different to other prevention strategies.

All data

Alterations in the analysis with all of the EOGBS data changed the results comparing no prevention or 'either prevention' areas with screening prevention areas. Analysing the most frequently reported GBS prevention strategy across the years instead of the most recently reported GBS prevention strategy (0.033 95% CI 0.013 to 0.054) and only including geographical areas with four or more years of data (0.051 95% CI 0.008 to 0.093) caused the difference in trends of annual EOGBS incidence between no prevention and screening prevention areas to become statistically significant. Both sensitivity analyses also made the difference in the trends of annual EOGBS incidence between screening prevention areas and 'either prevention' areas to lose statistical significance (0.028 95% CI -0.009 to 0.065 and 0.015 95% CI -0.0009 to 0.032, respectively). Similarly, removing outer fence box plot outliers (0.036 95% CI 0.011 to 0.060) and removing data from only centre (0.275 95% CI 0.136 to 0.414) caused the difference in the trends of annual EOGBS incidence between no prevention areas and screening prevention areas to become statistically significant, possibly due to the removal of St. Augustine data. Removing outer fence box plot outliers also caused the difference in the trends of annual EOGBS incidence in 'either prevention' or screening prevention areas to lose statistical significance (0.010 95% CI -0.004 to 0.023).

There were further sensitivity analyses that only caused the difference in the trends of annual EOGBS incidence between no prevention and 'either prevention' areas to lose statistical significance. One of these analyses was only including covariates with less than 10% of data imputed from another country (0.016 95% CI -0.001 to 0.0334). This also changed the direction of the trends in annual EOGBS incidence for no prevention areas (in line with the unadjusted analysis), although this did not reach statistical significance (-0.037 95% CI -0.099 to 0.0245). The other analyses were: only keeping data defining EOGBS as 5/6/7 days or less (0.020 95% CI -0.0002 to 0.041), only using survey data (0.012 95% CI -0.003 to 0.026), using alternative geographical areas where more than one data source was available (0.014 95% CI -0.004 to 0.033) and altering the maternal GBS colonisation rate to a lower rate where

ranges were available (0.016 95% CI, -0.0004 to 0.033). On the other hand, altering maternal GBS colonisation to a higher rate did not alter the results.

Excluding St. Augustine data

There were only three changes to the analysis excluding St Augustine that changed the results. All of the following changes caused a statistically significant difference in the trends of annual EOGBS incidence between screening prevention and 'either prevention' areas: only keeping data from more than one centre (0.025, 95% CI 0.010 to 0.040), only including variables with less than 10% of data imputed from other countries (0.015, 95% CI 0.0008 to 0.029), and only keeping the data defining EOGBS as 5/6/7 days or less (0.021, 95% CI 0.002 to 0.040). None of the other sensitivity analyses altered the results.

11.3.6 Unadjusted multi-level repeated measures analysis (objective g)

This section presents the results of the multi-level repeated measures regression analysis showing the unadjusted relationship between the most recently reported GBS prevention strategy and the trends of annual EOGBS incidence across time, accounting for the hierarchical structure of the data (objective g). Again, there was one analysis for all data and another for the data excluding St. Augustine.

All data

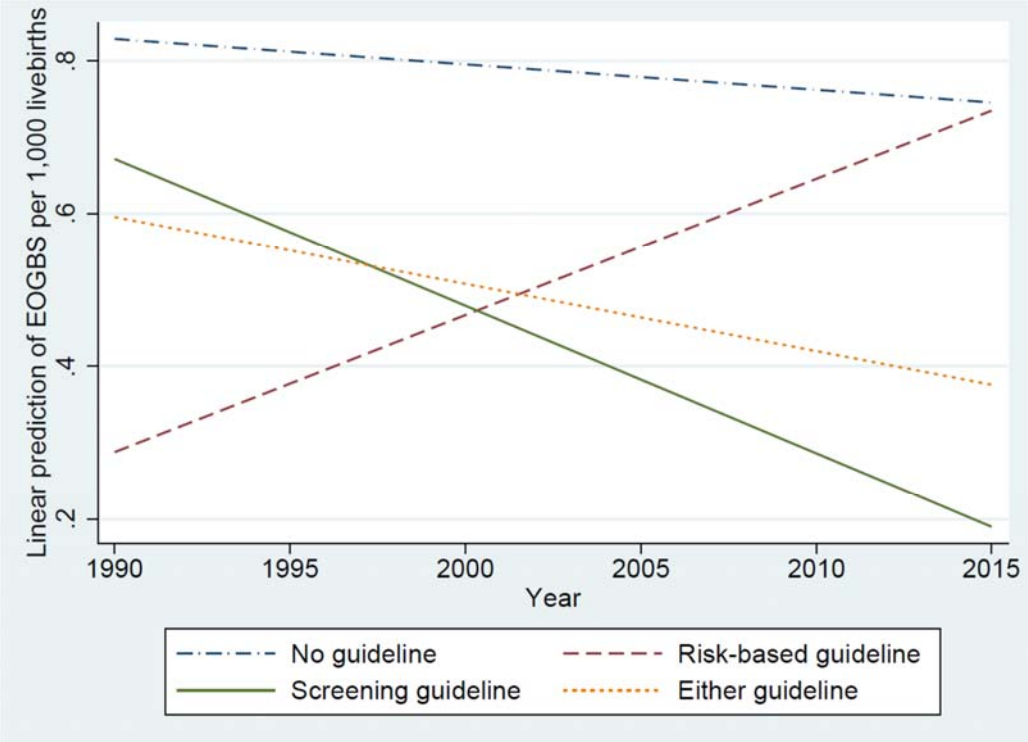
The results of the unadjusted multi-level analysis are summarised in Table 29 and Figure 17 for all data. In contrast to the linear regression analyses, there were no statistically significant differences in the trends of annual EOGBS incidence between screening prevention areas and other prevention areas. The between-geographical area intercept variance (σ^2_{u0}) indicated that the estimated variability in the EOGBS incidence at year 2005 between geographical areas was 0.163 (95% CI 0.087 to 0.304). The between-geographical area slope variance (σ^2_{u1}) indicated that the variability in the pattern of EOGBS incidence between geographical areas was 0.003, (95% CI 0.002 to 0.006). Finally, the joint intercept-slope (σ_{u01}) covariance was -0.013 (95% CI -0.024 to -0.002). The negative intercept-slope covariance with the positive estimates implies that geographical areas with lower than average EOGBS incidence at first year tended also to increase the most over the observation period. By contrast, geographical areas with higher than average EOGBS incidence at baseline tended to show above average decreases. The within geographical area variance (σ^2_e) shows that the variability in EOGBS incidence across the years within geographical areas was 0.067 (95% CI 0.057 to 0.079). The

statistically non-significant trends of EOGBS incidence shown in Figure 17 for each prevention area closely resembled each of the trends found in the adjusted linear regression.

Table 29. Multi-level growth curve analysis on annual EOGBS incidence per 1,000 livebirths by recently reported GBS prevention strategy

Variable	Coefficient (95% confidence intervals)
All data	
Fixed effects (Measures of association)	
GBS prevention strategy	
Screening prevention	(reference)
No prevention	0.016 (-0.048 to 0.080)
Risk-based prevention	0.037 (-0.008 to 0.082)
Either screening or risk-based prevention	0.010 (-0. 080 to 0.100)
Random effects (Measures of variation)	
Between geographical area	
Between intercept variance	0.163 (0.087 to 0.304)
Between slope variance	0.003 (0.002 to 0.006)
Between intercept-slope variance	-0.013 (-0.024 to -0.002)
Within geographical area	0.067 (0.057 to 0.079)
Excluding St Augustine data	
Fixed effects (Measures of association)	
GBS prevention strategy	
Screening prevention	(reference)
No prevention	0.078 (0.032 to 0.125)
Risk-based prevention	0.030 (0.009 to 0.069)
Either screening or risk-based prevention	0.009 (-0.051 to 0.069)
Random effects (Measures of variation)	
Between geographical Area	
Intercept variance	0.0815 (0.041 to 0.161)
Slope variance	0.001 (0.0006 to 0.003)
Intercept-slope variance	0.0008 (-0.005 to 0.006)
Within geographical area	0.066 (0.056 to 0.078)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Adjusted model adjusted for preterm births, maternal GBS, prolonged rupture of membranes, intrapartum fever, GBS serotype, surveillance type, geographical coverage, EOGBS definition



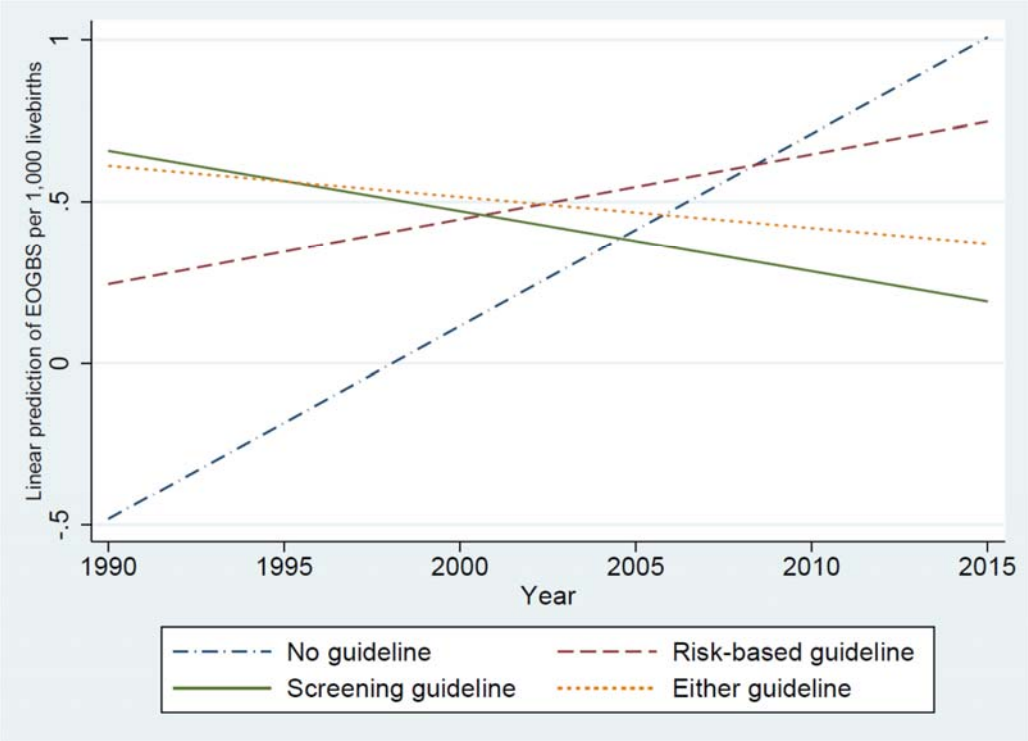
EOGBS early-onset group B *Streptococcus* disease

Figure 17. Unadjusted trends of annual EOGBS incidence for each recently reported GBS prevention strategy using multi-level analysis

Excluding St. Augustine data

The results excluding St. Augustine data from the analysis are shown in Table 29 and Figure 18. Similar to the adjusted linear regression analysis, there were decreases in annual EOGBS incidence in both screening and 'either prevention' areas, and increases in annual EOGBS incidence in both no prevention and risk-based prevention areas. Compared with screening prevention areas, the EOGBS incidence increased by 0.030 (95% CI 0.009 to 0.069) yearly in risk-based prevention areas and by 0.078 (95% CI 0.032 to 0.125) yearly in no prevention areas. The difference in the trends of annual EOGBS incidence between risk-based areas and no prevention areas was also statistically significant (p=0.0012). The difference in the trends of annual EOGBS incidence between screening prevention areas and 'either prevention' areas was not statistically significant. The between-geographical area intercept variance indicated that the estimated variability in the EOGBS incidence at year 2005 between geographical areas was 0.0815 (95% CI 0.041 to 0.161). The between-geographical area slope variance indicated that the variability in the pattern of EOGBS incidence between geographical areas was 0.001 95% (CI 0.0006 to 0.003). However, the joint intercept-slope covariance was not (0.0008 95% CI -0.005 to 0.006). The within-geographical area variance shows that the variability in

EOGBS incidence across the years within geographical areas was 0.066 (95% CI 0.056 to 0.078).



EOGBS early-onset group B *Streptococcus* disease

Figure 18. Unadjusted trends of annual EOGBS incidence for each recently reported GBS prevention strategy using multi-level analysis and excluding St Augustine

11.4 Discussion

In this discussion, I will summarise the findings of this study and compare the findings with previous literature. In Chapter 14, I will discuss the strengths and the limitations as well as the research and policy implications related to this study.

11.4.1 Principal findings

In this study, I aimed to investigate whether GBS prevention strategies, particularly universal screening, have an impact on the trends of annual EOGBS incidence across countries, adjusting for country differences. The findings suggest that the international trends of EOGBS may indeed be related to some prevention strategies. However, there was substantial uncertainty in the key results that follow. The most consistent trends across analyses were that

EOGBS incidence increased in areas that (most recently) reported risk-based prevention whereas it decreased in areas that reported universal screening. Based on the available evidence, the predicted values in risk-based prevention areas showed that EOGBS incidence increased from approximately 0.10 to 0.5 per 1,000 livebirths in the adjusted linear regression and approximately 0.3 to 0.75 per 1,000 livebirths in the unadjusted multi-level analysis during the study period. The predicted values in screening prevention areas showed that EOGBS incidence reduced from approximately 0.8 to 0.9 per 1,000 livebirths to 0.2 to 0.3 per 1,000 livebirths in the unadjusted linear regression and from approximately 0.66 to 0.19 per 1,000 livebirths in the unadjusted multi-level analysis during the study period. The difference in the trends of annual EOGBS incidence between risk-based and screening areas were statistically different in most analyses.

By contrast, there was little evidence for a difference in the trends of annual EOGBS incidence between areas that reported universal screening and those that reported 'either prevention' strategy, as EOGBS incidence decreased across time in both. Results comparing no prevention with universal screening were complicated by greater uncertainty across the analyses, as different assumptions and models revealed conflicting EOGBS trends. When the countries' contexts were not accounted for in the analyses, areas reporting no prevention showed a decrease in EOGBS incidence that was statistically different to those reporting universal screening. However, when countries' contexts or time were accounted for, areas reporting no prevention either had an upward (and not a downward) trend that was statistically different to areas reporting universal screening, or little change in annual EOGBS incidence, which was not statistically different to screening.

When comparing no prevention with screening, the inclusion of data from one centre in St. Augustine, that had no prevention, had a strong influence on the results. Including St. Augustine in the linear and multi-level regression analyses resulted in either a steeper decrease of EOGBS incidence in areas that most recently reported no prevention compared with universal screening, or no difference. In the adjusted linear regression and the unadjusted multi-level analysis, the predicted values identified the following trends in areas that reported no prevention during the study period, which were not different to universal screening: EOGBS incidence decreased from around 0.84 per 1,000 livebirths in 1990 to 0.74 in 2015, while in the adjusted linear regression EOGBS incidence decreased from 0.94 to 0.91 per 1,000 livebirths. On the other hand, removing St. Augustine from the analyses showed that EOGBS incidence increased over time in areas that reported no prevention. The predicted values indicated that EOGBS incidence increased from 0.44 in 1990 to 1.11 per 1,000 livebirths in 2015 in the adjusted linear regression and from -0.48 to 1.0 per 1,000 livebirths

in the unadjusted multi-level analysis, which were statistically significant compared with areas that reported universal screening. Data from St. Augustine were unusual as the rate of EOGBS incidence started at 5.41 per 1,000 livebirths in 1989. The EOGBS incidence in approximately 90% of the observations was below 1.00 per 1,000 livebirths. The rate of EOGBS then reduced dramatically to 2.79 per 1,000 livebirths by 2002; the magnitude of this change was also higher than that observed in any other area.

The findings on the EOGBS trends under risk-based *versus* universal screening prevention were more stable than no prevention compared with screening. All of the analyses showed that EOGBS incidence increased in areas that most recently reported risk-based prevention whereas it decreased under screening. However, in the multi-level analysis with all of the data (including St. Augustine), the difference between the increase under risk-based prevention was not statistically different to the decrease under screening. This may indicate that when accounting for the variance between geographical areas (which showed different intercepts, slopes and intercept-slopes for EOGBS incidence between areas), there is no difference between risk-based prevention and universal screening. On the other hand, when St. Augustine was removed from the multi-level analyses the difference in the trends of the two strategies were statistically different. Furthermore, if the multi-level analysis is re-run with the compositional covariates, the results might vary. Likewise, in general there was no difference in the downward trends in EOGBS incidence between screening and 'either prevention.' Although in the adjusted linear regression with all data, the difference in the trends of EOGBS incidence between universal screening and 'either prevention' reached statistical significance, in nine out of the 10 sensitivity analyses for this model, the statistical significance was lost.

The results in this study when including St. Augustine may also be due to a statistical phenomenon known as regression to the mean, where an outcome that is extreme on the first measurement will be closer to the centre of the distribution for subsequent measurements.[403, 404]The distribution of EOGBS incidence in this study was left-skewed with a group of observations that would be considered extreme. In this group was St. Augustine that had extreme incidence on the first measurement and then decreased closer towards the mean in subsequent years. Further supporting this possibility is the result in the multi-level unadjusted analysis including St. Augustine, which showed that geographical areas with lower than average EOGBS incidence at first year tended to increase the most over the observation period (which is the pattern found in risk-based prevention) and that areas with higher than average EOGBS incidence at baseline tended to show above average decreases (which is the pattern found in the remaining areas when all data are included). Therefore, it is possible that the

yearly changes that occurred over time were due to this statistical bias as opposed to a change as a result of any or no prevention strategy.

Given these inconsistent findings, it is difficult to reach a conclusion on the association between GBS prevention strategy and EOGBS incidence. On the one hand, there are results from linear regression analyses with a reasonable sample size that did not account for the data structure (time nested within geographical area). On the other hand, there are results from multi-level analysis that did account for the data structure but did not account for confounding factors. All of these analyses have revealed different results. Screening consistently showed a downward trend of EOGBS incidence across the analyses while risk-based prevention consistently showed an upward trend. It is also likely that there is no difference in the EOGBS trends between screening and 'either prevention'. However, there are important inconsistencies and uncertainties in these results.

11.4.2 Comparison with previous literature

As discussed in Chapter 2, there have been a number of studies comparing the rate of EOGBS incidence within an area, before and after changing the GBS prevention strategy from no or risk-based prevention to screening. Many of these studies show conflicting results; some in support of the findings from this study and some against. A meta-analysis in 2011 compared the rates of GBS sepsis during a screening period with a period of no or risk-based prevention that preceded screening, in eight studies published between 1994 and 2006. The authors found that across the US, Austria, Australia, Italy and Switzerland, there was less neonatal GBS sepsis compared with the periods of no (OR 0.43 95% CI 0.25 to 0.73) and risk-based prevention (OR 0.25, 95% CI 0.16 to 0.37).[16] Similarly, another meta-analysis of seven studies across Turkey, Australia and the US in 2013 also found that the odds of EOGBS under risk-based prevention were higher than under screening (OR 0.45, 95% CI 0.37 to 0.53).[17] One of the main limitations of the reviews is that these figures were not adjusted for other differences during the screening and non-screening periods. In the current study, I have attempted to adjust for differences between areas with different prevention strategies, and the results on risk-based prevention compared with screening are in line with the reviews. Risk-based prevention does indeed appear to have higher and increasing rates of EOGBS compared with screening. The results without St. Augustine are also in line with review findings that under screening there is a lower EOGBS incidence than under no prevention. The current results conflict with the review when including St. Augustine. This may imply that other factors, possibly wider than GBS prevention, such as health system or gynaecology and obstetrics care improvements, can

contribute to a decrease in EOGBS incidence, especially in countries that have previously had very high rates.

Related to this, a relatively recent study comparing EOGBS infections in a teaching hospital in Hungary between a period with no prevention and a period with screening, found decreases in the incidence of all EOGBS infections (0.36, 95% CI 0.26 to 0.49), GBS sepsis (0.27, 95% CI 0.12 to 0.58), and GBS pneumonia (0.19, 95% CI 0.11 to 0.32) (p=0.001 for all three comparisons).[173] The rate of EOGBS reduced from a high of 7.55 to 2.44 per 1,000 livebirths. The rates of EOGBS in the Hungarian study were closest to the trends found in St. Augustine in this study, which showed a high rate of EOGBS that drastically reduced over time. However, in St. Augustine, this was not a result of prevention intervention. In the Hungarian study, no adjustment was made for confounding variables, therefore factors beyond the screening programme could have contributed to the decrease, as opposed to screening. Likewise, the decrease under screening could be due to regression to the mean bias as explained above.[403, 404]

There has also been a recent review that pooled the rate of EOGBS incidence by the different GBS prevention strategies across countries, but did not statistically compare the rates. This review found that the pooled incidence of EOGBS across countries was 0.44 per 1,000 livebirths (95% CI 0.36 to 0.51), which is similar to the mean incidence of EOGBS found in this study, 0.49 per 1,000 livebirths. The authors found that countries that adopted both screening in addition to risk-based policies had the lowest incidence at 0.38 per 1,000 livebirths (95% CI 0.25 to 0.51), followed by screening only at 0.45 (95% CI 0.31 to 0.59), and the highest incidence was for countries adopting a risk-based prevention strategy (0.49, 95% CI 0.34 to 0.65).[405] Although, in this study, I did not categorise screening prevention into those that screen and offer IAP for risk factors, and those that only offer IAP based on the screening test, the results are comparable. Both screening or screen and risk-based prevention show lower rates than risk-based prevention alone.

Since the pooled meta-analytic comparisons on risk-based *versus* screening prevention, smaller studies have reported inconsistent findings. In line with this study, research in the US found lower odds of developing EOGBS in NICU admissions during the screening period compared with the either risk-based or screening period in adjusted analyses (OR 0.69 p<0.001).[172] This analysis was adjusted for gestational age, sex, race, inborn status, 5-min APGAR, ventilator support on first postnatal data, prenatal steroid expose, prenatal antibiotic exposure and mode of delivery. In contrast to the results of this study, unadjusted results from another US study found that the incidence of EOGBS decreased after the introduction of a

risk-based strategy (2.06 to 0.96 per 1,000 livebirths) but did not reduce further in the era of screening (1.11 per 1,000 livebirths).[170] Likewise, a recent study in one UK maternity unit found that EOGBS fell from 0.99 per 1,000 livebirths in the risk-based period to 0.33 per 1,000 livebirths during the screening period; however, this did not reach statistical significance in unadjusted analyses (p=0.08).[34] Recently, a model developed by the NSC based on the best available evidence and expert opinion, found that the number of EOGBS cases would be lower under screening and risk-based prevention as it would prevent 52 to 57 cases compared with risk based prevention alone.[177]

Overall, the existing evidence from literature on the impact of GBS prevention strategies on EOGBS incidence reflects the inconsistency and uncertainty found in this study. Trends in EOGBS incidence might indeed decrease under screening compared with risk-based and no prevention, however, whether this decrease is statistically or clinically significant is not clear.

11.5 Conclusions for this chapter

- Findings from this chapter have highlighted that GBS prevention strategies may be associated with international trends of EOGBS incidence per 1,000 livebirths.
- In areas that most recently reported a risk-based prevention, EOGBS incidence increased by around 0.4 to 0.5 per 1,000 livebirths in 27 years. In areas that most recently reported universal screening, there was a decrease in EOGBS incidence by approximately 0.5 to 0.6 per 1,000 livebirths in 27 years, while in those that reported 'either prevention' strategy, there was a decrease of around 0.2 per 1,000 livebirths.
- These trends were quite consistent across the analyses and, in general, the differences in the average change in annual EOGBS incidence between risk-based prevention and screening were statistically significant across most analyses. However, the difference between universal screening and 'either prevention' in the majority of the analyses was not.
- While the comparison of adopting universal screening with risk-based prevention was relatively consistent, the result must be treated with caution as it contains important limitations and some instability across the analyses.
- Areas that most recently reported no GBS prevention displayed conflicting findings, with some analyses showing an increase and others a decrease in EOGBS incidence. Further examination showed that this might be due to outlying data trends and/or other factors contributing to EOGBS incidence.

12. THE IMPACT OF UNIVERSAL GBS SCREENING ON THE TRENDS OF ANNUAL EARLY-ONSET SEPSIS INCIDENCE

This chapter presents the results for the ecological trend analysis study that combines international data to explore the impact of universal GBS screening on the trends of annual early-onset sepsis incidence, compared with other GBS prevention strategies. While I discussed the methodology used to address this study in Chapter 9, here I will first present the study specific aim and objectives followed by some study specific methodological procedures. I will then present the detailed results from the statistical analyses: first the MICE imputation results, then the descriptive statistics, followed by the unadjusted analyses and the main adjusted model. Finally, I will summarise the principal findings and how they relate to previous literature.

12.1 Aims and objectives

The aim of this chapter is to measure the effect of universal GBS screening on the trend of annual early-onset sepsis incidence across time, compared with other GBS prevention strategies, in a statistical model that combines data from geographical areas with different prevention strategies, and adjusts for compositional differences between the areas.

The research objectives are to:

a) Describe the frequency of the GBS prevention strategy as well as the mean or frequency of the compositional covariates in general (irrespective of early-onset sepsis incidence);
b) Describe the mean early-onset sepsis incidence across time, geographical areas, world regions and GBS prevention strategies;
c) Investigate the unadjusted relationship between universal GBS screening and the trend of annual early-onset sepsis incidence across time compared with other prevention strategies, using linear regression;
d) Investigate the unadjusted relationship between each compositional covariate and the mean early-onset sepsis incidence across time, using linear regression;

e) Investigate the relationship between universal GBS screening and the trend of annual early-onset sepsis incidence across time compared with other prevention strategies, using linear regression and adjusting for the compositional covariates; and
f) Examine the stability of the adjusted relationship between universal GBS screening and the trend of annual early-onset sepsis incidence across time compared with other prevention strategies in a range of sensitivity analyses, if the relationship was statistically significant.

12.2 Methods

I have detailed the methodology used for this study in Chapter 9. Here, I describe the data selected to perform the analysis.

12.2.1 Geographical data included

As discussed in Chapter 10, there were annual early-onset sepsis data from 28 geographical areas. However, some of them overlapped in terms of coverage, giving rise to the potential of double-counting. These were: Alberta that overlapped with multi-centre data across Canada; data from Barcelona *versus* multiple centres across Spain; and data from multiple centres *versus* enhanced surveillance in New Zealand. I chose Alberta as it had population-based and mandatory surveillance; multi-centres from Spain to have broader national coverage; and the enhanced surveillance for New Zealand. This left 25 geographical areas. However, after analysing the early-onset sepsis incidence across the years and countries, I excluded Mansoura City in Egypt where the rate of early-onset sepsis incidence for 2014 was 72.46 per 1,000 livebirths. This rate was an extreme outlier from the remaining data and was vastly different from the incidence rates in the UK. This left data from a total of 24 geographical areas in the analyses that follow.

Further descriptive analyses also revealed that Mexico City had a strong influence on the descriptive trends of early-onset sepsis incidence over time. However, as the incidence rates themselves were not extreme outliers, I ran the unadjusted and adjusted linear regression separately with and without Mexico City.

12.2.2 Compositional covariates included

The list of compositional covariates included in the analyses that follow was shorter than the list in the EOGBS analyses in Chapter 11, as the sample size was considerably smaller, thus, the model needed to be simplified. I prioritised the following compositional covariates that were theoretically most important and investigated them in the unadjusted analyses, while the others mentioned in Chapter 11 were excluded: preterm births, low birthweights, maternal GBS colonisation, prolonged rupture of membranes and intrapartum fever (as they are strong risk factors for neonatal sepsis and EOGBS) and the human development index, world region, surveillance type, geographical coverage and early-onset sepsis definition (to account for crucial limitations in the data collection methods).

12.3 Results

12.3.1 Multiple imputation for compositional covariates

There were 32 (20%) observations that had complete compositional covariate data for every year. The proportion of missing data is shown in Table 30. Of the covariates that were included in the analyses, preterm births had the largest amount of missing data with 63% of years missing. This was followed closely by the human development index, which had 60% of years missing, and, finally, low birthweights with 51% of years missing.

Table 30. Proportion of missing data in compositional covariates used for the early-onset sepsis dataset

Variable	Observed (%)	Missing (%)
Preterm births	60 (37)	102 (63)
Low birthweights	79 (49)	83 (51)
Human development index	64 (40)	98 (60)

There were multiple patterns of missing data. The most common pattern was for none of the covariates to be observed, followed by all covariates observed, only low birthweights to be observed, both only the human development index to be observed and only low birthweights and preterm births to be observed, low birthweights and the human development index to be observed, only preterm births to be observed and finally the human development index and preterm births to be observed. The logistic regression for whether the missingness of a variable was related to the other variables revealed that the missingness of preterm births was related to early-onset sepsis definition and multiple births. Missingness of low birthweights was

related to year and early-onset sepsis definition. Missingness of the human development index was related to year. As data were missing because they were available from some years but not others and their missingness was related to other data, the mechanism for the missing data was 'missing at random'.

As mentioned in Chapter 9, there were 100 imputations because of the large amount of missing data and the results showed that convergence was reached within these imputations. Additionally, the largest fraction of missing information ($_{FMI}$) from the analysis models was 68%, confirming that the number of imputations was sufficient. Below, I present the descriptive statistics for some of the imputed datasets and the original dataset for comparison.

12.3.2 Descriptive analysis (objectives a & b)

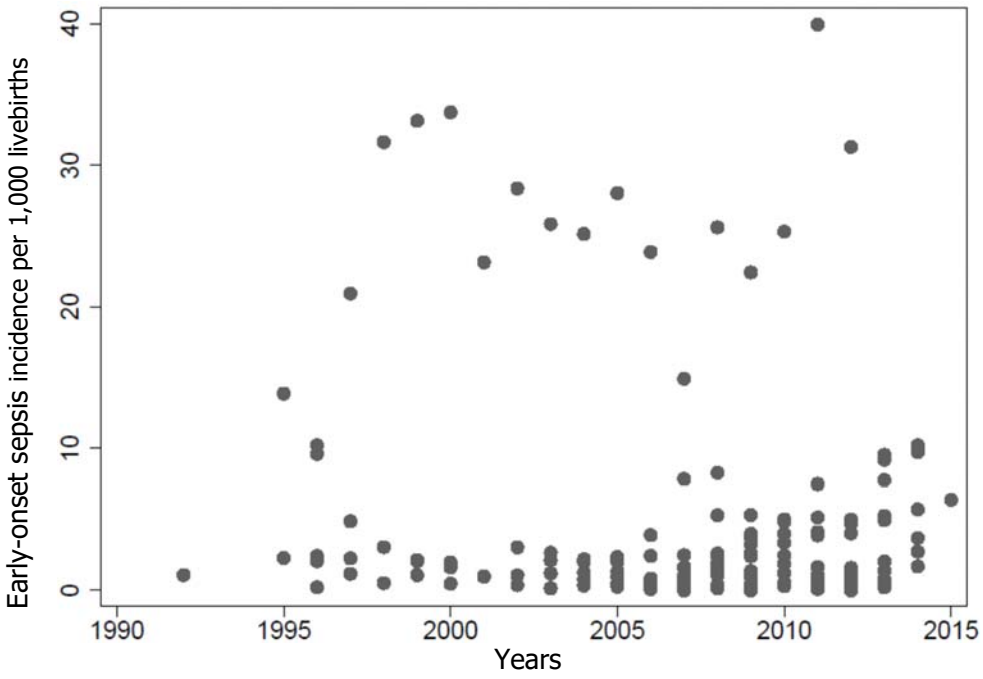
This section presents the mean early-onset sepsis incidence across time, geographical areas, world regions and GBS prevention strategies (objective b) as well as the frequency of the GBS prevention strategy and the mean or frequency of the compositional covariates in general (irrespective of early-onset sepsis incidence) (objective a).

Early-onset sepsis incidence

The mean early-onset sepsis incidence across 162 observations (24 geographical areas, 22 years between 1992 and 2015) was 4.87 per 1,000 livebirths (SD 8.00, range: 0.00 to 39.95). Across the years, early-onset sepsis incidence varied between 1.09 and 11.74 per 1,000 livebirths (see Table 31). Figure 19 shows the scatterplot of all early-onset sepsis incidence observations by year. It shows that there were two patterns within the data: one with rates of early-onset sepsis lower than 10 per 1,000 livebirths that increased over time, and another with a random scatter between 10 to 40 cases of early-onset sepsis per 1,000 livebirths. These patterns may be reflective of trends over time or a result of different geographical areas that contributed to each year (see Table 31).

Table 31. Early-onset sepsis incidence per 1,000 livebirths across the years

Year	Number of geographical areas	Mean livebirths (Standard deviation)	Mean early-onset sepsis incidence (Standard deviation)
1992	1	247,076.00	1.09
1995	2	4,425.50 (1,918.38)	8.06 (8.17)
1996	5	17,810.40 (29,733.50)	4.90 (4.64)
1997	4	24,977.75 (40,981.75)	7.31 (9.21)
1998	3	4,445.00 (1,410.28)	11.74 (17.28)
1999	4	24,303.75 (39,162.95)	9.60 (15.70)
2000	4	23,055.00 (36,951.62)	9.45 (16.20)
2001	2	37,205.67 (54,612.31)	8.37 (12.79)
2002	4	37,479.25 (46,222.46)	8.20 (13.48)
2003	6	29,135.00 (33,907.51)	5.53 (9.99)
2004	6	25,592.83 (29,493.70)	5.28 (9.75)
2005	10	64,389.90 (139,861.70)	3.88 (8.52)
2006	11	62,819.36 (135,090.70)	3.26 (6.94)
2007	12	59,229.42 (133,392.60)	2.62 (4.41)
2008	14	57,883.00 (124,832.50)	3.87 (6.63)
2009	13	25,184.85 (36,798.35)	3.59 (5.91)
2010	13	34,245.85 (45,417.99)	3.97 (6.62)
2011	12	33,406.92 (40,865.52)	5.47 (11.11)
2012	14	39,306.21 (48,879.46)	4.06 (8.06)
2013	13	20,173.77 (30,923.28)	4.02 (3.38)
2014	7	4,554.71 (3,458.30)	6.24 (3.68)
2015	1	2,195.00	6.38
Total	162 observations (24 areas, 22 years)	37,738.85 (78,582.26)	4.87 (8.00)



Each dot represents the early-onset sepsis incidence for one year for one geographical area

Figure 19. Scatterplot of early-onset sepsis incidence by year

Plotting the early-onset sepsis incidence by year reveals that the pattern of high early-onset sepsis incidence above 10 per 1,000 livebirths increasing to almost 40 per 1,000 livebirths is from one geographical area of Mexico City (see Appendix 16-A). Excluding Mansoura City, the highest mean early-onset sepsis incidence per 1,000 livebirths across years was found in Mexico City (24.52 [SD 8.20]) followed by St. Augustine in Trinidad and Tobago (10.18). Removing Mexico City gave a mean early-onset sepsis incidence of 2.26 per 1,000 livebirths (SD 2.34, range 0.00 to 10.18). The lowest mean early-onset sepsis incidence per 1,000 livebirths across years was found in Alberta (0.21 [SD 0.08]) followed by Switzerland (0.30 [SD 0.18]) (see Table 32). These are the averages across the years for each geographical area but, as can be seen in Appendix 16-A, for many areas the rates fluctuated from the first year to the last.

Table 32. Early-onset sepsis incidence per 1,000 livebirths by geographical area

Geographical area	Number of years	Mean number of livebirths (Standard deviation)	Mean early-onset sepsis incidence (Standard deviation)
Alberta	11	47,896.00 (4,854.45)	0.21 (0.08)
Australia	11	29,199.82 (4,802.03)	2.24 (0.51)
Bangalore	3	4,044.33 (1,605.77)	5.68 (0.72)
Buenos Aires	18	6,473.22 (923.54)	0.68 (0.32)
Cordoba	2	1,573.00 (152.74)	6.12 (4.80)
Emilia-Romagna	1	146,682.00	0.61
Guangzhou	3	2,109.33 (323.93)	7.24 (3.00)
Kaunas	7	3,524.86 (194.83)	5.18 (2.15)
Kingston	12	2,619.58 (326.72)	2.59 (1.03)
Kuala Terengganu	4	11,637.50 (755.55)	0.92 (0.42)
Kuwait	10	11,074.10 (486.46)	3.52 (1.14)
Macau	10	2,667.70 (697.83)	0.84 (0.77)
Manila	1	6,682.00	10.18
Mexico City	19	5,055.11 (593.10)	24.52 (8.20)
New Zealand	1	127,134.00	0.49
Panama City	1	247,076.00	1.09
Portugal	6	95,824.83 (8,100.16)	5.13 (0.20)
Riga	1	2,060.00	9.71
Spain	16	96,036.63 (13,710.00)	1.35 (0.51)
St Augustine	1	4,910.00	10.18
Switzerland	3	81,901.00 (988.11)	0.30 (0.18)
Tokyo	11	1,627.73 (192.71)	1.05 (0.93)
US	4	464.044.30 (9,575.21)	0.35 (0.06)
Zagreb	6	4,254.50 (102.26)	3.22 (2.52)
Total	162	37,738.85 (78,582.26)	4.87 (8.00)

US United States of America

Taking a broader global perspective, the highest mean early-onset sepsis per 1,000 livebirths was reported in Latin America and the Caribbean (10.05 [SD 12.08]), probably due to the influence of high incidences from Mexico City. The lowest incidence was reported in North America (0.25 per 1,000 livebirths [SD 0.10]), which was much lower than incidences found in the other regions. Asia, Europe, Oceania and North Africa and the Middle East had early-

onset sepsis rates around two to four per 1,000 livebirths (see Table 33). Again, these rates vary across time and, within regions, may fluctuate by year as shown in the scatterplots of early-onset sepsis incidence by world region presented in Appendix 16-B.

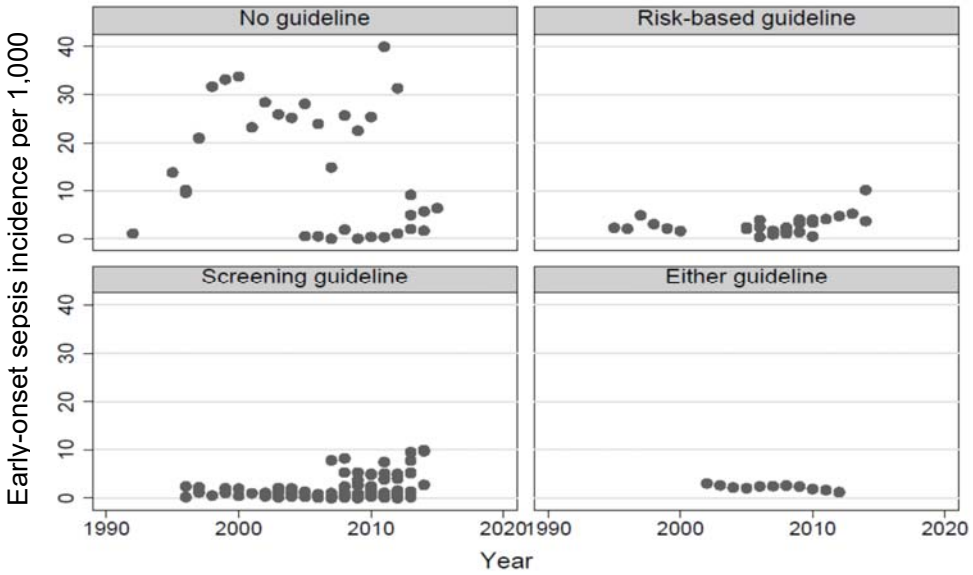
Table 33. Early-onset sepsis incidence per 1,000 livebirths by region

Region	Number of observations/ years (%)	Mean early-onset sepsis incidence (standard deviation)
Asia	32 (19.75)	2.27 (2.83)
Europe	40 (24.69)	2.98 (2.48)
Latin America and the Caribbean	53 (32.72)	10.05 (12.08)
North Africa and the Middle East	10 (6.17)	3.52 (1.14)
North America	15 (9.26)	0.25 (0.10)
Oceania	12 (7.41)	2.10 (0.70)
Total	162 (100.00)	4.87 (8.00)

Finally, the mean early-onset sepsis incidence by most recently reported GBS prevention strategy is presented in Table 34. The highest mean incidence per 1,000 livebirths was reported under no prevention (14.78 [SD 12.81]), which was much higher than the other prevention strategies. This was followed by risk-based prevention (2.88 [SD 1.95]), which was closely followed by either risk-based or screening prevention (2.24 [SD 0.51]) and finally screening prevention (2.03 [SD 2.46]), all of which had relatively similar incidences. Figure 20 shows the scatterplot for early-onset sepsis incidence by the most recently reported GBS prevention strategy. There was a potential increase in early-onset sepsis incidence in areas that reported universal screening and a potential decrease in areas that reported either risk-based or screening prevention. There was a mixed pattern in areas that reported risk-based prevention whereby incidence initially increased until 1998, then decreased until 2000, followed by a period of no data until approximately 2005, after which there was an increase. Under no prevention, there was a random pattern and a substantial impact of data from Mexico City as the plot shows the same pattern.

Table 34. Early-onset sepsis incidence per 1,000 livebirths by recently reported GBS prevention strategy

Most recent GBS prevention	Number of observations/years (%)	Mean early-onset sepsis incidence (standard deviation)
No prevention	34 (20.99)	14.78 (12.81)
Risk-based prevention	28 (17.28)	2.88 (1.95)
Screening prevention	89 (54.94)	2.03 (2.46)
Either risk-based and screening prevention	11 (6.79)	2.24 (0.51)
Total	162 (100)	4.87 (8.00)



Each dot represents the early-onset incidence for one year for one geographical area.

Figure 20. Scatterplot of early-onset sepsis by most recently reported GBS prevention strategy

Predictor and compositional covariates

Most geographical areas (and observations) recently reported a screening strategy (55% of observations and 54% of areas), followed by no prevention (21% of observations and 21% of areas), risk-based prevention (17% of observations and 21% of areas) and risk-based or screening prevention (7% of observations and 4% of areas) (see Table 35 and Table 17). With respect to world region, the majority of observations were from Latin America and the Caribbean (33%) and the fewest were from North Africa and the Middle East (6%). There were no data from Sub-Saharan Africa (see Table 33).

Table 35. Frequency of geographical areas for recently reported GBS strategy for the early-onset sepsis dataset

Most recent GBS prevention strategy	Frequency (%)
No prevention	5 (21)
Risk-based prevention	5 (21)
Screening prevention	13 (54)
Either risk-based and screening prevention	1 (4)
Total	24 (100)

The mean values for the compositional covariates that were multiple imputed are presented in Table 36. Averages are provided for the data with no imputation, followed by imputed datasets 1 and 100, to provide an indication of how well the multiple imputation performed. The mean percentage of preterm births across the years in the original dataset was 10.91% (SD 5.32) and the imputed means were around 9.27% to 9.37%, therefore, within 1.64% of the original mean. Similarly, the mean percentage of low birthweights was 12.93% (SD 8.39) and the imputed mean values were around 10.5% and 11%, thus, within 2.4% of the original mean. By contrast, the imputation for the human development index performed better, as the mean value was the same across the datasets at 0.81 (SD 0.08).

Table 36. Mean values for multiple imputed compositional covariates for the early-onset sepsis dataset

Covariate	Original dataset Mean (SD)	Imputed dataset 1 Mean (SD)	Imputed dataset 2 Mean (SD)
Preterm births (%)	10.91 (5.32)	9.27 (4.08)	9.37 (4.32)
Low birthweights (%)	12.93 (8.39)	10.49 (7.32)	11.02 (8.53)
Human development index	0.81 (0.08)	0.81 (0.08)	0.81 (0.08)

The mean values for compositional covariates that only had one value across all of the years and, thus, were not multiple imputed are presented in Table 37. Across the geographical areas, maternal colonisation of GBS was approximately 16.45% (SD 10.28), the mean percentage of prolonged rupture of membranes was 7.09% (SD 6.28) and intrapartum fever was 1.92% (SD 2.53). Most data covered one centre only (65.43%), followed by multiple centres (27.16%) and mandatory/enhanced surveillance (7.41%). After data from one centre, most data covered a country (25.31%), a region (7.41%) or were city or town-wide (1.85%). Finally, the most common definition of early-onset sepsis was 2/3/4 days or less (45.06%), followed by 5/6/7 days or less (41.36%) and vertical onset/mother-acquired (13.58%).

Table 37. Mean values for un-imputed compositional covariates for the early-onset sepsis dataset

Covariate	Descriptive statistic
Continuous variables	*Mean (SD)*
Maternal GBS colonisation	16.45 (10.28)
Prolonged rupture of membranes	7.09 (6.28)
Intrapartum fever	1.92 (2.53)
Categorical variables	*Frequency (%)*
Surveillance type	
Mandatory or enhanced population surveillance	12 (7.41)
Multiple centres/counties	44 (27.16)
One centre	106 (65.43)
Geographical coverage	
National	41 (25.31)
Regional	12 (7.41)
City/town wide	3 (1.85)
One centre in a city/town	106 (65.43)
Early-onset sepsis definition	
2/3/4 days or less	73 (45.06)
5/6/7 days or less	67 (41.36)
Vertical onset/Mother-acquired	22 (13.58)

12.3.3 Unadjusted linear regression analysis (objective c & d)

This section presents the results of the linear regression analysis showing the unadjusted relationship between most recently reported GBS prevention strategy and the trends of annual early-onset sepsis incidence across time for all data and then for the data excluding Mexico City (objective c). This is followed by the linear regression analysis showing the unadjusted relationship between each compositional covariate and mean early-onset sepsis incidence (objective d).

Most recently reported GBS prevention strategy

The results of the unadjusted analysis are summarised in Table 38 and Figure 21. Contrary to expectation, there was a continuous decrease in annual early-onset sepsis incidence in areas with no prevention. Compared with screening prevention areas, the early-onset sepsis incidence decreased by 0.706 (95% CI -1.130 to -0.281) yearly in no prevention areas. There were no statistically significant differences in the trends of annual early-onset sepsis incidence in screening prevention areas compared with risk-based prevention or 'either prevention' areas. When Mexico City was excluded, there was still a continuous decrease in annual early-onset sepsis incidence in no prevention areas compared with screening areas (-0.047 95% CI -0.419 to -0.003), however, the slope was no longer as steep as the analysis including Mexico City (see Figure 22).

Table 38. Unadjusted and adjusted linear regression analyses on the average annual change in early-onset sepsis incidence per 1,000 livebirths by recently reported GBS prevention strategy

Most recent reported GBS prevention	Average annual change in early-onset sepsis (95% confidence interval)	p-value
Unadjusted analysis		
Screening prevention	(reference)	
No prevention	-0.706 (-1.130 to -0.281)	0.001
Risk-based prevention	-0.051 (-0.559 to 0.456)	0.842
Either risk-based and screening prevention	-0.289 (-1.458 to 0.880)	0.626
Adjusted analysis		
Screening prevention	(reference)	
No prevention	0.002 (-0.834 to 0.838)	0.996
Risk-based prevention	-0.089 (-0.449 to 0.271)	0.625
Either risk-based and screening prevention	-0.244 (-0.597 to 0.110)	0.174

GBS group B *Streptococcus*
Adjusted analysis adjusted for region, preterm births, low birthweights, maternal GBS colonisation, human development index, geographical coverage, surveillance type, and early-onset sepsis definition.

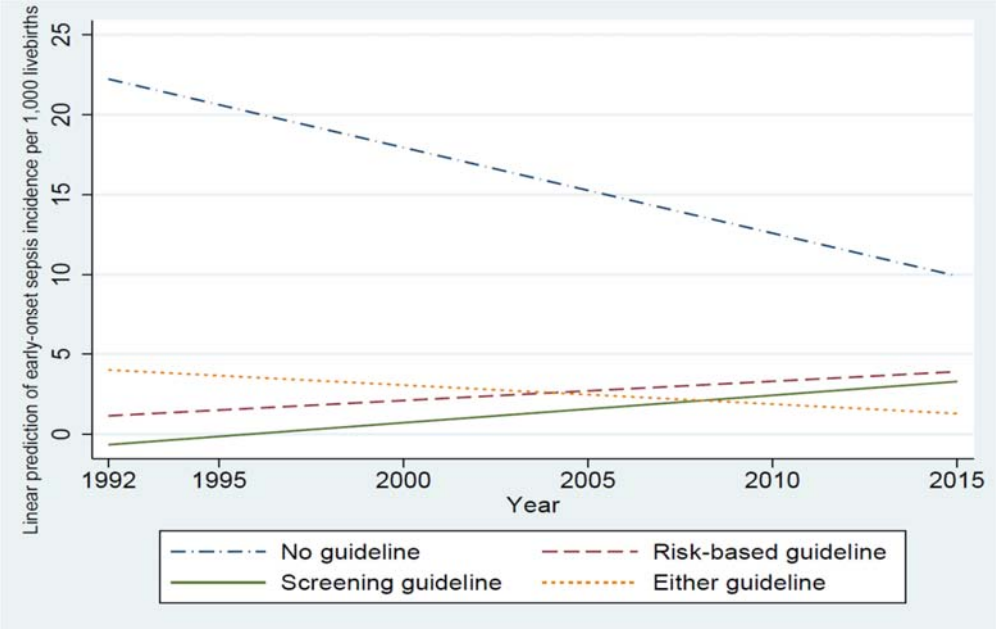


Figure 21. Unadjusted trends of annual early-onset sepsis incidence by recently reported GBS prevention strategy

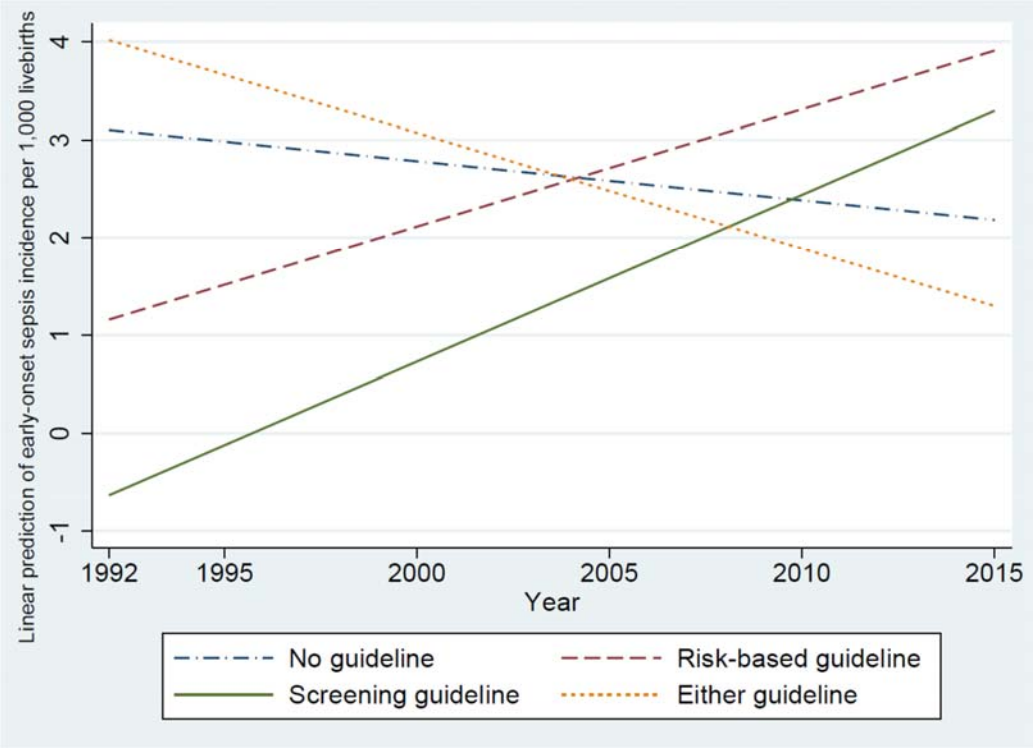


Figure 22. Unadjusted trends of annual early-onset sepsis incidence by recently reported GBS prevention strategy excluding Mexico City

Compositional covariates

Compared with North America, the early-onset sepsis incidence was 9.803 per 1,000 livebirths higher in Latin America and the Caribbean (95% CI 5.643 to 13.963), though this may have been because of Mexico City (see Table 39). As low birthweights increased by one percent, the rate of early-onset sepsis incidence increased by 0.641 per 1,000 livebirths (95% CI 0.446 to 0.836). Compared with national and mandatory surveillance, early-onset sepsis incidence per 1,000 livebirths was higher in surveillance from one centre (4.550 95% CI 1.745 to 7.354 and 6.235 95% CI 1.615 to 10.892, respectively). By contrast, early-onset sepsis incidence decreased by 44.525 as the human development index increased by one unit (95% CI -59.338 to -29.711). Early-onset sepsis also decreased by 0.311 per 1,000 livebirths (95% CI -0.423 to -0.200) when maternal GBS colonisation increased by one percent; by 0.483 per 1,000 livebirths (95% CI -0.667 to -0.299) when prolonged rupture of membranes increased by one percent; and by 0.846 (95% CI -1.322 to -0.370) when intrapartum fever increased by one percent. This may be a result of antibiotics given for these factors. Finally, compared with 2/3/4 days or less, early-onset sepsis was lower when defined as 5/6/7 days or less (-6.446 95% CI -8.936 to -3.996) or vertical onset/mother-acquired (-5.972 CI -9.522 to -2.422). The percentage of preterm births was not statistically associated with early-onset sepsis incidence.

Table 39. Unadjusted linear regression analyses of early-onset sepsis incidence per 1,000 livebirths by compositional covariate

Covariate	Early-onset sepsis coefficient (95% confidence interval)	p-value
Region		
North America	(reference)	
Asia	2.019 (-2.432 to 6.470	0.372
Europe	2.731 (-1.576 to 7.037)	0.212
Latin America and the Caribbean	9.803 (5.643 to 13.963)	0.000
North Africa and the Middle East	3.267 (-2.541 to 9.074)	0.268
Oceania	1.848 (-3.661 to 7.357)	0.509
Preterm births	-0.015 (-0.488 to 0.183)	0.370
Low birthweights	0.641 (0.446 to 0.836)	0.000
Human development index	-44.525 (-59.338 to -29.711)	0.000
Maternal GBS colonisation	-0.311 (-0.423 to -0.200)	0.000
Prolonged rupture of membranes	-0.483 (-0.667 to -0.299)	0.000
Intrapartum fever	-0.846 (-1.322 to -0.370)	0.001
Surveillance type		
Mandatory population surveillance	(reference)	
Multiple centres/counties	1.958 (-3.002 to 6.917)	0.437
One centre	6.253 (1.615 to 10.892)	0.009
Geographical coverage		
National	(reference)	
Regional	-1.703 (-6.707 to 3.301)	0.502
City/town wide	3.727 (-5.391 to 12.846)	0.421
One centre in a city/town	4.550 (1.745 to 7.354)	0.002
Early-onset sepsis definition		
2/3/4 days or less	(reference)	
5/6/7 days or less	-6.466 (-8.936 to -3.996)	0.000
Vertical onset/Mother-acquired	-5.972 (-9.522 to -2.422)	0.001

12.3.4 Adjusted linear regression analysis (objective e)

This section presents the results of the linear regression analysis showing the relationship between the most recently reported GBS prevention strategy and the trends of annual early-onset sepsis incidence adjusted for the compositional covariates (objective e).

Given the results of the unadjusted linear regressions and the *a priori* list, I included all of the covariates in the initial adjusted analysis. As I removed statistically non-significant covariates ($p>0.05$), this left the minimal and final model shown in Table 38. As shown in Figure 23, when the compositional covariates were accounted for, the trends in annual early-onset sepsis incidence decreased in 'either prevention' areas, increased in no prevention and screening prevention areas and remained stable in risk-based prevention areas. However, compared with screening prevention areas, the trends of annual early-onset sepsis in any of the other prevention areas were not statistically different (see Table 38). In contrast with the unadjusted analysis, the difference in the trends in annual early-onset sepsis incidence between no prevention and screening prevention areas lost statistical significance (0.002 95% CI -0.834

to 0.838). These results did not change when Mexico City was removed from the model. The mean adjusted R^2 for this model was 82%. As the adjusted relationship between universal GBS screening and the trend of annual early-onset sepsis incidence across time was not statistically different to other prevention strategies, I did not explore sensitivity analyses (objective f).

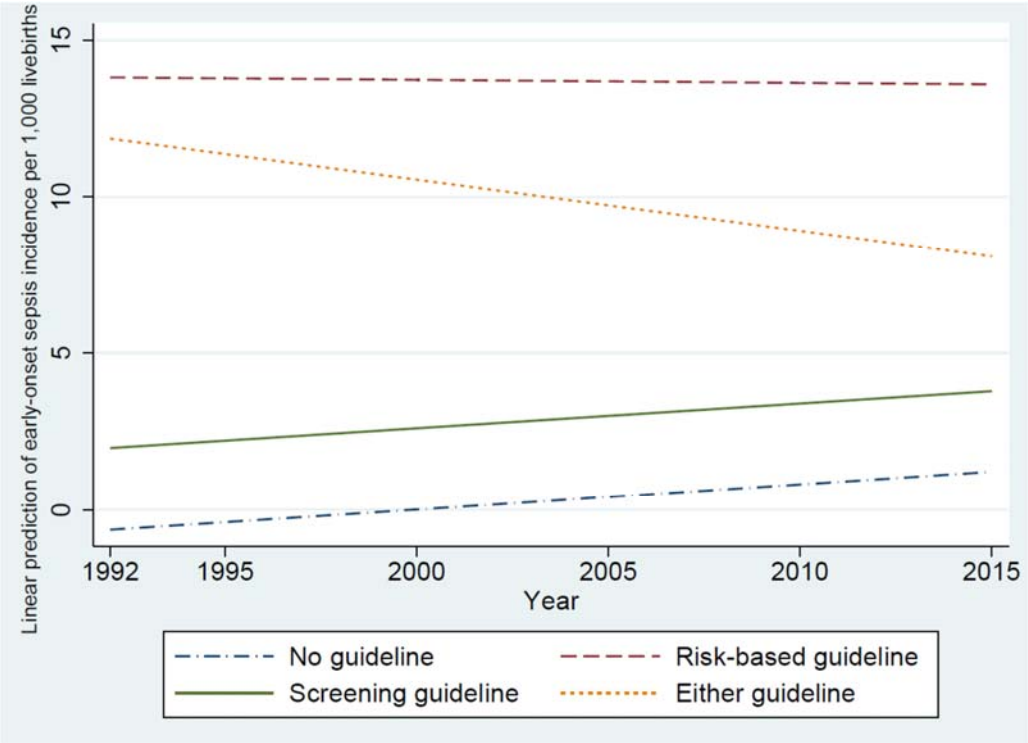


Figure 23. Adjusted trends of annual early-onset sepsis incidence by recently reported GBS prevention strategy

12.4 Discussion

In this discussion, I will summarise the findings of this study and compare the findings with previous literature. In Chapter 14, I will discuss the strengths and the limitations as well as the research and policy implications related to this study.

12.4.1 Principal findings

In this study, I aimed to investigate whether GBS prevention strategies, particularly universal screening, have an impact on the trends of annual early-onset sepsis incidence across countries. The key finding was that international trends of early-onset sepsis are unrelated to

any of the prevention strategies. Based on the available evidence, the predicted values showed that early-onset sepsis increased from approximately 2.5 to 4 per 1,000 livebirths in screening prevention areas and from approximately -0.1 to 1.0 per 1,000 livebirths in no prevention areas, during the study period. In risk-based prevention areas, the predicted values showed that early-onset sepsis remained stable across time whereas in 'either prevention' areas it decreased. However, none of these differences between the different prevention areas and screening areas were statistically significant.

There are many possible explanations for the findings of this study. It could be that the most recent GBS prevention strategy has no relationship with the trends in early-onset sepsis. While universal GBS screening might reduce EOGBS incidence (as shown in some of the results in Chapter 11), it may have no impact on the trends of overall early-onset sepsis. The differences in the findings between the two chapters may reflect IAP in the blood preventing GBS detection or IAP producing changes in the organisms causing early-onset sepsis. However, the results of this study could equally be due to the lack of statistical power from the small sample size in this study. As identified in Chapter 9, 63 observations in each prevention strategy provided an 80% chance to detect a change of 0.1 per 1,000 livebirths. Accounting for the imbalances between the observations in each prevention group, the sample size achieved was 20 per prevention group for the comparison of either *versus* screening prevention, 41 for risk-based *versus* screening prevention, and 48 for no *versus* screening prevention. With these sample sizes, there was only a 50% or lower chance of observing a statistically significant annual difference of 0.1 per 1,000 livebirths between the interventions. The annual differences found between the prevention strategies were below 0.1 per 1,000 livebirths and, therefore, the sample size may have been too small for these differences to be statistically significant.

To conclude, the results indicate that universal GBS screening or other prevention strategies may not have an impact on the trends of annual early-onset sepsis incidence. However, this study was statistically under-powered and the resulting findings are uncertain. Alternative results might be found if a larger sample size were analysed.

12.4.2 Comparison with previous literature

While the results of this study are uncertain, there is evidence that supports the finding that GBS screening does not reduce neonatal sepsis. For example, the first multi-state population study exploring the rates of early-onset sepsis in the US under universal screening found that the incidence remained constant at around 0.77 per 1,000 livebirths between 2005 and 2008.[175]

Another multi-state US study published in 2016 also reported a stable incidence of early-onset sepsis between 0.77 and 0.79 per 1,000 livebirths from 2005 to 2014.[87] In the UK, a multi-centre study reported that the incidence of early-onset sepsis in NICU admissions was steady under risk-based prevention, with no differences across 2006, 2007, and 2008 ($p>0.1$).[99] Earlier multi-centre studies in the US during the 1990s where either risk-based or screening strategies were implemented have shown similar stable trends.[406, 407] However, all of these neonatal sepsis trends were during periods of one particular GBS prevention strategy and did not compare the trends in one strategy with another.

One of the early multi-centre studies compared a period of no prevention (1991 to 1993) with risk-based or screening prevention (1998 to 2000) in 15 centres across the US, finding no difference between the periods (19.3 per 1,000 livebirths in the no prevention period and 15.4 per 1,000 livebirths in the risk-based or screening period).[20] However, this study was only in the low birthweight population (<1500g) and may not be generalisable to all neonates. On the other hand, another study in the US found that the average annual percent decrease in neonatal sepsis hospitalisations for term infants during 1996 to 2001 when risk-based prevention or universal screening were recommended was statistically significant (-3.6%, 95% CI -5.1 to 2.0%), however after 2002 when only universal screening was introduced the average annual percent change was not statistically significant.[174] Although these results are in contrast to those found in this study, the authors of this study did not adjust the analysis for any confounding factors beyond the GBS prevention strategy which might be the reason for the differences in results. Alternatively, it may because this study lacked statistical power in this study or that this analysis was on all neonatal sepsis. Indeed, when using a proxy for early-onset sepsis (term infants diagnosed with sepsis during delivery with admission and discharge within ten days of birth), the authors found no difference in the average annual percent change between 1988 and 2006. However, when comparing the average incidence of 'early-onset' sepsis hospitalisations per 1,000 livebirths in the periods of universal screening (10.3) and 'either prevention' (11.4) with no prevention (14.3), the differences were statistically significant. Annual differences in early-onset sepsis may not be large enough to reach statistical significance.

Trends in early-onset sepsis might not reduce in screening programmes as widespread IAP may decrease EOGBS, yet increase gram-negative organisms causing sepsis, resulting in no overall reduction. There has been increasing evidence investigating sepsis caused by *E. coli,* the largest gram-negative organism causing neonatal sepsis. Two US studies in neonates with low birthweight found that early-onset *E. coli* increased in risk-based and screening periods compared with no prevention,[20, 187] For example, early-onset *E. coli* increased from 2.83 per

1,000 NICU admissions during no prevention to 7.12 during risk-based prevention and 10.22 during screening.[187] By contrast, studies in all (term and preterm) neonates have not found changes in early-onset *E. coli* incidence during the period of GBS prevention,[406, 407] [87] while others have found that IAP usage increased the odds of early-onset *E. coli* infection in unadjusted but not adjusted analyses when factors such as gestational age were accounted for.[188, 189] To inform whether early-onset sepsis trends are unaffected by GBS prevention strategies as a result of increasing gram-negative organisms, an investigation comparing *E. coli* in countries with different GBS prevention strategies will be investigated in Chapter 13.

The results of this study did not clarify the conflicting findings in the literature on the impact of universal GBS screening or other GBS prevention strategies on the trends of annual early-onset sepsis. As shown in this study and in the literature, it is possible that early-onset sepsis incidence may not decrease under screening, however, this could be due to factors beyond the GBS prevention strategy.

12.5 Conclusions for this chapter

- The findings in this chapter are suggestive of universal GBS screening having no impact on the trends of annual early-onset sepsis incidence compared with risk-based prevention, 'either prevention' and no prevention.
- One of the reasons for a stable trend in early-onset sepsis under GBS screening may be that, although EOGBS incidence decreases, the incidence of gram-negative sepsis, such as *E. coli,* increases.
- Importantly, this study did not have a sufficient sample size, therefore the findings might be a result of low statistical power as opposed to no true difference.
- The evidence remains inconclusive and larger sample sizes are required to determine the impact of universal GBS screening on early-onset sepsis trends across countries.

13. THE IMPACT OF UNIVERSAL GBS SCREENING ON THE TRENDS OF LOGBS, EARLY-ONSET *E. COLI*, AND NEONATAL GBS ANTIBIOTIC RESISTANCE

This chapter presents the results for the final ecological trend analysis study that combines international data to explore the impact of universal GBS screening and widespread IAP on the trends of six potential harms, compared with other GBS prevention strategies. While I discussed the methodology used to address this study in Chapter 9, here I will first present the study specific aim and objectives followed by some study specific methodological procedures. I will then present the detailed results from the statistical analyses: first the MICE imputation results, then the descriptive statistics, followed by the unadjusted analyses, the main adjusted model and the sensitivity analyses. Finally, I will summarise the principal findings and how they relate to previous literature.

13.1 Aims and objectives

The aim of this chapter is to measure the effect of universal GBS screening and widespread IAP on the trend of six potential harms across time, compared with other GBS prevention strategies, in a statistical model that combines data from geographical areas with different prevention strategies, and adjusts for compositional differences between the areas. The six potential harmful outcomes investigated were: annual LOGBS incidence, annual early-onset *E. coli* incidence, the annual percentage of EOGBS cases resistant to clindamycin, the annual percentage of EOGBS cases resistant to erythromycin, the annual percentage of neonatal GBS cases resistant to clindamycin and the annual percentage of neonatal GBS cases resistant to erythromycin.

The research objectives are to:

a) Describe the frequency of the GBS prevention strategy as well as the mean or frequency of the compositional covariates in general (irrespective of harms);
b) Describe the mean for each harmful outcome across time, geographical areas, world regions and GBS prevention strategies;

c) Investigate the unadjusted relationship between universal GBS screening and the trend of each harmful outcome across time compared with other prevention strategies, using linear regression;
a) Investigate the unadjusted relationship between each compositional covariate and the mean of each harmful outcome across time, using linear regression;
b) Investigate the relationship between universal GBS screening and the trend of each harmful outcome across time compared with other prevention strategies, using linear regression and adjusting for the compositional covariates; and
d) Examine the stability of the adjusted relationship between universal GBS screening and the trend of each harmful outcome across time compared with other prevention strategies in a range of sensitivity analyses, if the relationships were statistically significant.

13.2 Methods

I have detailed the methodology used for this study in Chapter 9. Here, I describe the data selected to perform the analysis.

13.2.1 Geographical data included

As discussed in Chapter 10, there were annual LOGBS data from 47 geographical areas. However, some of them overlapped in terms of coverage, giving rise to the potential of double-counting. These were: voluntary *versus* enhanced surveillance from England, two hospitals from Johannesburg *versus* data from the township of Soweto, data from multiple centres *versus* enhanced surveillance in New Zealand, two different multi-centre sources for Australia, and enhanced surveillance from the United Kingdom and Republic of Ireland *versus* data from each of the five countries in the British Isles. I included the following data sources (and excluded the others): the enhanced surveillance for England and individual UK countries instead of the United Kingdom and Republic of Ireland overall as the EOGBS incidence across the countries varies; Soweto over the two hospitals in Johannesburg for population and wider coverage; the enhanced surveillance for New Zealand; and the multi-centre data that had a larger number of years for Australia. This left 42 geographical areas. However, after analysing the LOGBS incidence across the years and countries, I excluded Mansoura City in Egypt where the rate of LOGBS incidence for 2014 was 24.15 per 1,000 livebirths. This was an

extreme outlier and was vastly different from the incidence rates in the UK. This left data from a total of 41 geographical areas in the analyses.

For early-onset *E. coli,* data were available from 28 geographical areas and the areas with overlapping data were: Ontario that overlapped with multi-centre data across Canada and data from multiple centres *versus* enhanced surveillance in New Zealand. I included Ontario as it had population-based surveillance, and the enhanced surveillance for New Zealand (and excluded the others). This left 26 geographical areas. However, I excluded Mansoura City in Egypt where the rate of early-onset *E. coli* incidence for 2014 was 28.99 per 1,000 livebirths as it was an extreme outlier from the remaining data and was vastly different from the incidence rates in the UK. This left data from a total of 25 geographical areas in the analyses.

For EOGBS cases resistant to clindamycin, data were available from 23 areas and for EOGBS cases resistant to erythromycin, data were available from 24 areas. For neonatal GBS cases resistant to clindamycin, data were available from 17 areas and for neonatal GBS cases resistant to erythromycin data were available from 19 areas. For all, data only overlapped for Alberta and one or two sources of multi-centre data across Canada. I chose Alberta as the data were from mandatory population surveillance. This left data from a total of 21 geographical areas for EOGBS cases resistant to erythromycin, 22 areas for EOGBS cases resistant to erythromycin, 16 areas for neonatal GBS cases resistant to clindamycin and 18 areas for neonatal GBS cases resistant to erythromycin.

13.3 Results

13.3.1 Multiple imputation for compositional covariates

Table 40 summarises of the proportion of missing data for each compositional covariate in each dataset. There were 63 (20%) observations that had complete compositional covariate for every year in the dataset on LOGBS, 43 (21%) on *E. coli,* 19 (16%) on EOGBS clindamycin resistance, 19 (17%) on EOGBS erythromycin resistance, 18 (16%) on neonatal GBS clindamycin resistance and 19 (17%) on neonatal GBS erythromycin resistance. The covariate with the largest number of missing years across the datasets was the human development index, which had 51% to 61% of years missing. The covariate with the least amount of missing data was skilled delivery which had 11% to 17% of years missing. There were more than 20 different patterns of missing data for the LOGBS dataset, 18 for *E. coli*, 22

for EOGBS clindamycin resistance and erythromycin resistance, and 15 for neonatal GBS clindamycin and erythromycin resistance.

Across all datasets, the missingness of each compositional covariate was related to at least one variable. The exception was for the missingness of skilled delivery which did not converge in the early-onset *E. coli* dataset or the EOGBS and neonatal clindamycin and erythromycin datasets, and caesarean section which did not converge in the EOGBS and neonatal clindamycin and erythromycin datasets. As data were missing because they were available from some years but not others and their missingness was related to other data, the mechanism for the missing data was 'missing at random'.

As mentioned in Chapter 9, I set the number of imputations at 100 because of the large amount of missing data and the results showed that convergence was reached within these imputations. Additionally, the largest fraction of missing information ($_{FMI}$) from of the analysis models was below 79%, confirming that the number of imputations was sufficient. Below, I present the descriptive statistics of some of the imputed datasets and the original dataset for comparison.

Table 40. Proportion of missing data in compositional covariates for each dataset

Variable	LOGBS		Early-onset *E. coli*		Early-onset GBS				Neonatal GBS			
					clindamycin resistance		erythromycin resistance		clindamycin resistance		erythromycin resistance	
	Observed (%)	Missing (%)	Observed (%)	Missing (%)	Observed (%)	Missing (%)	Observed (%)	Missing (%)	Observed (%)	Missing (%)	Observed (%)	Missing (%)
Preterm births	153 (49)	158 (51)	100 (48)	109 (52)	59 (49)	62 (51)	59 (51)	56 (49)	63 (57)	47 (43)	64 (59)	45 (41)
Low birthweights	153 (51)	158 (49)	119 (57)	90 (43)	78 (64)	43 (36)	72 (63)	43 (37)	77 (70)	33 (30)	75 (69)	34 (31)
Caesarean section	257 (83)	54 (17)	165 (79)	44 (21)	108 (89)	13 (11)	102 (89)	13 (11)	102 (93)	8 (7)	100 (92)	9 (8)
Skilled attendance at delivery	276 (89)	35 (11)	178 (85)	31 (15)	102 (84)	19 (16)	96 (83)	19 (17)	92 (84)	18 (16)	91 (83)	18 (17)
Human development index	120 (39)	191 (61)	84 (40)	125 (60)	55 (45)	66 (55)	50 (43)	65 (57)	44 (40)	66 (60)	43 (39)	66 (61)

E. coli Escherichia coli, GBS group B *Streptococcus*, LOGBS late-onset GBS

13.3.2 Descriptive analysis for the predictor and compositional covariates (objective a)

This section presents the frequency of the GBS prevention strategy and the mean or frequency of the compositional covariates for each outcome dataset (objective a).

The majority of geographical areas across the datasets most recently reported a universal screening strategy 39% to 56% areas), followed by risk-based prevention (21% to 37%), no prevention (12% to 19%) and 'either prevention' (0% to 4%) (see Table 41). With respect to world region, the majority of observations were from Europe across all datasets (36.84% to 51.44%). The world region with the fewest observations varied across datasets. For LOGBS incidence, the fewest observations were from North Africa and the Middle East (0.96%); for early-onset *E. coli* incidence, there were no data from Sub-Saharan Africa followed by Oceania (5.74%); for EOGBS resistance, the fewest observations were also from Oceania (0.83% for clindamycin and 0.87% for erythromycin); and for neonatal GBS resistance they were from North Africa and the Middle East (0.91% for clindamycin and 0.92% for erythromycin).

Table 41. Frequencies of recently reported GBS prevention strategy for each dataset

Most recent reported GBS prevention strategy	Dataset				
	LOGBS (%)	***E. coli* (%)**	**EOGBS clindamycin and erythromycin (%)**	**Neonatal GBS clindamycin (%)**	**Neonatal GBS erythromycin (%)**
No prevention	6 (13.04)	4 (12.12)	4 (18.18)	3 (18.75)	3 (16.67)
Risk-based prevention	17 (36.96)	7 (21.21)	8 (36.36)	4 (25.00)	5 (27.78)
Screening prevention	21 (45.65)	13 (39.39)	10 (45.45)	9 (56.25)	10 (55.56)
Either prevention	2 (4.35)	1 (3.03)	0 (0.00)	0 (0.00)	0 (0.00)
Total	46 (100.00)	33 (100.00)	22 (100.00)	16 (100.00)	18 (100.00)

E. coli Escherichia coli, GBS group B *Streptococcus*, LOGBS late-onset GBS

The mean values for the compositional covariates that were multiple imputed for each dataset are presented in Table 42. Averages are provided for the data with no imputation, followed by imputed datasets 1 and 100. The mean percentage of preterm births across the years in the original datasets ranged from 9.39% (SD 4.22) to 10.32% (SD 4.61) and the imputed means were from 8.71% (SD 3.52) to 9.40% (SD 3.89). The mean percentage of low birthweights ranged from 9.88% (SD 7.26) to 11.94% (SD 7.86) and the imputed mean values were from 8.56% (SD 6.09) to 10.46% (SD 7.36). The mean percentage of caesarean section deliveries

ranged from 26.62% (SD 8.74) to 28.33% (SD 9.31) and the imputed mean values ranged from 25.97% (SD 8.87) to 27.78% (SD 8.57). The mean skilled attendance at delivery ranged from 96.58% (SD 6.26) to 97.54% (SD 5.77) and the imputed mean values ranged from 96.71% (SD 5.81) to 97.67% (SD 5.47). Finally, the mean human development index across the data ranged from 0.82 (SD 0.08) to 0.83 (0.09) and the imputed mean values also ranged from 0.82 to 0.83. Similar to the previous multiple imputations, in general, the imputed mean values for the human development index were the closest to the original mean, whereas, the mean values for low birthweights were the furthest from the original mean.

Table 42. Mean values for multiple imputed compositional covariates for each dataset

Dataset	Mean % preterm births (SD)	Mean % low birthweights (SD)	Mean % Caesarean delivery (SD)	Mean % skilled attendance at delivery (SD)	Mean HDI (SD)
LOGBS					
Original	9.39 (4.22)	9.88 (7.26)	27.09 (10.11)	97.54 (5.77)	0.83 (0.09)
Imputed 1	8.71 (3.52)	8.63 (5.92)	26.72 (10.11)	97.60 (5.49)	0.83 (0.08)
Imputed 100	8.83 (3.49)	8.56 (6.09)	26.55 (10.12)	97.67 (5.47)	0.83 (0.08)
E. coli					
Original	9.83 (4.30)	10.29 (7.53)	28.33 (9.31)	97.28 (6.74)	0.82 (0.08)
Imputed 1	9.12 (3.53)	9.24 (6.58)	26.75 (9.30)	97.56 (6.26)	0.82 (0.07)
Imputed 100	9.09 (3.34)	8.96 (6.63)	26.70 (9.29)	97.62 (6.27)	0.82 (0.08)
EOGBS clindamycin resistance					
Original	9.93 (4.46)	11.94 (7.86)	27.87 (8.42)	97.04 (5.70)	0.82 (0.08)
Imputed 1	9.08 (3.53)	10.30 (7.37)	27.34 (8.15)	97.32 (5.33)	0.82 (0.08)
Imputed 100	9.11 (3.61)	10.33 (7.30)	27.49 (8.21)	97.42 (5.32)	0.83 (0.08)
EOGBS erythromycin resistance					
Original	10.02 (4.46)	11.65 (7.91)	27.84 (8.76)	96.58 (6.26)	0.82 (0.08)
Imputed 1	9.24 (3.72)	10.06 (7.27)	27.78 (8.57)	96.71 (5.81)	0.83 (0.07)
Imputed 100	9.28 (3.72)	10.13 (7.17)	27.33 (8.79)	97.03 (5.84)	0.83 (0.08)
Neonatal GBS clindamycin resistance					
Original	10.28 (4.63)	11.46 (7.88)	26.62 (8.74)	97.18 (5.45)	0.82 (0.08)
Imputed 1	9.28 (3.91)	9.93 (7.44)	25.97 (8.87)	97.57 (5.07)	0.82 (0.08)
Imputed 100	9.18 (4.02)	10.46 (7.36)	27.01 (8.85)	97.25 (0.11)	0.82 (0.08)
Neonatal GBS erythromycin resistance					
Original	10.32 (4.61)	11.81 (7.92)	26.77 (8.97)	97.15 (5.48)	0.82 (0.08)
Imputed 1	9.38 (4.14)	10.18 (7.60)	26.90 (9.11)	96.94 (5.23)	0.82 (0.09)
Imputed 100	9.40 (3.89)	10.19 (7.52)	26.65 (8.94)	97.33 (5.04)	0.82 (0.09)

E. coli Escherichia coli, GBS group B *Streptococcus*, HDI human development index, LOGBS late-onset GBS

The mean values for compositional covariates that only had one value across all of the years and were not multiple imputed are presented in Table 43. Across the datasets, on average mothers were 26 years old at first child and had an average of 1.83 (SD 0.48) to 1.92 (SD

0.47) children. Multiple or twin births ranged from 16.41% (SD 7.96) to 18.16% (SD 14.34), maternal colonisation of GBS from 16.48% (SD 9.70) to 21.02% (SD 10.68), mean percentage of prolonged rupture of membranes from 5.85% (SD 6.17) to 6.80% (SD 6.20) and intrapartum fever from 1.21% (SD 1.70) to 1.58% (SD 2.32). Mean government expenditure on health *per capita* ranged from $2,610.91 (SD 1,523.16) to $3,348.76 (SD 2,163.51). The most frequent GBS serotype was serotype III (72.73% to 84.45%) and most data covered one centre only (44.53% to 62.68%). The commonest definition for LOGBS was 5/6/7/8 days onwards (67.19%), for *E. coli* it was <5/6/7/8 days (51.20%), for EOGBS it was <5/6/7 days (74.38% for clindamycin and 72.17% for erythromycin), and for neonatal GBS definition it was <89/90/132 days (50% for clindamycin and 51.38% for erythromycin).

Table 43. Mean values for un-imputed compositional covariates for each dataset

Covariate	Dataset					
	LOGBS (%)	***E. coli* (%)**	**EOGBS clindamycin (%)**	**EOGBS erythromycin (%)**	**Neonatal GBS clindamycin (%)**	**Neonatal GBS erythromycin (%)**
Continuous variables	*Mean (SD)*					
Fertility rate	1.86 (0.44)	1.86 (0.49)	1.89 (0.47)	1.92 (0.47)	1.83 (0.48)	1.84 (0.47)
Average maternal age	26.11 (3.41)	25.71 (3.82)	25.86 (3.38)	25.83 (3.45)	26.02 (3.50)	26.10 (3.48)
Multiple or twin births (per 1,000 livebirths)	18.16 (14.34)	17.59 (9.56)	16.41 (7.96)	16.85 (7.94)	16.86 (9.54)	17.03 (9.48)
Per capita government expenditure on health (PPP int $)	3,348.76 (2,163.51)	2,697.45 (1,694.25)	2610.91 (1523.16)	2,694.58 (1,516.29)	2,631.35 (1,627.50)	2,668.64 (1,609.80)
Maternal GBS colonisation	21.02 (10.68)	17.28 (9.38)	16.48 (9.70)	17.52 (9.34)	16.88 (9.39)	17.34 (9.41)
Prolonged rupture of membranes	6.80 (6.20)	5.85 (6.17)	6.13 (5.98)	5.98 (5.95)	6.41 (6.80)	6.10 (6.56)
Intrapartum fever	1.28 (1.95)	1.58 (2.32)	1.25 (1.73)	1.29 (1.78)	1.21 (1.70)	1.22 (1.71)
Categorical variables	*Frequency (%)*					
Most prevalent GBS serotype						
Ia	31 (8.07)	10 (4.78)	8 (6.61)	8 (6.96)	8 (7.27)	8 (7.34)
Ib	65 (16.93)	36 (17.22)	23 (19.01)	17 (14.78)	7 (6.36)	7 (6.42)
III	285 (74.22)	160 (76.56)	88 (72.73)	87 (75.65)	94 (85.45)	93 (85.32)
V	3 (0.78)	3 (1.44)	2 (1.65)	3 (2.61)	1 (0.91)	1 (0.92)
Surveillance type						
Mandatory or enhanced population surveillance	70 (18.23)	1 (0.48)	12 (9.92)	13 (11.30)	11 (10.00)	12 (11.01)
Voluntary population surveillance	74 (19.27)	52 (24.88)	32 (26.45)	32 (27.83)	32 (29.09)	32 (29.36)
Multiple centres/counties	69 (17.79)	25 (11.96)	2 (1.65)	2 (1.74)	1 (0.91)	2 (1.83)
One centre	171 (44.53)	131 (62.68)	75 (61.98)	68 (59.13)	66 (60.00)	63 (57.80)
Geographical coverage						
National	165 (42.97)	57 (27.27)	34 (28.10)	35 (30.43)	33 (30.00)	35 (32.11)
Regional	39 (10.16)	18 (8.61)	12 (9.92)	12 (10.43)	11 (10.00)	11 (10.09)
City/town wide	9 (2.34)	3 (1.44)	-	-	-	-
One centre in a city/town	171 (44.53)	131 (62.68)	75 (61.98)	68 (59.13)	66 (60.00)	63 (57.80)
EOGBS definition						
1	108 (28.13)	96 (45.93)	31 (25.62)	32 (27.83)	17 (15.45)	17 (15.60)
2	258 (67.19)	107 (51.12)	90 (74.38)	83 (72.17)	55 (50.00)	56 (51.38)
3	16 (4.17)	6 (2.87)	-	-	-	-
4	2 (0.52)	-	-	-	38 (34.55)	36 (33.03)

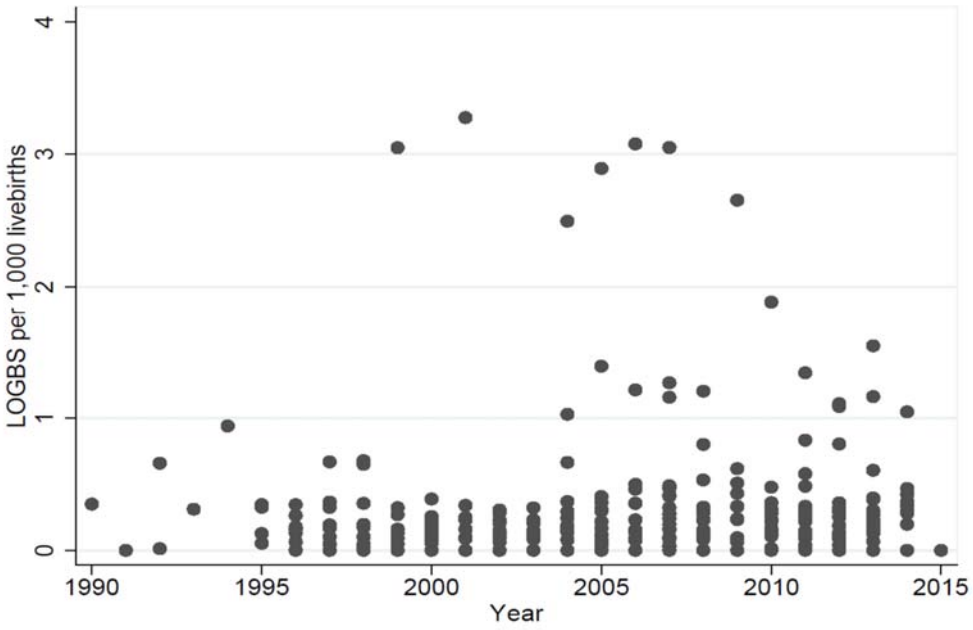
E. coli Escherichia coli, GBS group B *Streptococcus*, LOGBS late-onset GBS
LOGBS definition: 1, 2/3/4 days onwards, 2, 5/6/7/8 days onwards, 3, 48 hours to 6 days, 4, Not stated; *E. coli* definition 1, <2/3/4 days, 2, <5/6/7/8 days, 3, mother infected; Early-onset GBS definition 1, 2/3 days or less, 2, 5/6/7 days or less; Neonatal GBS definition 1, <28/30/31/44 days, 2, <89/90/132 days, 4 Not stated

13.3.3 Descriptive analysis for outcomes (objective b)

This section presents the mean values for each outcome across time, geographical areas, world regions and GBS prevention strategies (objective b).

LOGBS incidence

The mean LOGBS incidence across 311 observations (41 geographical areas, 26 years between 1990 and 2015) was 0.29 per 1,000 livebirths (SD 0.49, range 0.00 to 3.28). Across the years, LOGBS incidence varied between 0.00 and 0.94 per 1,000 livebirths (see Table 44). Figure 24 demonstrates that LOGBS increased across the years and that the range of LOGBS incidence was wider during the latter years. As evident from Table 44, this could be as a result of more geographical areas contributing to the later years compared with the earlier years.



LOGBS early-onset group B *Streptococcus* disease
Each dot represents the LOGBS incidence for one year for one geographical area

Figure 24. Scatterplot of LOGBS incidence by year

Table 44. LOGBS incidence per 1,000 livebirths across the years

Year	Number of geographical areas	Mean livebirths (Standard deviation)	Mean LOGBS incidence (Standard deviation)
1990	1	-	0.35
1991	1	3,048.00	0.00
1992	2	125,055.00 (172,563.80)	0.34 (0.46)
1993	1	3,211.00	0.31
1994	1	3,187.00	0.94
1995	4	65,607.75 (87,745.83)	0.21 (0.15)
1996	7	108,579.50 (160,043.80)	0.16 (0.12)
1997	9	135,492.70 (158,860.40)	0.21 (0.21)
1998	9	139,151.10 (165,591.30)	0.25 (0.26)
1999	10	141,895.10 (178,065.50)	0.42 (0.93)
2000	15	148,593.60 (200,307.40)	0.16 (0.11)
2001	10	163,196.50 (190,437.60)	0.48 (0.99)
2002	11	149,873.00 (181,548.60)	0.14 (0.10)
2003	14	125,212.30 (174,464.60)	0.13 (0.10)
2004	15	120,529.90 (176,675.60)	0.42 (0.63)
2005	17	107,191.90 (170,848.10)	0.38 (0.73)
2006	18	103,838.50 (170,763.30)	0.40 (0.73)
2007	19	98,528.13 (165,631.40)	0.48 (0.71)
2008	19	94,391.52 (169,069.50)	0.25 (0.31)
2009	19	92,636.31 (166,652.50)	0.31 (0.60)
2010	21	89,700.53 (157,026.10)	0.24 (0.40)
2011	25	75,302.52 (142,072.70)	0.24 (0.31)
2012	23	81,802.23 (147,133)	0.25 (0.33)
2013	25	72,104.59 (139,968.60)	0.24 (0.38)
2014	14	98,904.64 (210,339.50)	0.27 (0.28)
2015	1	2,195.00	0.00
Total	311	103,862.30 (162,717.10)	0.29 (0.49)

LOGBS early-onset group B *Streptococcus* disease

The highest mean LOGBS incidence was found in Soweto in South Africa at 1.20 per 1,000 livebirths (SD 0.13), followed by Mexico City at 0.99 per 1,000 livebirths (1.40), and Guangzhou in China at 0.97 per 1,000 livebirths (SD 0.45). Brno, Cordoba, Manila, Podgorica, Riga, Slovenia, Tunis and Zagreb reported zero cases, which may be a result of diagnostic related issues, small sample size or voluntary surveillance. Besides these areas, the lowest LOGBS incidences were in New Zealand at 0.02 per 1,000 livebirths, Kuala Terengganu at 0.03 per 1,000 livebirths (0.04), and Ho Chi Minh at 0.04 per 1,000 livebirths (0.03) (see Table 45). These are averages across the years but as shown in Appendix 17-A, for many areas the rates of LOGBS fluctuated from year to year.

Table 45. LOGBS incidence per 1,000 livebirths by geographical area

Geographical area	Number of years	Mean number of livebirths (Standard deviation)	Mean LOGBS incidence (Standard deviation)
Alberta	11	47,896.00 (4,854.45)	0.24 (0.09)
Australia	11	29,199.82 (4,802.03)	0.20 (0.12)
Bangalore	3	4,044.33 (1,605.77)	0.07 (0.11)
Barcelona	18	1,776.89 (247.95)	0.10 (0.23)
Brno	1	6,415	0.00
Cordoba	2	1,573 (152.74)	0.00
Denmark	15	63,296.47 (3,182.94)	0.13 (0.05)
Emilia-Romagna	11	38,686.09 (2,477.04)	0.30 (0.10)
England	2	668,696.00 (68,071.76)	0.31 (0.08)
Finland	19	58,658.79 (1,898.58)	0.25 (0.09)
France	18	570,199.50 (57,015.64)	0.22 (0.04)
Guangzhou	4	2,097.50 (265.54)	0.97 (0.45)
Ho Chi Minh	3	45,589.33 (5,934.90)	0.04 (0.03)
Kaunas	7	3,524.86 (194.83)	0.79 (0.90)
Kingston	16	2,744.69 (360.63)	0.33 (0.32)
Kuala Terengganu	9	12,578.56 (1,141.11)	0.03 (0.04)
Macau	10	2,667.70 (697.83)	0.68 (0.66)
Manila	1	6,682.00	0.00
Mauritius	1	12,986.00	0.39
Mexico City	19	5,055.11 (594.00)	0.99 (1.40)
Netherlands	19	190,186.70 (9,833.44)	0.10 (0.04)
New Zealand	1	127,134.00	0.02
Northern Ireland	2	24,866 (2,207.59)	0.26 (0.12)
Norway	18	59,343.44 (1,936.13)	0.22 (0.08)
Ontario	17	135,098.60 (4,563.32)	0.08 (0.03)
Panama City	2	131,288.00 (163,749.00)	0.30 (0.40)
Podgorica	2	3,216.00 (52.33)	0.00
Portugal	7	109,472.90 (4,266.57)	0.13 (0.08)
Republic of Ireland	2	65,921.50 (10,129.30)	0.27 (0.02)
Riga	1	2,060.00	0.00
Santo Domingo	1	18,000.00	0.83
Sao Paolo	1	13,749.00	0.15
Scotland	2	59,475.50 (2,795.19)	0.32 (0.15)
Slovenia	5	21,839.00 (448.82)	0.00
Soweto	6	29,064.50 (2,561.84)	1.20 (0.13)
Switzerland	3	81,901.00 (988.11)	0.13 (0.10)
Tokyo	11	1,627.73 (192.70)	0.12 (0.26)
Tunis	3	3,818.00 (115.17)	0.00
US	19	422,561.70 (52,171.40)	0.33 (0.03)
Wales	2	35,126.00 (1,715.44)	0.35 (0.16)
Zagreb	6	4,254.50 (102.26)	0.00
Total	311	103862.30 (162717.10)	0.29 (0.49)

LOGBS late-onset group B *Streptococcus* disease, US United States of America

The highest mean LOGBS incidence was reported in Sub-Saharan Africa (1.09 per 1,000 livebirths [SD 0.33]) whereas the lowest was reported in Oceania (0.19 per 1,000 livebirths [SD 0.12]) after North Africa and the Middle East that both reported zero cases (Table 46). Again, these are the averages but they vary across the years (as shown in Appendix 17-B) as do the number of geographical areas contributing to each region.

Table 46. LOGBS incidence per 1,000 livebirths by region

Region	Number of observations/ years (%)	Mean LOGBS incidence (standard deviation)
Asia	41 (13.18)	0.30 (0.50)
Europe	160 (51.44)	0.20 (0.25)
Latin America and the Caribbean	41 (13.18)	0.63 (1.02)
North Africa and the Middle East	3 (0.96)	0.00 (0.00)
North America	47 (15.11)	0.22 (0.12)
Oceania	12 (3.86)	0.19 (0.12)
Sub-Saharan Africa	7 (2.25)	1.09 (0.33)
Total	311	0.29 (0.49)

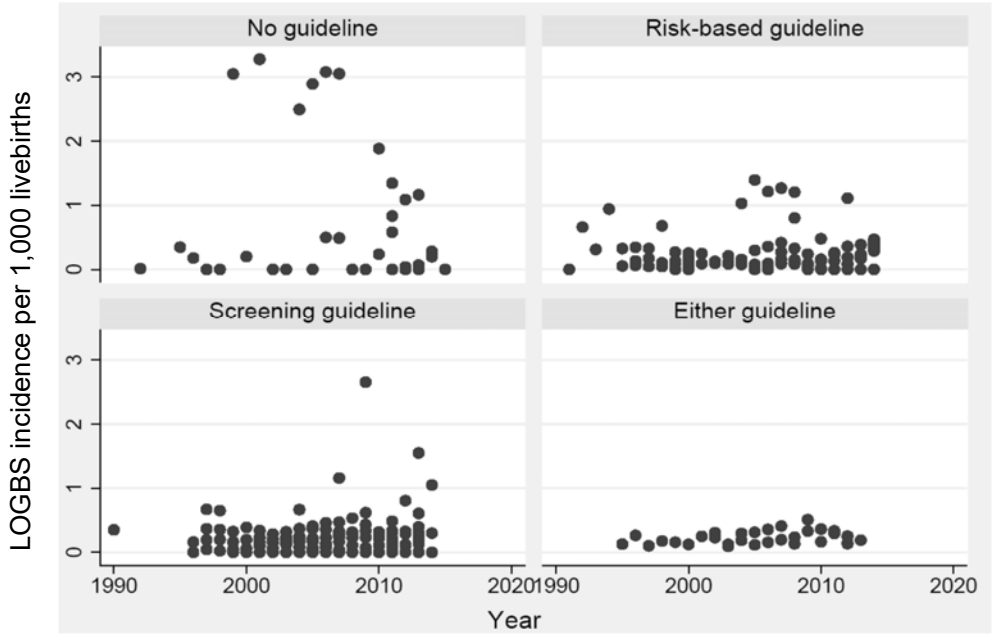
LOGBS late-onset group B *Streptococcus* disease

The mean LOGBS incidence by most recently reported GBS prevention strategy is presented in Table 47. The highest mean LOGBS incidence per 1,000 livebirths was reported under no prevention 0.72 [SD 1.09]), followed by risk-based prevention (0.24 [SD 0.30]), either risk-based or screening prevention (00.23 [SD 0.10]), and the lowest mean rate was reported under screening prevention (0.22 [SD 0.31]). In Figure 25, it is clear that under no prevention there is a random scatter of LOGBS, which is similar to risk-based prevention and 'either prevention', while under screening it appears that there may be an increase.

Table 47. LOGBS incidence per 1,000 livebirths by recently reported GBS prevention strategy

Most recent reported GBS prevention	Number of observations/ years (%)	Mean LOGBS incidence (standard deviation)
No prevention	38 (12.22)	0.72 (1.09)
Risk-based prevention	99 (31.83)	0.24 (0.30)
Screening prevention	144 (46.30)	0.22 (0.31)
Either risk-based and screening prevention	30 (9.65)	0.23 (0.10)
Total	311	0.29 (0.49)

GBS group B *Streptococcus*, LOGBS late-onset GBS

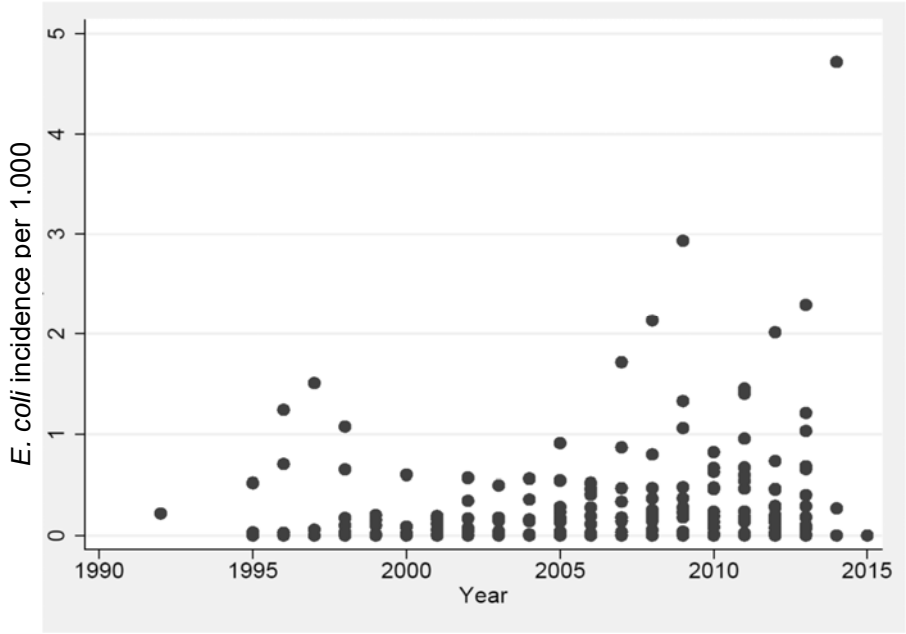


LOGBS Late-onset group B *Streptococcus*
Each dot represents the LOGBS incidence for one year for one geographical area

Figure 25. LOGBS incidence by recently reported GBS prevention strategy

Early-onset *E. coli* incidence

The mean early-onset *E. coli* incidence across 209 observations (25 geographical areas, 22 years between 1992 and 2015) was 0.29 per 1,000 livebirths (SD 0.54, range 0.00 to 4.72). Across the years, *E. coli* incidence varied between 0.00 and 0.71 per 1,000 livebirths. Figure 26 demonstrates that *E. coli* increased across the years, which could be as a result of more geographical areas contributing to the later years compared with the earlier years (see Table 48).



E. coli Escherichia coli
Each dot represents the *E. coli* incidence for one year for one geographical area

Figure 26. Scatterplot of *E. coli* incidence by year

Table 48. Early-onset *E. coli* incidence per 1,000 livebirths across the years

Year	Number of geographical areas	Mean livebirths (Standard deviation)	Mean *E. coli* incidence (Standard deviation)
1992	1	247,076.00	0.22
1995	3	66,454.67 (107,446.20)	0.18 (0.29)
1996	5	40,823.00 (83,140.41)	0.39 (0.56)
1997	6	56,736.67 (84,232.87)	0.26 (0.61)
1998	7	135,572.70 (220,338.60)	0.29 (0.41)
1999	7	133,834.70 (215,842.30)	0.07 (0.09)
2000	7	131,765.90 (210,393.30)	0.10 (0.22)
2001	6	151,850.70 (218,136.90)	0.09 (0.08)
2002	7	134,902.70 (204,644.20)	0.17 (0.21)
2003	8	120,915.60 (203,303.20)	0.11 (0.17)
2004	8	121,319.00 (209,308.10)	0.15 (0.21)
2005	11	90,234.91 (184,481.30)	0.21 (0.29)
2006	12	85,995.75 (183,266.90)	0.17 (0.20)
2007	13	81,349.69 (181,923.50)	0.29 (0.50)
2008	16	127,689.90 (25,7438.50)	0.30 (0.54)
2009	15	77,910.47 (174,012.70)	0.48 (0.79)
2010	16	82,513.25 (172,096.10)	0.25 (0.27)
2011	17	74,571.06 (167,350.00)	0.40 (0.48)
2012	18	78,634.39 (164,302.60)	0.28 (0.48)
2013	18	66,908.00 (157,958.20)	0.39 (0.60)
2014	7	5,643.27 (5,065.38)	0.71 (1.77)
2015	1	2,195.00	0.00
Total	209	91,558.10 (17,7403.70)	0.28 (0.54)

E. coli Escherichia coli

The highest mean *E. coli* incidence was reported in Guangzhou in China at 2.30 per 1,000 livebirths (SD 1.66), followed by Mexico City at 0.96 per 1,000 livebirths (SD 0.84) and Kaunas in Lithuania at 0.81 per 1,000 livebirths (SD 0.30). Bangalore, Cordoba and Riga reported zero cases (could be a result of diagnostic related issues, small sample size or voluntary surveillance). Besides these areas, the lowest LOGBS incidences were in Ontario at 0.003 per 1,000 livebirths (SD 0.004), Kuala Terengganu at 0.02 per 1,000 livebirths (SD 0.03) and the Netherlands at 0.03 per 1,000 livebirths (SD 0.02) (see Table 49). For some areas such as Barcelona, Guangzhou and Mexico City, the incidence of *E. coli* fluctuated from year to year whereas for other areas such as Bangalore, Cordoba, Kuala Terengganu and the Netherlands, they were stable across time (see Appendix 18-A).

Table 49. Early-onset *E. coli* incidence per 1,000 livebirths by geographical area

Geographical area	Number of years	Mean number of livebirths (Standard deviation)	Mean *E. coli* incidence (Standard deviation)
Australia	11	29,199.82 (4,802.03)	0.34 (0.12)
Bangalore	3	4,044.33 (1,605.77)	0.00 (0.00)
Barcelona	18	1,776.89 (247.95)	0.25 (0.33)
Buenos Aires	18	6,473.22 (923.54)	0.13 (0.12)
Cordoba	2	1,573.00 (152.74)	0.00 (0.00)
Emilia-Romagna	1	146,682.00	0.13
England	16	629,548.60 (46,740.74)	0.14 (0.04)
Guangzhou	4	2,097.50 (265.54)	2.30 (1.66)
Kaunas	7	3,524.86 (194.83)	0.81 (0.30)
Kingston	12	2,619.58 (326.72)	0.18 (0.40)
Kuala Terengganu	9	12,578.56 (1,141.11)	0.02 (0.03)
Kuwait	10	11,074.10 (486.46)	0.43 (0.28)
Macau	10	2,667.70 (697.83)	0.08 (0.17)
Mexico City	19	5,055.11 (594.00)	0.96 (0.84)
Netherlands	19	190,186.70 (9,833.44)	0.03 (0.02)
New Zealand	1	127,134.00	0.09
Ontario	17	135,098.60 (4,563.32)	0.003 (0.004)
Panama city	1	247,076.00	0.22
Portugal	6	95,824.83 (8,100.16)	0.21 (0.03)
Riga	1	2,060.00	0.00
Switzerland	3	81,901.00 (988.11)	0.04 (0.05)
Tokyo	11	1627.73 (192.71)	0.12 (0.26)
Tunis	3	3,818.00 (115.17)	0.18 (0.31)
US	1	858,000.00	0.19
Zagreb	6	4,254.50 (102.26)	0.27 (0.23)
Total	209	91,558.10 (177,403.70)	0.28 (0.54)

E. coli Escherichia coli, US United States of America

The highest mean *E. coli* incidence was reported in Latin America and the Caribbean (0.44 per 1,000 livebirths [SD 0.67]) and the lowest was reported in North America (0.01 per 1,000 livebirths [SD 0.04]) (see Table 50). Again, the rates vary across the years (in Appendix 18-B) and the number of geographical areas contributing to them.

Table 50. Early-onset *E. coli* incidence per 1,000 livebirths by region

Region	Number of observations/ years (%)	Mean *E. coli* incidence (standard deviation)
Asia	37 (17.70)	0.31 (0.87)
Europe	77 (36.84)	0.21 (0.28)
Latin America and the Caribbean	52 (24.88)	0.44 (0.67)
North Africa and the Middle East	13 (6.22)	0.37 (0.30)
North America	18 (8.61)	0.01 (0.04)
Oceania	12 (5.74)	0.32 (0.14)
Total	209 (100.00)	0.28 (0.54)

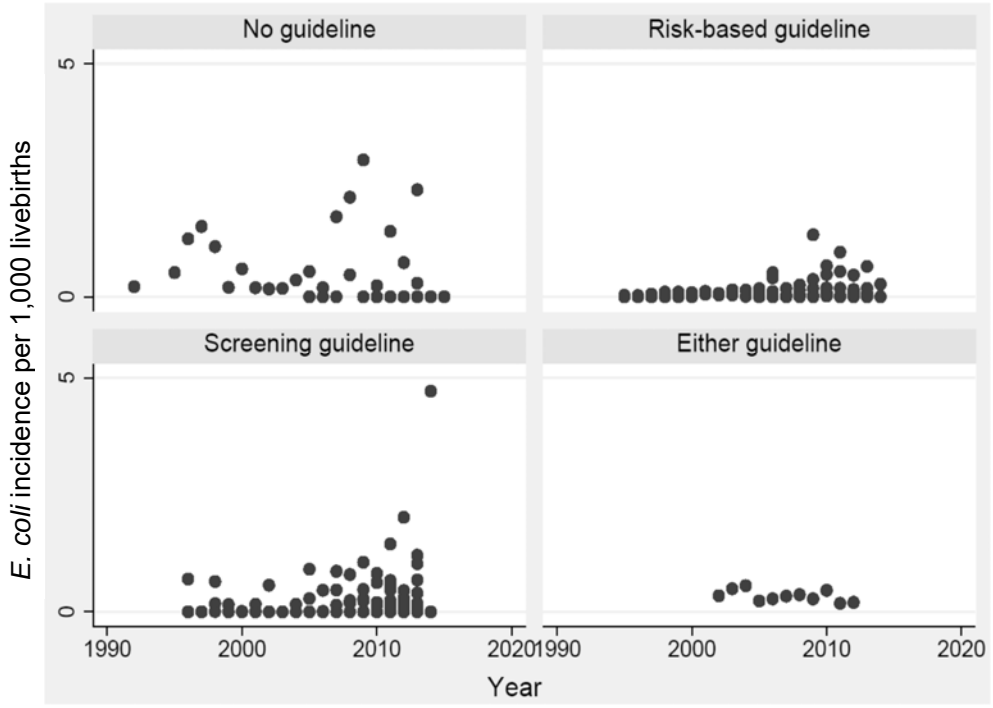
E. coli Escherichia coli

With respect to the most recent GBS prevention strategy, the highest mean *E. coli* incidence per 1,000 livebirths was reported under no prevention 0.58 [SD 0.78]), followed by 'either prevention' (0.34 [SD 0.12]), screening prevention (0.28 [SD 0.59]) and the lowest mean rate was reported under risk-based prevention (0.15 [SD 0.24]) (see Table 51). Under all strategies besides 'either prevention', early-onset *E. coli* incidence increased across time (see Figure 27).

Table 51. Early-onset *E. coli* incidence per 1,000 livebirths by recently reported GBS prevention strategy

Most recently reported GBS prevention	Number of observations/ years (%)	Mean *E. coli* incidence (sta38ndard deviation)
No prevention	33 (15.79)	0.58 (0.78)
Risk-based prevention	70 (33.49)	0.15 (0.24)
Screening prevention	95 (45.45)	0.28 (0.59)
Either risk-based and screening	11 (5.26)	0.34 (0.12)
Total	209 (100.00)	0.28 (0.54)

E. coli Escherichia coli, GBS group B *Streptococcus*



E. coli Escherichia coli, GBS group B *Streptococcus*
Each dot represents the *E. coli* incidence for one year for one geographical area

Figure 27. *E. coli* incidence by recently reported GBS prevention strategy

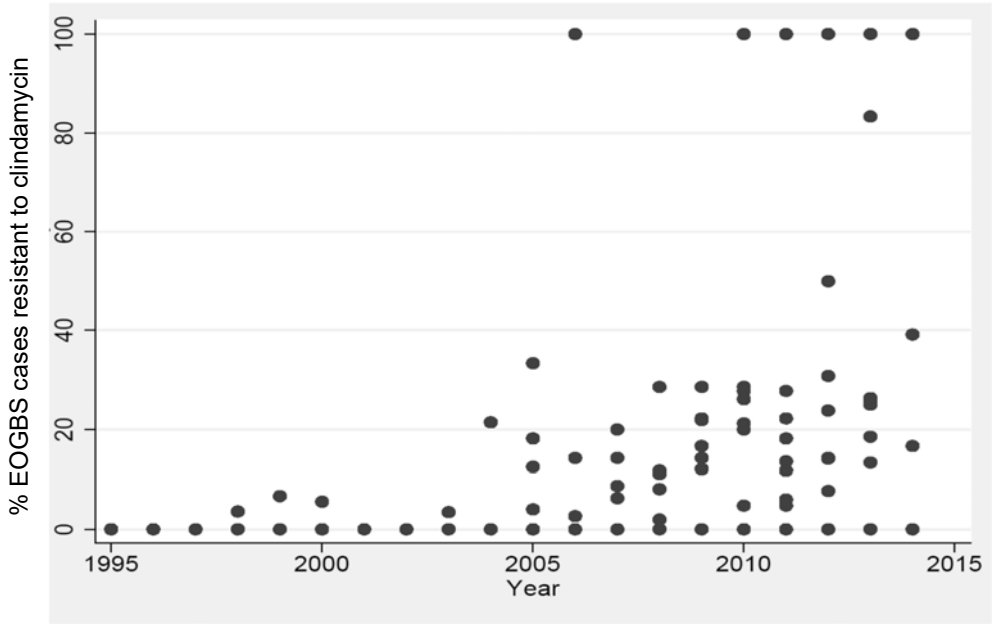
EOGBS resistance

The mean percentage of EOGBS cases resistant to clindamycin across 121 observations (22 geographical areas, 20 years between 1995 and 2014) was 13.83% (SD 24.90), while the mean percentage of EOGBS cases resistant to erythromycin across 115 observations (22 geographical areas, 20 years between 1995 and 2014) was 16.21 (SD 24.45). Both outcomes ranged from 0% to 100%, which could be a result of small numbers of the EOGBS cases in the denominator. Across the years, the percentage of EOGBS cases resistant to clindamycin varied between 0.00% and 36.54% (SD 45.57) while erythromycin resistance varied between 0.00% and 31.09% (SD 40.99). From Table 52, Figure 28 and Figure 29 it is clear that EOGBS resistance to clindamycin and erythromycin increased across the years, although this could be a result of more geographical areas contributing to the later years compared with the earlier years.

Table 52. Percentage of EOGBS resistance across the years

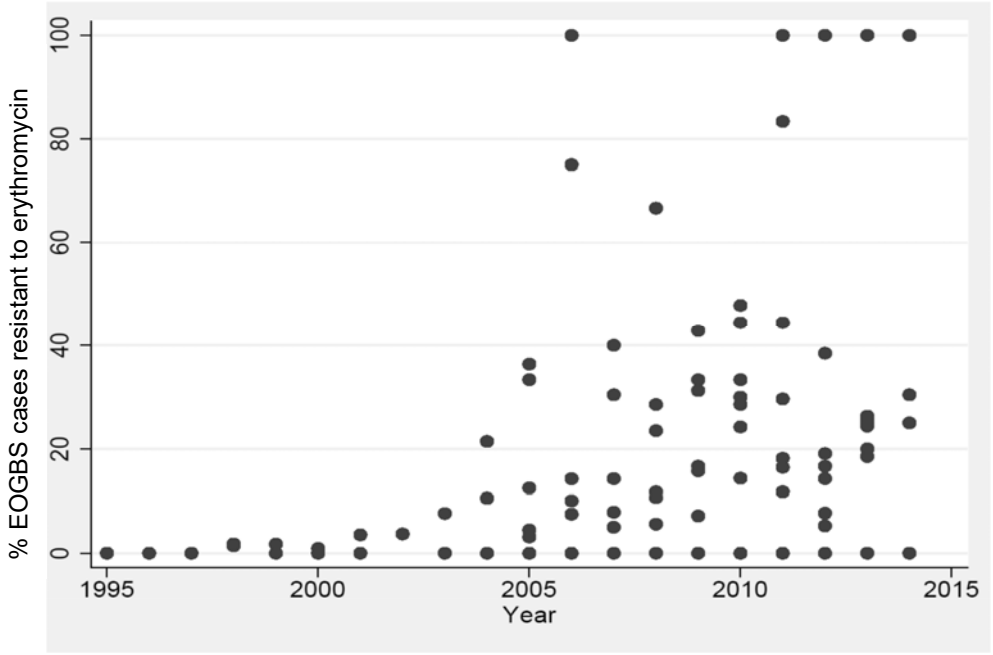
Year	Clindamycin resistance			Erythromycin resistance		
	No. areas	Mean EOGBS cases (SD)	Mean % cases resistant (SD)	No. areas	Mean EOGBS cases (SD)	Mean % cases resistant (SD)
1995	1	3.00	0.00	1	3.00	0.00
1996	2	4.00 (1.41)	0.00 (0.00)	2	4.00 (1.41)	0.00 (0.00)
1997	2	1.00 (0.00)	0.00 (0.00)	2	1.00 (0.00)	0.00 (0.00)
1998	2	35.50 (28.99)	1.79 (2.53)	2	98.00 (59.40)	1.61 (0.25)
1999	3	5.67 (8.08)	2.22 (3.85)	3	38.67 (65.24)	0.58 (1.01)
2000	2	10.00 (11.31)	2.78 (3.93)	2	55.50 (75.66)	0.46 (0.65)
2001	3	3.67 (4.62)	0 .00 (0.00)	3	38.33 (64.66)	1.18 (2.04)
2002	1	19.00	0.00	1	135.00	3.70
2003	4	9.75 (13.00)	0.86 (1.73)	4	38.50 (70.36)	1.91 (3.82)
2004	4	20.00 (15.49)	5.36 (10.71)	4	51.50 (68.65)	7.99 (10.24)
2005	8	12.00 (15.57)	8.50 (12.16)	8	24.88 (44.17)	11.22 (15.17)
2006	7	13.14 (17.81)	16.70 (37.10)	7	30.43 (58.41)	29.55 (40.57)
2007	9	13.56 (14.50)	5.47 (0.50)	9	28.33 (52.81)	10.84 (14.83)
2008	11	17.09 (21.97)	5.59 (8.96)	11	27.64 (52.42)	13.34 (20.35)
2009	9	15.33 (18.68)	12.85 (10.78)	8	31.00 (57.43)	18.38 (16.04)
2010	13	20.38 (31.44)	17.57 (27.58)	12	31.00 (57.75)	18.56 (18.46)
2011	11	18.55 (24.54)	18.57 (28.61)	10	29.60 (53.18)	30.38 (35.44)
2012	11	16.73 (25.99)	21.88 (30.35)	10	27.10 (53.22)	20.15 (30.47)
2013	11	17.27 (26.05)	26.58 (34.18)	11	24.91 (45.91)	21.83 (28.43)
2014	7	6.43 (8.32)	36.54 (45.57)	5	7.60 (9.84)	31.09 (40.99)
Total	121	14.83 (20.60)	13.83 (24.90)	115	30.57 (51.49)	16.21 (24.45)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*



EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Each dot represents the % of EOGBS cases resistant to clindamycin for one year for one geographical area

Figure 28. Scatterplot of EOGBS cases resistant to clindamycin by year



EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Each dot represents the % of EOGBS cases resistant to erythromycin for one year for one geographical area

Figure 29. Scatterplot of EOGBS cases resistant to erythromycin by year

Tunis had the highest mean percentage of EOGBS cases resistant to clindamycin (100%), however, this was only for one case. Thereafter, the highest mean resistance for clindamycin was 66.67% in Guangzhou (SD 57.74) and Macau (SD 42.49) followed by 27.10% (SD 28.39) in the Denmark. The highest mean resistance for erythromycin was 83.33% in Santo Domingo, 66.67% (SD 57.74) in Guangzhou and 60.00% (SD 56.57) in Tunis. No clindamycin resistance was reported in Barcelona, Brno, Mexico City, Riga and Zagreb and no erythromycin resistance was reported in Barcelona, Brno, Mauritius, Mexico City, Riga, Tokyo and Zagreb. Some of this may be the result of the small number of cases in the denominator (see Table 53). Besides these areas, the lowest resistances were reported in Soweto (0.39% [SD 0.88]), Sofia (1.00 [SD 2.45]) and Kuala Terengganu (3.17% [SD 8.40]) for clindamycin resistance, and New Zealand (1.79%), Soweto (5.74% [SD 4.32]) and England (9.46 [SD 7.04]) for erythromycin resistance. Resistance fluctuated yearly in some countries while it remained stable in others (see Appendix 19-A).

Table 53. Percentage of EOGBS resistance across geographical areas

Area	Clindamycin resistance			Erythromycin resistance		
	No. years	Mean EOGBS cases (SD)	Mean % cases resistant (SD)	No. years	Mean EOGBS cases (SD)	Mean % cases resistant (SD)
Alberta	11	12.18 (5.10)	20.85 (11.05)	11	12.18 (5.10)	36.17 (18.41)
Barcelona	8	1.25 (0.71)	0.00 (0.00)	8	1.25 (0.71)	0.00 (0.00)
Brno	1	2.00	0.00	1	2.00	0.00
Denmark	9	9.44 (5.36)	27.10 (28.39)	9	9.44 (5.36)	26.80 (29.26)
Emilia-Romagna	1	21.00	23.81	1	24.00	16.67
England	16	43.31 (29.66)	5.91 (5.07)	16	151 (26.13)	9.46 (7.04)
France	7	27.86 (5.55)	14.90 (8.42)	7	27.86 (5.55)	23.27 (9.16)
Guangzhou	3	1.33 (0.58)	66.67 (57.74)	3	1.33 (0.58)	66.67 (57.74)
Kuala Terengganu	7	4.14 (2.85)	3.17 (8.40)	7	4.14 (2.85)	19.05 (26.23)
Kuwait	10	12.40 (8.04)	16.31 (12.20)	10	12.40 (8.04)	15.44 (10.60)
Macau	5	3.40 (2.30)	66.67 (42.49)	3	2.00 (1.73)	33.33 (57.74)
Mansoura City	1	12.00	16.67	1	12.00	25.00
Mauritius	-	-	-	1	19.00	0.00
Mexico City	16	1.69 (1.14)	0.00 (0.00)	16	1.69 (1.14)	0.00 (0.00)
New Zealand	1	56.00	3.57	1	56.00	1.79
Riga	1	1.00	0.00	1	1.00	0.00
Santo Domingo	1	42.00	4.76	1	42.00	83.33
Sofia	6	3.00 (0.89)	1.00 (2.45)	-	-	-
Soweto	5	42.80 (5.26)	0.39 (0.88)	5	42.80 (5.26)	5.74 (4.32)
Tokyo	4	1.75 (0.96)	25.00 (50.00)	4	1.75 (0.96)	0.00 (0.00)
Tunis	1	2.00	100.00	2	4.00 (1.41)	60.00 (56.57)
US	1	92.00	26.09	1	92.00	47.83
Zagreb	6	1.50 (0.55)	0.00 (0.00)	6	1.50 (0.55)	0.00 (0.00)
Total	121	14.83 (20.60)	13.83 (24.90)	115	30.57 (51.49)	16.21 (24.45)

EOGBS early-onset GBS disease, GBS group B *Streptococcus,* US United States of America

Asia reported the highest percentage of cases resistant to clindamycin (34.50% [SD 45.53]) while Latin America and the Caribbean reported the lowest (0.28 [SD 1.15]). North America reported the highest percentage of cases resistant to erythromycin (37.14 [SD 17.87]) and Oceania reported the lowest (1.79%) (see Table 54). As shown in Appendix 19-B, resistance increased across time in North America, Europe, Asia and North Africa and the Middle East, was stable in Sub-Saharan Africa and Latin America and the Caribbean, while Oceania only had one observation from 1998.

Table 54. Percentage of EOGBS resistance by region

Region	Clindamycin resistance		Erythromycin resistance	
	Number of observations/ years (%)	Mean % cases resistant (SD)	Number of observations/ years (%)	Mean % cases resistant (SD)
Asia	19 (15.70)	34.50 (45.53)	17 (14.78)	25.49 (40.02)
Europe	55 (45.45)	8.59 (15.23)	49 (42.61)	11.68 (16.69)
Latin America and the Caribbean	17 (14.05)	0.28 (1.15)	17 (14.78)	4.90 (20.21)
North Africa and the Middle East	12 (9.92)	23.32 (26.55)	13 (11.30)	23.03 (25.04)
North America	12 (9.92)	21.29 (10.64)	12 (10.43)	37.14 (17.87)
Oceania	1 (0.83)	3.57	1 (0.87)	1.79
Sub-Saharan Africa	5 (4.13)	0.39 (0.88)	6 (5.22)	4.78 (4.52)
Total	121	13.83 (24.90)	115	16.21 (24.45)

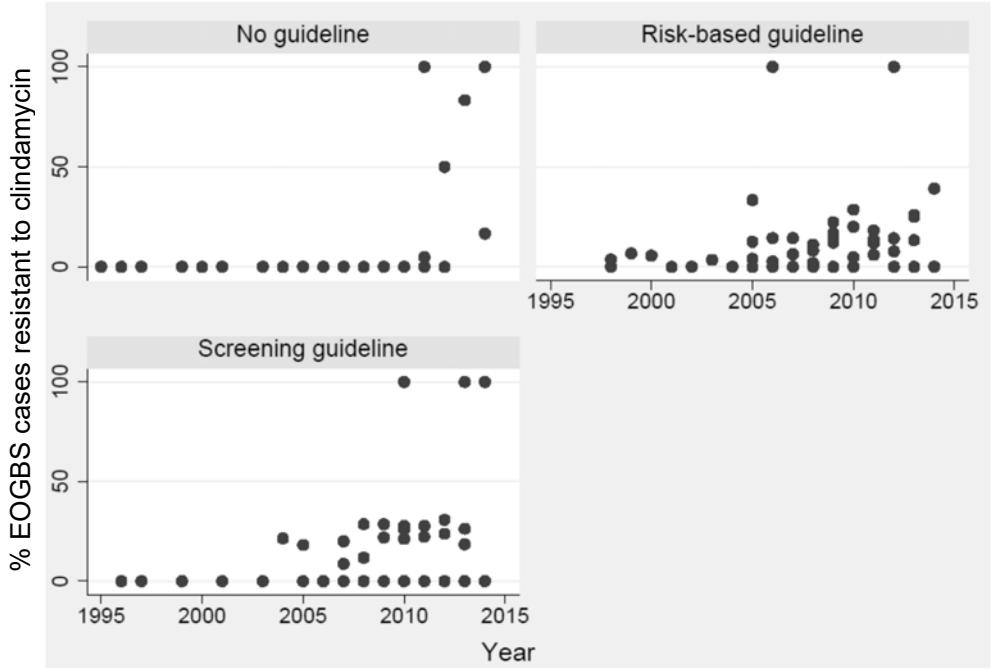
EOGBS early-onset GBS disease, GBS group B *Streptococcus*

With respect to the most recently reported GBS prevention strategy, the highest mean resistance to clindamycin was reported under screening (15.90% [SD 25.99]), followed by no prevention (15.42% [SD 33.22]) and finally risk-based prevention (11.55 [SD 19.82]). The highest mean resistance to erythromycin was also under screening (19.19% [26.19]), followed by risk-based prevention (16.29% [SD 21.29]) and no prevention (9.92% [SD 27.84]) (see Table 55). From Figure 30 and Figure 31 it is clear that there were increases in antibiotic resistance under all prevention strategies. It is also evident that the rates go from 0% in the earlier years to 100% in the later years, probably a result of small numbers.

Table 55. Percentage of EOGBS resistance by recently reported GBS prevention strategy

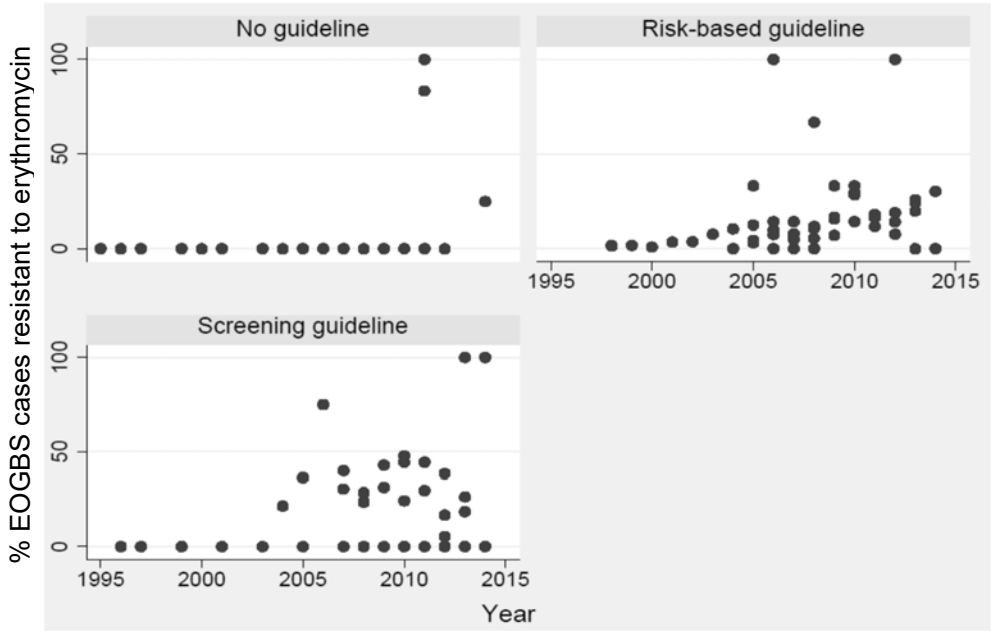
Most recent reported GBS prevention	Clindamycin resistance		Erythromycin resistance	
	Number of observations/ years (%)	Mean % cases resistant (SD)	Number of observations/ years (%)	Mean % cases resistant (SD)
No prevention	23 (19.00)	15.42 (33.22)	21 (18.26)	9.92 (27.84)
Risk-based prevention	55 (45.45)	11.55 (19.82)	51 (44.35)	16.29 (21.29)
Screening prevention	43 (35.54)	15.90 (25.99)	43 (37.39)	19.19 (26.19)
Total	121 (100.00)	13.83 (24.90)	115	16.21 (24.45)

EOGBS early-onset GBS disease, GBS group B *Streptococcus*



EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Each dot represents the % of EOGBS cases resistant to clindamycin for one year for one geographical area

Figure 30. EOGBS clindamycin resistance by recently reported GBS prevention strategy



EOGBS early-onset GBS disease, GBS group B *Streptococcus*
Each dot represents the % of EOGBS cases resistant to erythromycin for one year for one geographical area

Figure 31. EOGBS erythromycin resistance by recently reported GBS prevention strategy

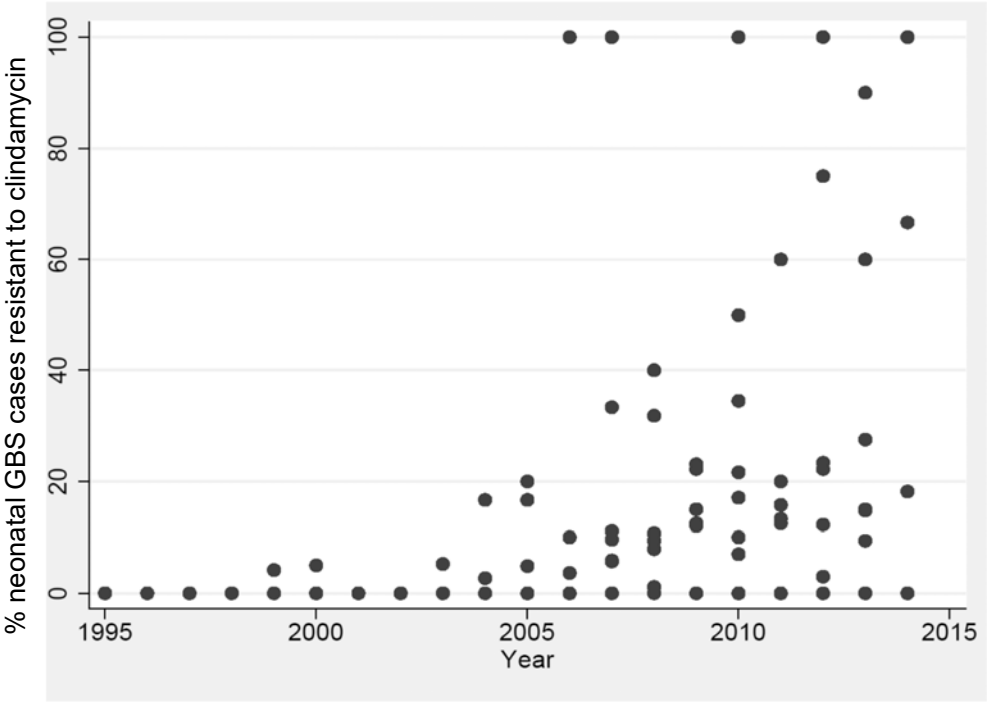
Neonatal GBS resistance

The mean percentage of neonatal GBS cases resistant to clindamycin across 110 observations (16 geographical areas, 20 years between 1995 and 2014) was 14.45% (SD 25.39) while the mean percentage of neonatal GBS cases resistant to erythromycin across 109 observations (18 geographical areas, 20 years between 1995 and 2014) was 13.83% (SD 20.55). Similar to EOGBS resistance, the ranges went from 0 to 100% which could be a result of small denominators. Across years, the mean percentage of neonatal GBS cases resistant to clindamycin varied between 0.00% and 36.97% (SD 44.56) while erythromycin resistance varied between 0.00% and 25.62% (SD 39.40). From Table 56, Figure 32 and Figure 33, it is clear that neonatal GBS resistance to clindamycin and erythromycin increased across the years, although this could be a result of more geographical areas contributing to the later years compared with the earlier years, as mentioned previously.

Table 56. Percentage of neonatal GBS resistance across the years

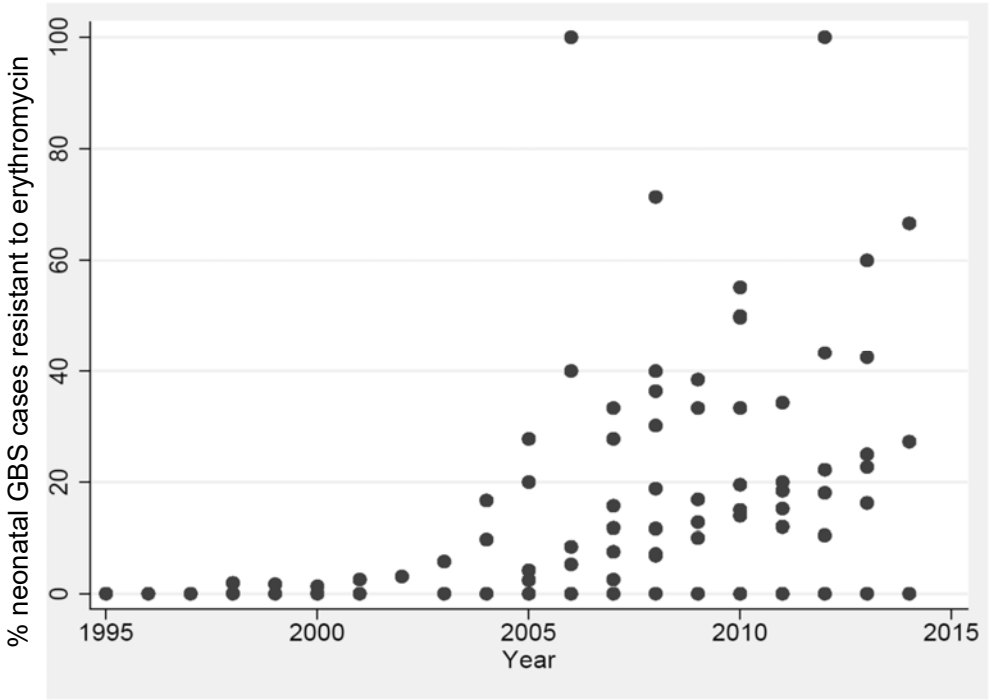
Year	Clindamycin resistance			Erythromycin resistance		
	No. areas	Mean GBS cases (SD)	Mean % cases resistant (SD)	No. areas	Mean GBS cases (SD)	Mean % cases resistant (SD)
1995	1	5.00	0.00	1	5.00	0.00
1996	2	4.50 (2.12)	0.00 (0.00)	2	4.50 (2.12)	0.00 (0.00)
1997	2	1.50 (0.71)	0.00 (0.00)	2	1.50 (0.71)	0.00 (0.00)
1998	2	10.50 (3.44)	0.00 (0.00)	2	104.00 (145.66)	0.97 (1.36)
1999	3	13.67 (11.68)	1.39 (2.41)	3	64.33 (97.00)	0.57 (0.98)
2000	2	11.50 (12.02)	2.50 (3.54)	2	77.00 (104.65)	0.66 (0.93)
2001	3	11.67 (9.29)	0.00 (0.00)	3	59.00 (86.16)	0.84 (1.46)
2002	1	27.00	0.00	1	195.00	3.08
2003	4	13.50 (17.02)	1.32 (2.63)	4	55.75 (100.95)	1.45 (2.90)
2004	5	28.40 (23.80)	3.87 (7.25)	5	66.20 (92.32)	5.28 (7.63)
2005	7	23.71 (29.19)	5.94 (8.71)	7	48.43 (78.26)	7.78 (11.34)
2006	6	26.83 (31.22)	18.94 (39.90)	6	61.17 (101.83)	25.62 (39.40)
2007	10	28.50 (32.13)	16.56 (31.00)	9	54.44 (88.91)	10.96 (12.50)
2008	11	31.09 (39.87)	9.18 (13.97)	12	49.50 (85.14)	18.54 (22.16)
2009	9	27.33 (38.96)	9.43 (9.70)	9	48.78 (96.72)	12.38 (14.83)
2010	12	38.58 (60.19)	24.18 (30.27)	12	53.58 (98.69)	22.50 (21.24)
2011	8	36.00 (50.27)	15.20 (19.76)	8	56.00 (102.66)	12.49 (12.20)
2012	8	35.63 (53.00)	29.48 (37.55)	8	52.25 (97.76)	24.26 (34.02)
2013	9	37.89 (3.72)	24.08 (31.17)	9	51.56 (83.21)	18.49 (21.60)
2014	5	6.60 (8.85)	36.97 (44.56)	4	6.75 (10.21)	23.48 (31.53)
Total	110	27.00 (38.78)	14.45 (25.39)	109	52.54 (85.54)	13.83 (20.55)

GBS group B *Streptococcus*



GBS group B *Streptococcus*
Each dot represents the % of neonatal GBS cases resistant to clindamycin for one year for one geographical area

Figure 32. Scatterplot of neonatal GBS cases resistant to clindamycin by year



GBS group B *Streptococcus*
Each dot represents the % of neonatal GBS cases resistant to erythromycin for one year for one geographical area

Figure 33. Scatterplot of neonatal GBS cases resistant to erythromycin by year

Macau had the highest mean percentage of neonatal GBS resistant to clindamycin (79.17% [SD 21.08]), followed by Guangzhou (43.75% [SD 33.69]) and Denmark (23.48% [SD 29.13]). The highest mean resistance for erythromycin was 49.71% in the US, followed by 43.75% (SD 33.69) in Guangzhou and 32.94% (SD 14.80) in Alberta (see Table 57). No clindamycin resistance was reported in Barcelona, Brno, Mexico City, Riga and Zagreb, and no erythromycin resistance was reported in Barcelona, Brno, Mauritius, Mexico City, Riga, Tokyo and Zagreb (probably a result of small denominator). Besides these areas, the lowest resistances were reported in Soweto (0.23% [SD 0.51]), Kuala Terengganu (3.17% [SD 8.40]) and England (6.44% [SD 4.88]) for clindamycin, and Soweto (3.41% [SD 2.66)]), England (8.79% [SD 6.48)]) and France (16.58% [SD 3.04]) for erythromycin. Resistance fluctuated across time in some countries and was stable across time in others (Appendix 20-A).

Table 57. Percentage of neonatal GBS resistance across geographical areas

Area	Clindamycin resistance			Erythromycin resistance		
	No. years	Mean EOGBS cases (SD)	Mean % cases resistant (SD)	No. years	Mean EOGBS cases (SD)	Mean % cases resistant (SD)
Alberta	11	24.00 (9.26)	19.51 (10.12)	11	24.00 (9.26)	32.94 (14.80)
Barcelona	9	1.44 (0.73)	0.00 (0.00)	9	1.44 (0.73)	0.00 (0.00)
Brno	1	2.00	0.00	1	2.00	0.00
Denmark	9	17.00 (8.65)	23.48 (29.13)	9	17.11 (8.75)	24.79 (28.82)
England	16	71.19 (51.79)	6.44 (4.88)	16	239.69 (55.25)	8.79 (6.48)
France	7	82.86 (16.51)	10.35 (5.09)	7	82.86 (16.51)	16.58 (3.04)
Guangzhou	8	3.00 (1.41)	43.75 (33.69)	8	3.00 (1.41)	43.75 (33.69)
Kuala Terengganu	7	4.57 (2.82)	3.17 (8.40)	7	4.57 (2.82)	19.73 (27.69)
Macau	6	6.00 (3.03)	79.17 (21.08)	3	6.33 (1.53)	17.78 (16.78)
Mansoura city	1	22.00	18.18	1	22.00	27.27
Mauritius	-	-	-	1	24.00	0.00
Mexico City	16	7.88 (7.31)	0.00 (0.00)	16	7.88 (7.31)	0.00 (0.00)
Portugal	-	-	-	1	53.00	30.19
Riga	1	1.00	0.00	1	1.00	0.00
Soweto	5	77.80 (8.90)	0.23 (0.51)	5	77.80 (8.90)	3.41 (2.66)
Tokyo	6	1.50 (0.84)	16.67 (40.82)	6	1.50 (0.84)	0.00 (0.00)
US	1	171.00	21.64	1	171.00	49.71
Zagreb	6	1.50 (0.55)	0.00 (0.00)	6	1.50 (0.55)	0.00 (0.00)
Total	110	27.00 (38.78)	14.45 (25.39)	109	52.54 (85.54)	13.83 (20.55)

EOGBS early-onset GBS, GBS group B *Streptococcus*, US United States of America

Asia reported the highest percentage of cases resistant to clindamycin (35.08% [SD 39.39]) and Latin America and the Caribbean reported the lowest (0.00% [0.00]) followed by Sub-Saharan Africa (0.23% [SD 0.51]). North America reported the highest percentage of cases resistant to erythromycin (34.34% [SD 14.92]) and Latin America and the Caribbean (0.00% [SD 0.00]) followed by Sub-Saharan Africa (2.84% [SD 2.76]) reported the lowest (see Table 58). As shown in Appendix 20-B, the rates fluctuated by year in North America and Europe, were relatively stable in Sub-Saharan Africa and Latin America and the Caribbean. There was only one observation from North Africa and the Middle East in 2015.

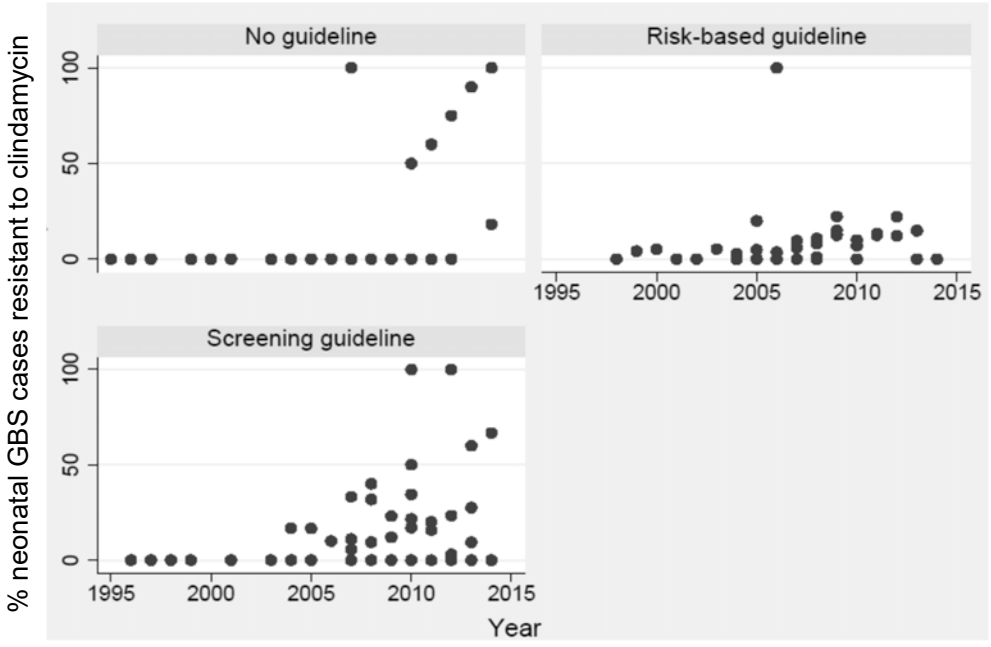
Table 58. Percentage of neonatal GBS resistance by region

Region	**Clindamycin resistance**	**Erythromycin resistance**		
	Number of observations/ years (%)	**Mean % cases resistant (SD)**	**Number of observations/ years (%)**	**Mean % cases resistant (SD)**
Asia	27 (24.55)	35.08 (39.39)	24 (22.02)	22.56 (29.40)
Europe	49 (44.55)	7.89 (14.88)	50 (45.87)	10.20 (15.47)
Latin America and the Caribbean	16 (14.55)	0.00 (0.00)	16 (15.09)	0.00 (0.00)
North Africa and the Middle East	1 (0.91)	18.18	1 (0.92)	27.27
North America	12 (10.91)	19.69 (9.67)	12 (11.01)	34.34 (14.92)
Sub-Saharan Africa	5 (4.55)	0.23 (0.51)	6 (5.50)	2.84 (2.76)
Total	110 (100.00)	14.45 (25.39)	109 (100.00)	13.83 (20.55)

With respect to the most recent prevention strategy, the highest mean resistance to clindamycin was reported under no prevention (21.44% [SD 36.67]), followed by screening (15.17% [SD 24.04]) and risk-based prevention (9.13% [SD 16.82]). The highest mean resistance to erythromycin was under screening (17.81% [SD 23.05]), followed by risk-based prevention (13.66% [SD 19.71]) and no prevention (4.03 ([SD 0.08]) (see Table 59). Figure 34 and Figure 35 show that resistance increased in all prevention strategies. Similar to EOGBS resistance rates, the neonatal GBS resistance rates also go from 0% in the earlier years to 100% in the later years, probably a result of small numbers.

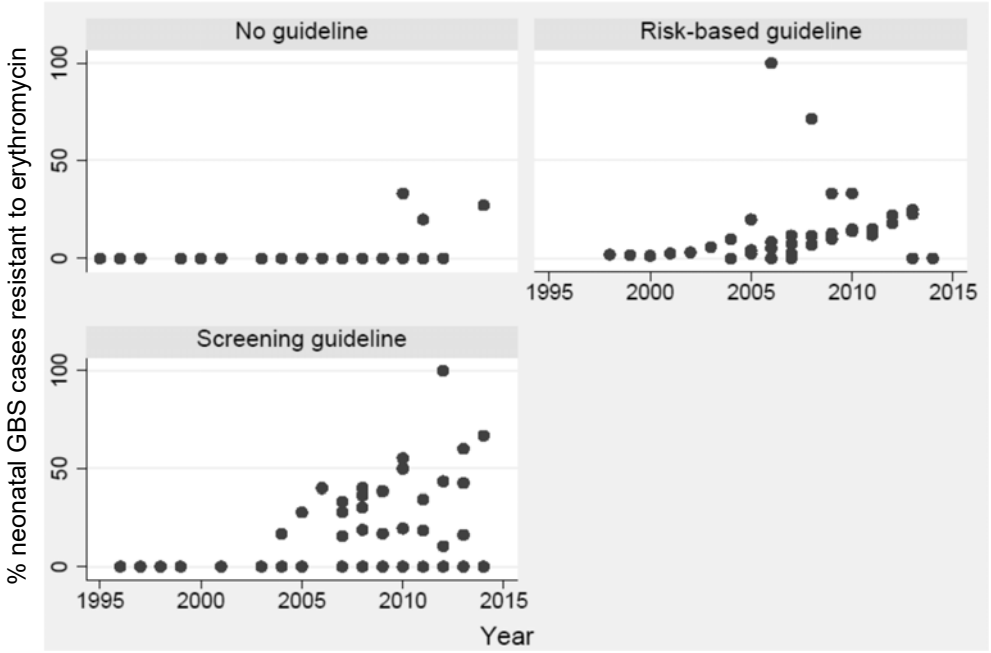
Table 59. Percentage of neonatal GBS resistance by recently reported GBS prevention strategy

Most recent reported GBS prevention	Clindamycin resistance		Erythromycin resistance	
	Number of observations/ years (%)	Mean % cases resistant (SD)	Number of observations/ years (%)	Mean % cases resistant (SD)
No prevention	23 (20.90)	21.44 (36.67)	20 (18.35)	4.03 (10.08)
Risk-based prevention	37 (33.64)	9.13 (16.82)	38 (34.86)	13.66 (19.71)
Screening prevention	50 (45.45)	15.17 (24.04)	51 (46.79)	17.81 (23.05)
Total	110 (100.00)	14.45 (25.39)	109 (100.00)	13.83 (20.55)



GBS group B *Streptococcus*
Each dot represents the % of neonatal GBS cases resistant to clindamycin for one year for one geographical area

Figure 34. Neonatal GBS clindamycin resistance by recently reported GBS prevention strategy



GBS group B *Streptococcus*
Each dot represents the % of neonatal GBS cases resistant to erythromycin for one year for one geographical area

Figure 35. Neonatal GBS clindamycin resistance by recently reported GBS prevention strategy

13.3.4 Unadjusted linear regression analysis (objectives c & d)

This section presents the results of the linear regression analysis showing the unadjusted relationship between the most recently reported GBS prevention strategy and the trends of each harmful outcome across time. This is followed by the linear regression analysis showing the unadjusted relationship between each compositional covariate and mean EOGBS incidence (objective d).

Most recently reported GBS prevention strategy

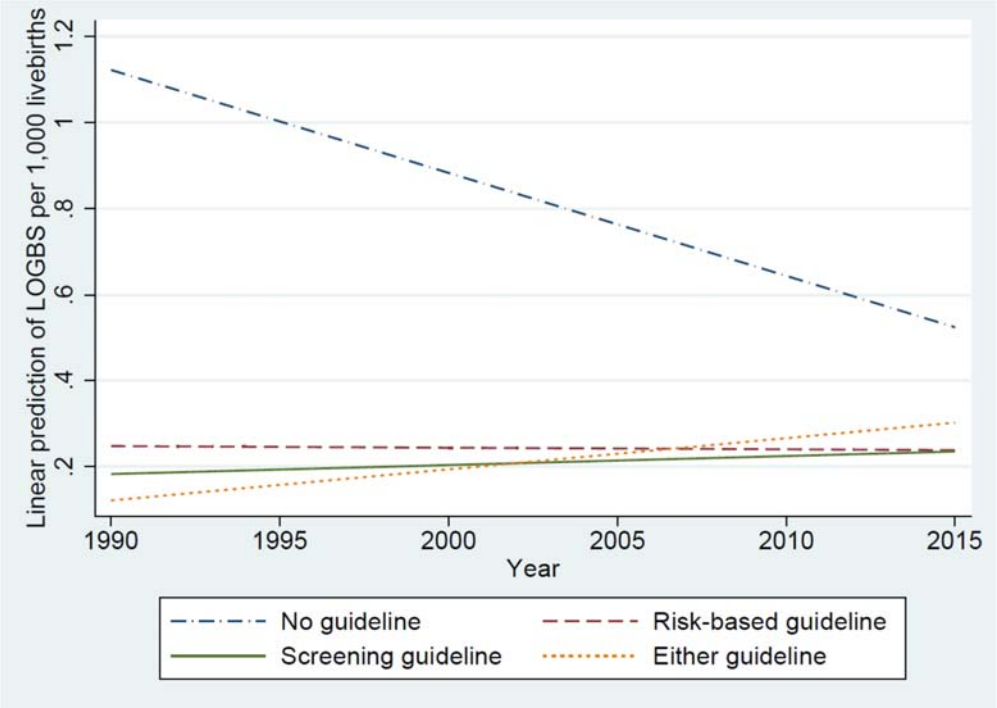
The results of the unadjusted analyses are summarised in Table 60 and Figure 36 to Figure 41. Again, contrary to expectation, there was a decrease in annual early-onset *E. coli* incidence in areas with no prevention. Compared with screening prevention areas, the early-onset *E. coli* incidence decreased by 0.036 (95% CI -0.072 to -0.001) yearly in no prevention areas. Similarly, compared with screening prevention areas, the percentage of neonatal GBS cases resistant to clindamycin increased yearly by 2.71% (95% CI -5.25 to -0.18) lower in risk-based prevention areas. There were no other statistically significant differences in the trends of harms between different prevention areas.

The statistically non-significant trends in harmful outcomes were as follows. LOGBS incidence remained relatively stable in screening and risk-based prevention areas, while it increased yearly in 'either prevention' areas and decreased yearly in no prevention areas. Early-onset *E. coli* incidence increased yearly in screening prevention and risk-based prevention areas whereas it decreased under 'either prevention' areas. With the exception of the trend in EOGBS resistant to clindamycin in risk-based prevention areas, the percentage of EOGBS cases resistant to clindamycin and erythromycin increased across time at similar rates in all areas. Likewise, the percentage of neonatal GBS cases resistant to clindamycin increased steeper under no prevention and screening prevention areas compared with risk-based prevention areas. Finally, the percentage of neonatal GBS cases resistant to erythromycin increased steeper under screening compared with risk-based and no prevention areas.

Table 60. Unadjusted linear regression analyses on the average annual change for the harms by recently reported GBS prevention strategy

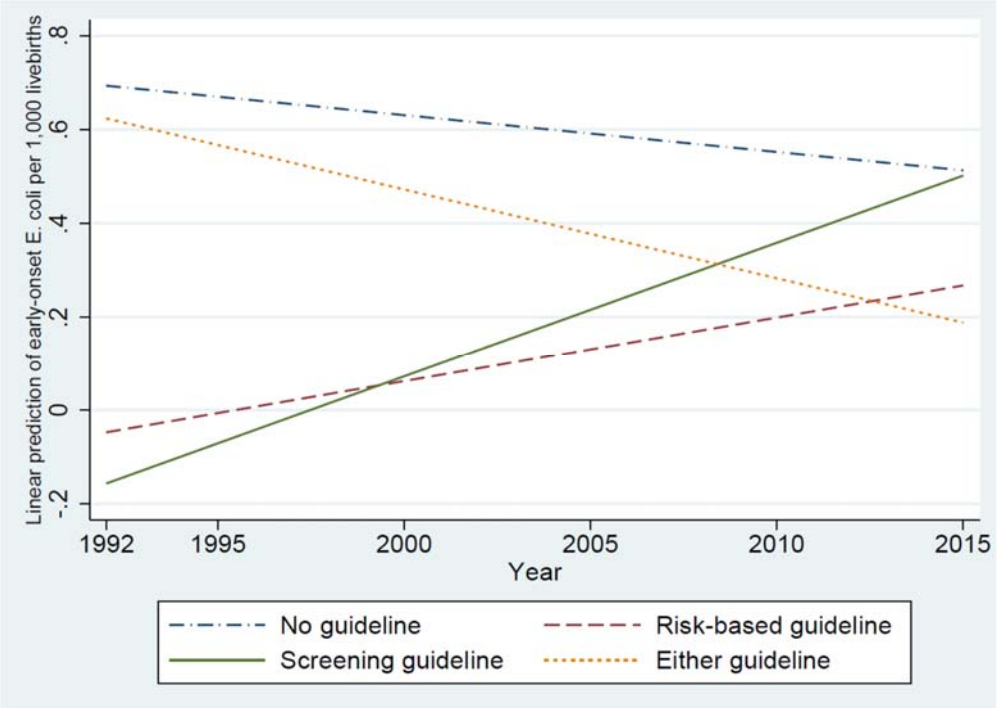
Most recent reported GBS prevention	Average annual change in outcome (95% confidence interval)	p-value
LOGBS per 1,000 livebirths		
Screening prevention	(reference)	
No prevention	-0.034 (-0.055 to 0.003)	0.080
Risk-based prevention	-0.002 (-0.024 to 0.019)	0.822
Either screening or risk-based prevention	.0051308 (-0.032 to 0.042)	0.785
Early-onset *E. coli* per 1,000 livebirths		
Screening prevention	(reference)	
No prevention	-0.036 (-0.072 to -0.001)	0.044
Risk-based prevention	-0.015 (-0.046 to 0.016)	0.339
Either screening or risk-based prevention	-0.048 (-0.147 to 0.052)	0.346
EOGBS clindamycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	-1.829 (-4.083 to 0.425)	0.111
Screening prevention	-0.654 (-2.976 to 1.667)	0.578
EOGBS erythromycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	-0.352 (-2.742 to 2.038)	0.771
Screening prevention	0.044 (-2.386 to 2.475)	0.971
Neonatal GBS clindamycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	-2.712 (-5.247 to -0.177)	0.036
Screening prevention	-1.371 (-3.617 to 0.876)	0.229
Neonatal GBS erythromycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	0.096 (-2.056 to 2.247)	0.930
Screening prevention	1.188 (-0.765 to 3.141)	0.230

E. coli Escherichia coli, EOGBS early-onset GBS, GBS group B *Streptococcus*, LOGBS late-onset GBS



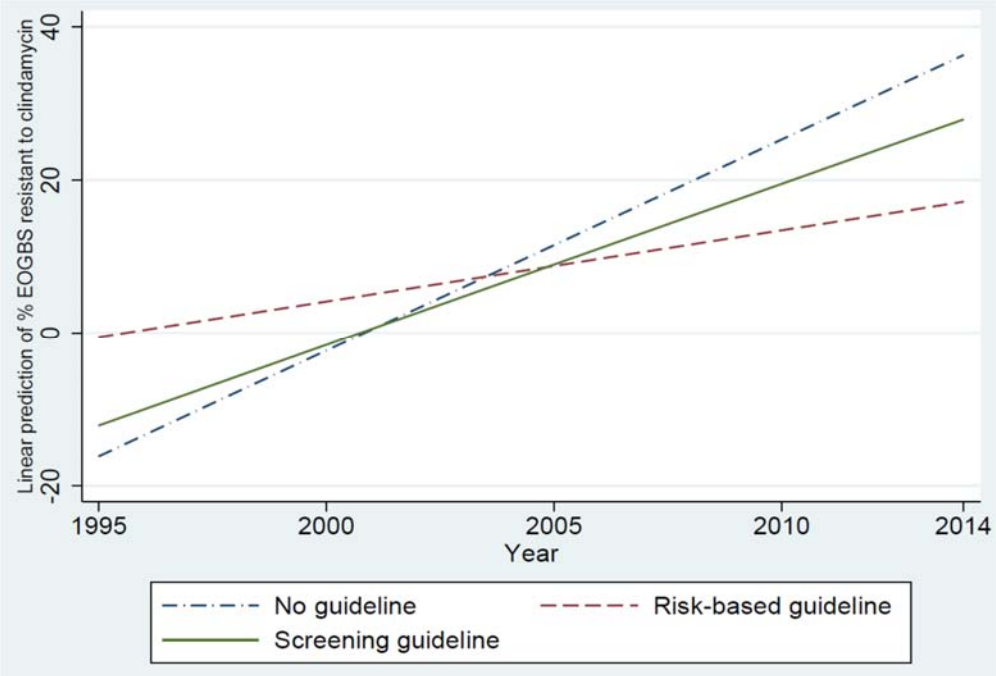
LOGBS Late-onset group B *Streptococcus*

Figure 36. Unadjusted trends of annual LOGBS incidence for each recently reported GBS prevention strategy using linear regression analysis



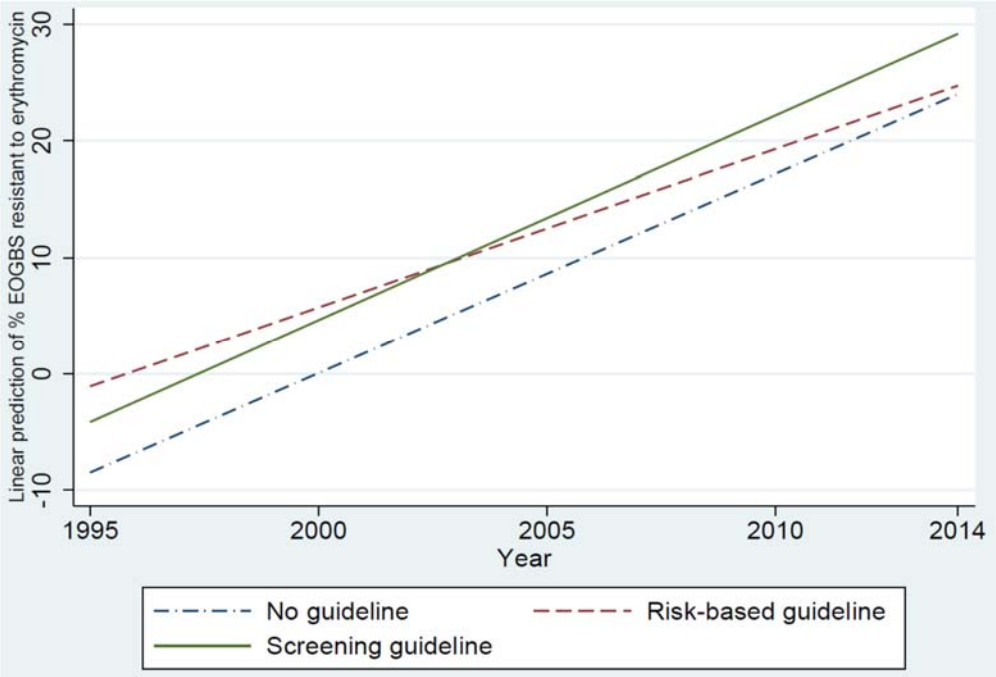
E. coli Escherichia coli, GBS group B *Streptococcus*

Figure 37. Unadjusted trends of annual early-onset *E. coli* incidence for each recently reported GBS prevention strategy using linear regression analysis



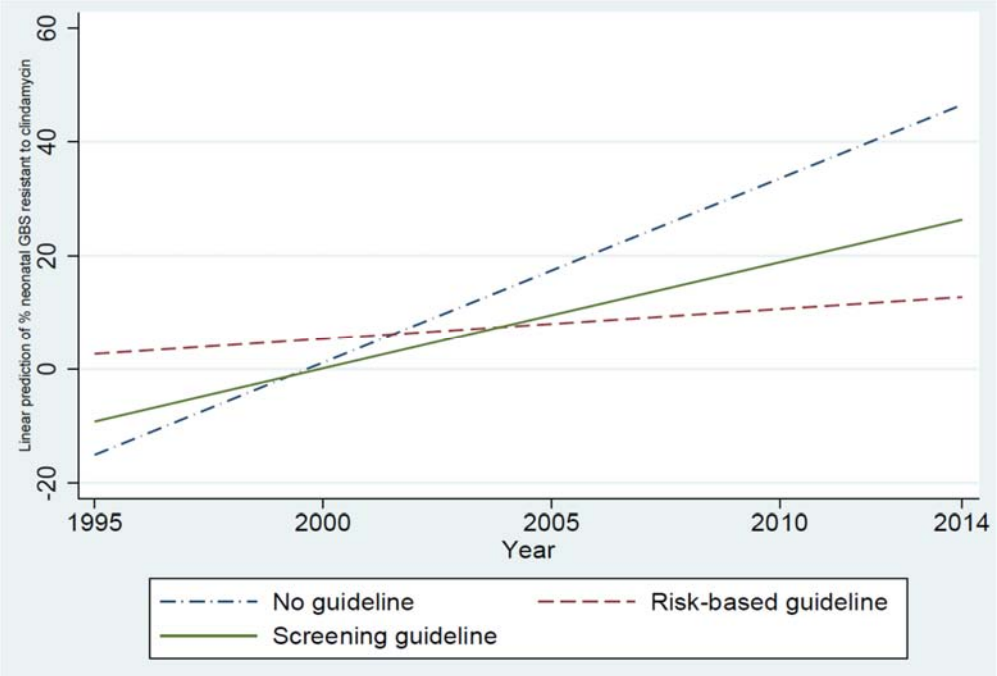
EOGBS early-onset group B *Streptococcus*

Figure 38. Unadjusted trends of the annual percentage of EOGBS cases resistant to clindamycin for each recently reported GBS prevention strategy using linear regression analysis



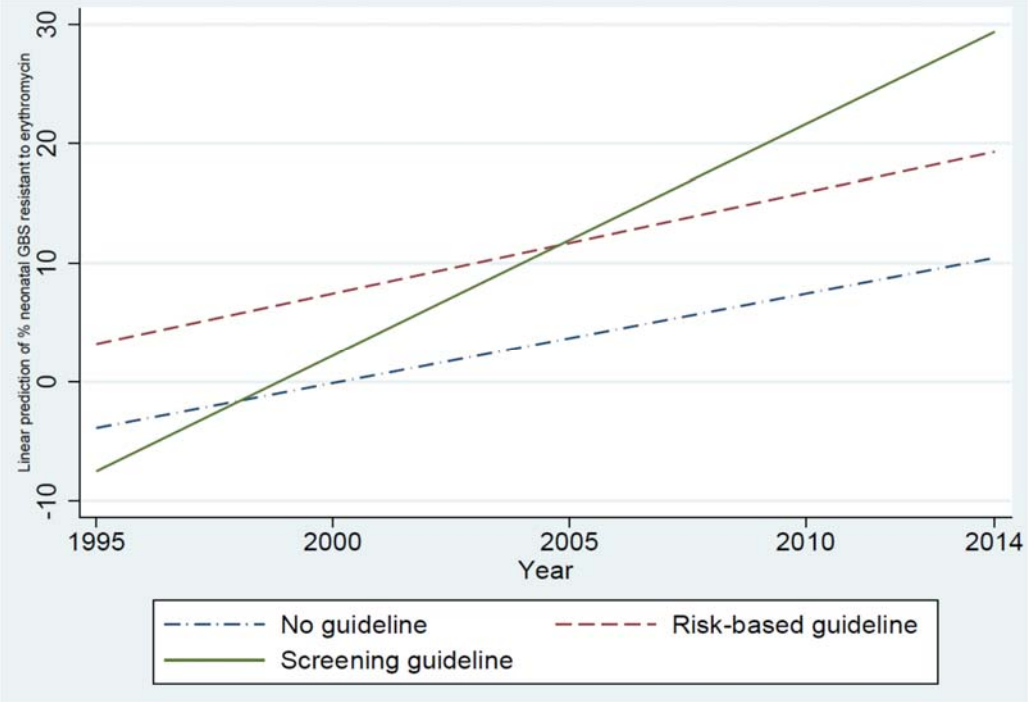
EOGBS early-onset group B *Streptococcus*

Figure 39. Unadjusted trends of the annual percentage of EOGBS cases resistant to erythromycin for each recently reported GBS prevention strategy using linear regression analysis



GBS group B *Streptococcus*

Figure 40. Unadjusted trends of the annual percentage of neonatal GBS cases resistant to clindamycin for each recently reported GBS prevention strategy using linear regression analysis



GBS group B *Streptococcus*

Figure 41. Unadjusted trends of the annual percentage of neonatal GBS cases resistant to erythromycin for each recently reported GBS prevention strategy using linear regression analysis

Compositional covariates

The results of the unadjusted linear regression analyses between the compositional covariates and the six outcomes are shown in Table 61. To summarise the key relationships, Latin America and the Caribbean reported a higher incidence of LOGBS (0.409, 95% CI 0.217 to 0.601) and early-onset *E. coli* per 1,000 livebirths (0.428, 95% CI 0.142 to 0.715) compared with North America. Sub-Saharan Africa had also had a higher incidence of LOGBS (0.870, 95% CI 0.505 to 1.234). Compared with North America, Latin America and the Caribbean reported a lower percentage of antibiotic resistance across those tested, and Europe and Sub-Saharan Africa had a lower percentage of erythromycin resistance for both EOGBS and neonatal GBS cases tested. A one percent increase in preterm births was associated with: a 0.033 (95% CI 0.015 to 0.051) per 1,000 livebirths increase in LOGBS incidence; a 0.061 (95% CI 0.038 to 0.084) per 1,000 livebirths increase in early-onset *E. coli* incidence; a 2.87% (95% CI 1.698 to 4.0458) increase in the percentage of EOGBS cases resistant to erythromycin; and a 1.62% (95% CI 0.578 to 2.653) increase in the percentage of neonatal GBS cases resistant to erythromycin. A one percent increase in low birthweights was associated with an increase of 0.027 (95% CI 0.018 to 0.035) per 1,000 livebirths in LOGBS incidence and a 0.034 per 1,000 livebirths (95% CI 0.023 to 0.044) in early-onset *E. coli* incidence. A one percent increase in caesarean sections was also associated with an increase in LOGBS (0.007, 95% CI 0.002 to 0.013) and early-onset *E. coli* (0.019, 95% CI 0.011 to 0.026) incidence.

By contrast, a unit increase in the human development index reduced LOGBS by 2.112 (-95% CI -2.761 to -1.464) and early-onset *E. coli* by 1.815 (95% CI -2.778 to -0.853) per 1,000 livebirths. Likewise, a percentage increase in maternal GBS colonisation reduced LOGBS by 0.007 (95% CI -0.012 to -0.002) and early-onset *E. coli* by 0.016 (95% CI -0.024 to -0.009) per 1,000 livebirths. A one unit increase in the percentage of prolonged rupture of membranes was associated with increases in all resistance outcomes. Similar to other findings, data from one centre compared with national surveillance and/or mandatory surveillance influenced the results.

Table 61. Unadjusted linear regression analyses of the harms by compositional covariates

Covariate	LOGBS/1000 coefficient (95% confidence interval [CI])	Early-onset *E. coli*/1000 coefficient (95% CI)	% EOGBS resistance to clindamycin (95% CI)	% EOGBS resistance to erythromycin (95% CI)	% Neonatal GBS resistance to clindamycin (95% CI)	% Neonatal GBS resistance to erythromycin (95% CI)
Region						
North America	(reference)	(reference)	(reference)	(reference)	(reference)	(reference)
Asia	0.088 (-0.105 to 0.280)	0.296 (-0.005 to 0.597)	13.213 (-3.390 to 29.815)	-11.653 (-28.776 to 5.471)	15.391 (-26.083 to 2.486)	-11.778 (-24.538 to 0.982)
Europe	-0.021 (-0.170 to 0.128)	0.196 (-0.078 to 0.470)	-12.700 (-27.045 to 1.646)	**-25.468 (-40.096 to -10.839)**	-11.799 (-26.083 to 2.486)	**-24.137 (-35.739 to -12.536)**
Latin America and the Caribbean	**0.409 (0.217 to 0.601)**	**0.428 (0.142 to 0.715)**	**-21.010 (-37.986 to -4.034)**	**-32.241 (-49.364 to -15.117)**	**-19.691 (-36.628 to -2.755)**	**-34.337 (-48.120 to -20.555)**
North Africa and the Middle East	-0.217 (-0.753 to 0.319)	0.361 (-0.020 to 0.742)	2.0264 (-16.355 to 20.408)	-14.109 (-32.290 to 4.072)	-1.509 (-47.670 to 44.652)	-7.064 (-44.629 to 30.500)
Oceania	-0.031 (-0.322 to 0.260)	0.306 (-0.085 to 0.696)	-17.719 (-64.583 to 29.145)	-35.357 (-82.628 to 11.914)	-	-
Sub-Saharan Africa	**0.870 (0.505 to 1.234)**	-	-20.898 (-44.864 to 3.069)	**-32.358 (-55.066 to -9.650)**	-19.464 (-43.071 to 4.143)	**-31.495 (-49.541 to -13.450)**
Preterm births	**0.033 (0.015 to 0.051)**	**0.061 (0.038 to 0.084)**	1.262 (-0.137 to 2.66)	**2.872 (1.698 to 4.0458)**	1.077 (-0.251 to 2.405)	**1.616 (0.578 to 2.653)**
Low birthweights	**0.027 (0.018 to 0.035)**	**0.034 (0.023 to 0.044)**	-0.561 (-1.170 to 0.048)	-0.521 (-1.150 to 0.108)	-0.517 (-1.162 to 0.127)	-0.449 (-0.988 to 0.091)
Caesarean section	**0.007 (0.002 to 0.013)**	**0.019 (0.011 to 0.026)**	0.296 (-0.248 to 0.839)	0.230 (-.293 to 0.753)	0.232 (-0.331 to 0.795)	-0.045 (-0.488 to 0.398)
Fertility rate	**0.277 (0.148 to 0.405)**	0.126 (-0.023 to 0.274)	**-13.293 (-22.598 to -3.988)**	-5.420 (-15.143 to 4.304)	**-18.890 (-28.407 to -9.373)**	-5.742 (-14.131 to 2.647)
Skilled attendance at delivery	-0.008 (-0.018 to 0.002)	-0.003 (-0.015 to 0.009)	0.094 (-0.758 to 0.945)	-0.003 (-0.787 to 0.7806)	**0.999 (0.079 to 1.918)**	0.652 (-0.108 to 1.412)
Average maternal age	**-0.046 (-0.062 to -0.031)**	**-0.032 (-0.051 to -0.013)**	-0.246 (-1.583 to 1.092)	-0.193 (-1.514 to 1.127)	-0.879 (-2.255 to 0.497)	-0.155 (-1.284 to 0.975)
Multiple or twin birth	**-0.008 (-0.015 to -0.002)**	-0.005 -(0.013 to 0.003)	0.007 (-0.561 to 0.575)	-0.421 (-0.990 to 0.147)	-0.330 (-0.834 to 0.174)	-0.389 (-0.798 to 0.0199)
Per capita government expenditure on health	-0.00004 (-0.00007 to 0.00002)	**-0.00009 (-0.0001 to -0.00005)**	0.0009 (-0.002 to 0.004)	**0.003 (0.0001 to 0.006)**	-0.001 (-0.004 to 0.002)	**0.003 (0.0002 to 0.005)**
Human development index	**-2.112**	**-1.815**	-9.981	14.032	-51.141	32.865

Covariate	LOGBS/1000 coefficient (95% confidence interval [CI])	Early-onset *E. coli*/1000 coefficient (95% CI)	% EOGBS resistance to clindamycin (95% CI)	% EOGBS resistance to erythromycin (95% CI)	% Neonatal GBS resistance to clindamycin (95% CI)	% Neonatal GBS resistance to erythromycin (95% CI)
	(-2.761 to -1.464)	**(-2.778 to -0.853)**	(-68.338 to 48.377)	(-48.942 to 77.006)	(-111.49 to 9.208)	(-22.842 to 88.573)
Maternal GBS colonisation	**-0.007 (-0.012 to -0.002)**	**-0.016 (-0.024 to -0.009)**	0.118 (-0.348 to 0.583)	0.404 (-0.079 to 0.886)	-0.204 (-0.718 to 0.311)	0.193 (-0.224 to 0.610)
Prolonged rupture of membranes	-0.0002 (-0.009 to 0.009)	0.006 (-0.006 to 0.017)	**2.098 (1.445 to 2.750)**	**1.153 (0.418 to 1.888)**	**2.141 (1.557 to 2.725)**	**0.915 (0.341 to 1.489)**
Intrapartum fever	-0.016 (-0.043 to 0.011)	-0.009 (-0.041 to 0.023)	1.695 (-0.900 to 4.290)	-0.727 (-3.286 to 1.833)	1.419 (-1.414 to 4.252)	-0.825 (-3.117 to 1.468)
Most prevalent GBS serotype						
Ia	(reference)	(reference)	(reference)	(reference)	(reference)	(reference)
Ib	-0.198 (-0.485 to 0.089)	0.196 (-0.184 to 0.575)	1.315 (-18.400 to 21.030)	-13.561 (-33.945 to 6.824)	-5.482 (-31.452 to 20.487)	**-23.475 (-44.326 to -2.624)**
III	0.113 (-0.066 to 0.292)	0.280 (-0.066 to 0.626)	9.183 (-8.554 to 26.920)	-6.739 (-24.305 to 10.826)	10.768 (-7.712 to 29.248)	-9.575 (-24.419 to 5.269)
V	-0.206 (-0.784 to 0.372)	0.147 (-0.553 to 0.846)	**52.295 (14.323 to 90.267)**	25.688 (-6.500 to 57.876)	12.699 (-40.522 to 65.921)	3.797 (-38.935 to 46.529)
Surveillance type						
Mandatory population surveillance	(reference)	(reference)	(reference)	(reference)	(reference)	(reference)
Voluntary population surveillance	-0.139 (-0.296 to 0.018)	-0.073 (-1.115 to 0.968)	-7.267 (-24.088 to 9.554)	-14.532 (-30.162 to 1.098)	-7.429 (-25.211 to 10.354)	**-15.201 (-28.251 to -2.151)**
Multiple centres/counties	-0.032 (-0.213 to 0.149)	0.086 (-0.966 to 1.138)	-6.271 (-44.224 to 31.682)	-7.083 (-43.178 to 29.012)	2.123 (-51.017 to 55.264)	9.753 (-19.692 to 39.199)
One centre	**0.168 (0.027 to 0.308)**	0.260 (-0.776 to 1.295)	-8.459 (-23.909 to 6.991)	**-19.465 (-33.850 to -5.080)**	-4.870 (-21.439 to 11.700)	**-20.897 (-33.040 to -8.754)**
Geographical coverage						
National	(reference)	(reference)	(reference)	(reference)	(reference)	(reference)
Regional	-0.015 (-0.185 to 0.156)	-0.135 (-0.414 to 0.145)	7.208 (-9.405 to 23.822)	**17.260 (1.521 to 32.998)**	7.139 (-10.500 to 24.779)	**16.948 (3.613 to 30.284)**
City/town wide	-0.137 (-0.687 to 0.414)	-0.145 (-0.757 to 0.467)	-	-	-	-
One centre in a city/town	**0.225 (0.109 to 0.340)**	**0.245 (0.081 to 0.408)**	-1.251 (-11.480 to 8.978)	-4.863 (-14.650 to 4.925)	2.270 (-8.532 to 13.072)	-6.694 (-14.827 to 1.439)
Definition						

Covariate	LOGBS/1000 coefficient (95% confidence interval [CI])	Early-onset *E. coli*/1000 coefficient (95% CI)	% EOGBS resistance to clindamycin (95% CI)	% EOGBS resistance to erythromycin (95% CI)	% Neonatal GBS resistance to clindamycin (95% CI)	% Neonatal GBS resistance to erythromycin (95% CI)
1	(reference)	(reference)	(reference)	(reference)	(reference)	(reference)
2	**-0.278 (-0.409 to -0.148)**	**-0.307 (-0.451 to -0.163)**	3.743 (-6.545 to 14.032)	1.674 (-8.449 to 11.797)	-7.422 (-21.448 to 6.604)	-4.358 (-15.388 to 6.673)
3	-0.493 (-1.442 to 0.456)	-0.241 (-0.672 to 0.190)	-	-	-	-
4	-0.508 (-1.185 to 0.168)	-	-	-	-7.025 (-21.773 to 7.723)	**-13.675 (-25.3972 to -1.952)**

E. coli Escherichia coli, GBS group B *Streptococcus*, LOGBS late-onset GBS
LOGBS definition: 1, 2/3/4 days onwards, 2, 5/6/7/8 days onwards, 3, 48 hours to 6 days, 4, Not stated
E. coli definition 1, <2/3/4 days, 2, <5/6/7/8 days, 3, mother infected
Early-onset GBS definition 1, 2/3 days or less, 2, 5/6/7 days or less
Neonatal GBS definition 1, <28/30/31/44 days, 2, <89/90/132 days, 4 Not stated
Numbers in bold are p<0.05

13.3.5 Adjusted linear regression analysis (objective e)

This section presents the results of the adjusted linear regression analysis showing the relationship between the most recently reported GBS prevention strategy and the trends of each harmful outcome across time adjusted for the compositional covariates (objective e).

As statistically non-significant covariates were removed, this left the minimal final models shown in Table 62, Figure 42 to Figure 47. In contrast with the unadjusted analysis, when compositional covariates were adjusted for, there was an increase in annual LOGBS incidence in all prevention areas. However, compared with screening prevention areas, LOGBS incidence increased by 0.079 (95% CI 0.013 to 0.146) yearly in no prevention areas and 0.016 (95% CI 0.001 to 0.031) yearly in risk-based prevention areas. The trends in annual LOGBS incidence between risk-based areas and no prevention areas were also statistically different (p=0.0241). There were no other statistically significant differences in the trends of other harmful outcomes between different prevention areas.

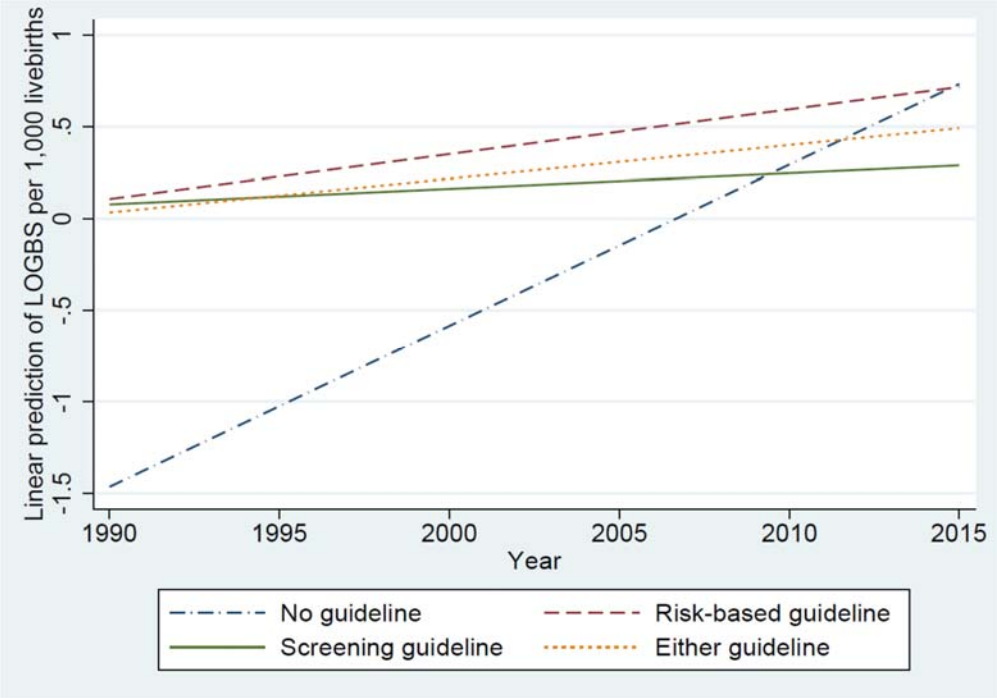
The statistically non-significant trends in the harmful outcomes were as follows. Early-onset *E. coli* incidence increased in no, risk-based and screening prevention areas whereas it decreased in 'either prevention' areas. The percentage of EOGBS cases resistant to clindamycin increased at a similar rate in all prevention areas, while the percentage of EOGBS cases resistant to erythromycin increased more steeply in no prevention areas followed by screening and risk-based prevention areas. The percentage of neonatal GBS cases resistant to clindamycin remained steady in no prevention areas, whereas it increased in risk-based prevention areas and screening areas. The percentage of neonatal GBS cases resistant to erythromycin remained steady in risk-based prevention areas, whereas it increased in no prevention and screening prevention areas.

Table 62. Adjusted linear regression analyses on the average annual change for the harms by recently reported GBS prevention strategy

Most recent reported GBS prevention	Coefficient by year (95% confidence interval)	p-value
LOGBS per 1,000 livebirths		
Screening prevention	(reference)	
No prevention	0.079 (0.013 to 0.146)	0.020
Risk-based prevention	0.016 (0.001 to 0.031)	0.035
Either prevention	0.010 (-0.008 to 0.028)	0.277
Early-onset *E. coli* per 1,000		
Screening prevention	(reference)	
No prevention	0.021 (-0.029 to 0.072)	0.406
Risk-based prevention	0.005 (-0.012 to 0.023)	0.564
Either prevention	-0.036 (-0.075 to 0.003)	0.072
EOGBS clindamycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	0.207 (-1.857 to 2.272)	0.842
Screening prevention	0.175 (-2.780 to 3.130)	0.907
EOGBS erythromycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	-1.401 (-3.668 to 0.865)	0.219
Screening prevention	-0.608 (-2.578 to 1.361)	0.536
Neonatal GBS clindamycin resistance		
No prevention	(reference)	
Risk-based prevention	0.449 (-2.655 to 3.553)	0.774
Screening prevention	1.429 (-1.571 to 4.428)	0.346
Neonatal GBS erythromycin resistance (%)		
No prevention	(reference)	
Risk-based prevention	-0.209 (-1.684 to 1.266)	0.777
Screening prevention	0.812 (-0.877 to 2.501)	0.339

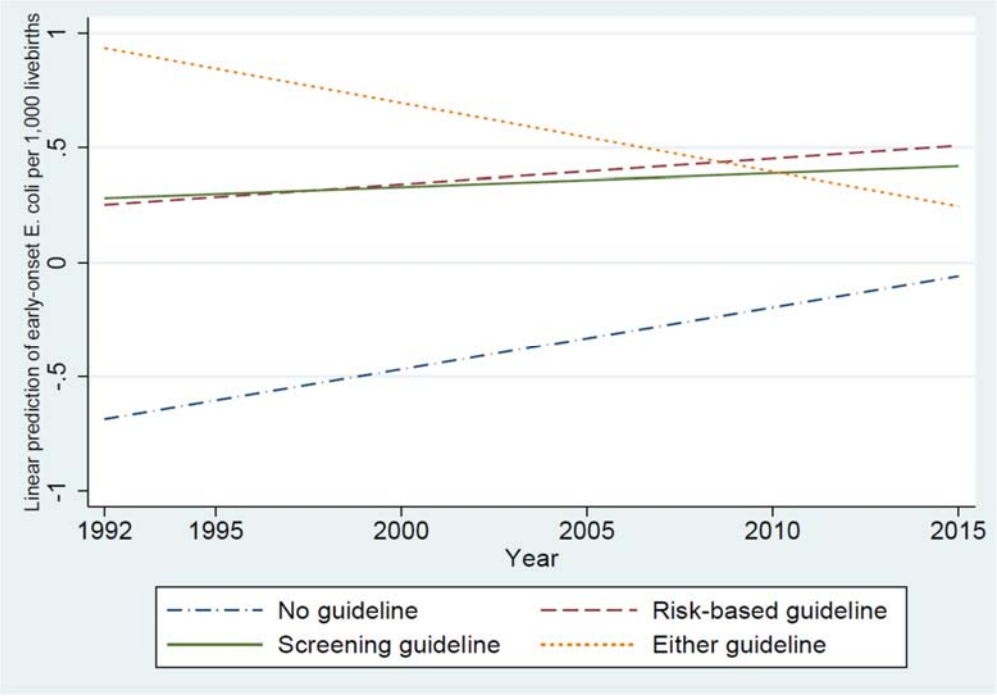
LOGBS late-onset group B *Streptococcus, E. coli Escherichia coli,*

a. LOGBS adjusted for percentage preterm births, low birthweights, human development index, region, prolonged rupture of membranes, maternal GBS colonisation, geographical coverage, surveillance type, and definition
b. *E. coli* adjusted for percentage preterm births, low birthweights, human development index, region, maternal GBS colonisation, geographical coverage, surveillance type, and definition
c. EOGBS clindamycin resistance adjusted for region, human development index, percentage preterm births, low birthweight, surveillance type, geographical coverage, and definition
d. EOGBS erythromycin resistance adjusted for region, human development index, percentage preterm births, low birthweight, surveillance type, geographical coverage, and definition
e. Neonatal GBS clindamycin resistance adjusted for region, human development index, percentage preterm births, prolonged rupture of membranes, low birthweight, surveillance type, and definition
f. Neonatal GBS clindamycin resistance adjusted for region, human development index, percentage preterm births, low birthweight, surveillance type, and definition



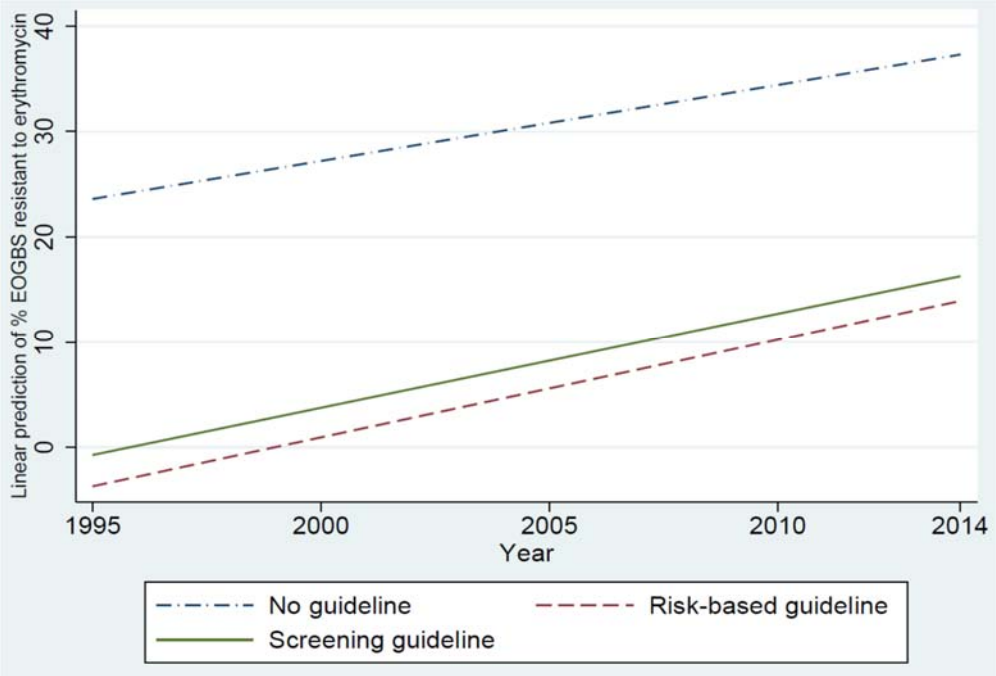
LOGBS Late-onset group B *Streptococcus*

Figure 42. Adjusted trends of annual LOGBS incidence for each recently reported GBS prevention strategy using linear regression analysis



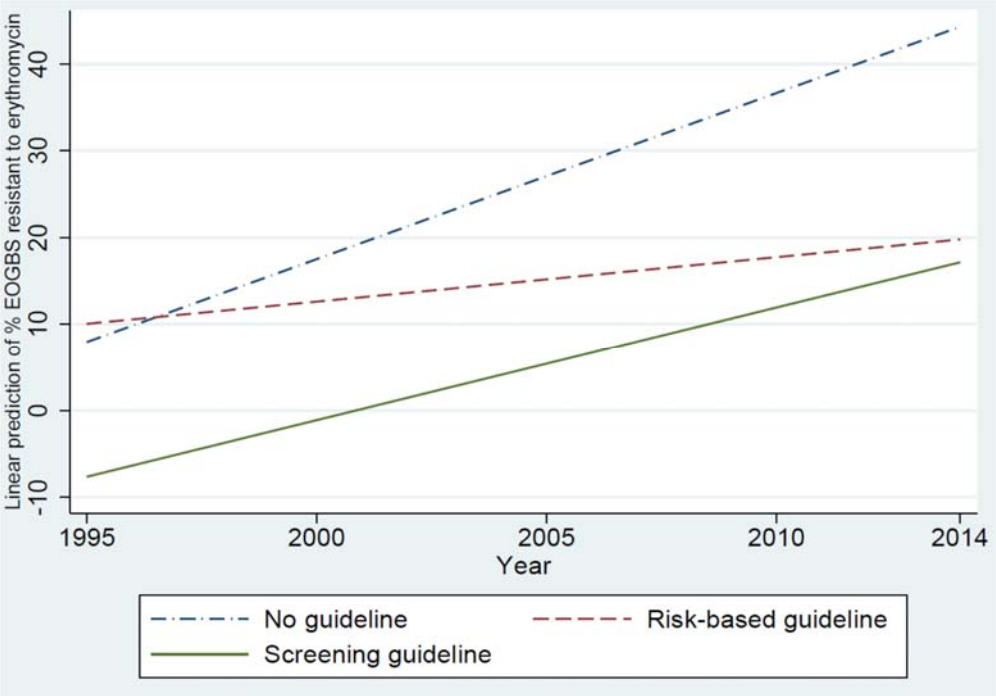
E. coli Escherichia coli, GBS group B *Streptococcus*

Figure 43. Adjusted trends of annual early-onset *E. coli* incidence for each recently reported GBS prevention strategy using linear regression analysis



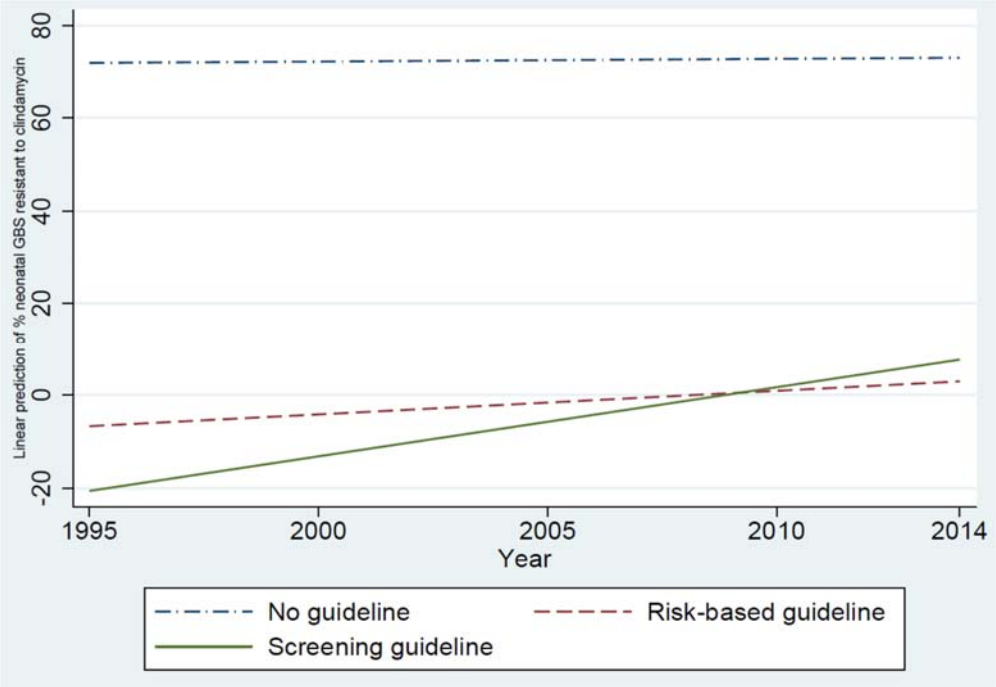
EOGBS early-onset group B *Streptococcus*

Figure 44. Adjusted trends of the annual percentage of EOGBS cases resistant to clindamycin for each recently reported GBS prevention strategy using linear regression analysis



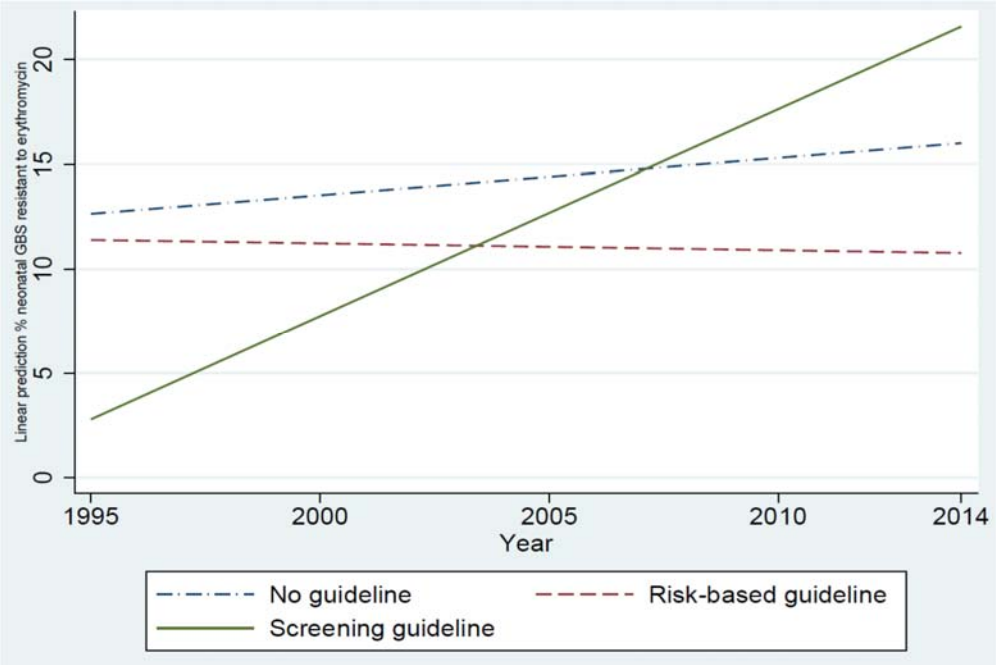
EOGBS early-onset group B *Streptococcus*

Figure 45. Adjusted trends of the annual percentage of EOGBS cases resistant to erythromycin for each recently reported GBS prevention strategy using linear regression analysis



GBS group B *Streptococcus*

Figure 46. Adjusted trends of the annual percentage of neonatal GBS cases resistant to clindamycin for each recently reported GBS prevention strategy using linear regression analysis



GBS group B *Streptococcus*

Figure 47. Adjusted trends of the annual percentage of neonatal GBS cases resistant to erythromycin for each recently reported GBS prevention strategy using linear regression analysis

13.3.6 Sensitivity analysis for LOGBS trends (objective f)

The results from final model on the trends of annual LOGBS incidence were sensitive to alterations in the assumptions and data in the model. The difference in the yearly trends of LOGBS incidence between risk-based prevention and screening prevention areas lost statistical significance when using the most frequently reported GBS prevention across the years instead of the most recently reported GBS prevention (0.011, 95% CI -.001 to 0.024). This comparison also lost statistical significance when only including geographical areas with four or more years of data (0.015, 95% CI -0.002 to 0.033) and when removing outer fence box plot outliers (0.007, 95% CI -0.002 to 0.017). Removing data from only one centre caused the difference in trends between no prevention and screening prevention areas to lose statistical significance (0.021, 95% CI -0.087 to 0.129). Removing outer fence box plot outliers (0.012, 95% CI 0.0003 to 0.023) and data from only one centre (0.009, 95% CI 0.0003 to 0.0174) also caused the trends in annual LOGBS incidence between 'either prevention' areas to become statistically different to screening prevention areas.

Altering the maternal GBS colonisation rate to a lower rate (where ranges were available) caused the trends in annual LOGBS incidence in both risk-based (0.014, 95% CI -0.0003 to 0.029) and no prevention areas (0.055, 95% CI -0.003 to 0.114) compared with screening prevention areas to lose statistical significance. Altering maternal GBS colonisation to a higher rate only caused the difference in trends between no prevention and screening prevention areas to just miss statistical significance (0.059, 95% CI -0.0005 to 0.118). Similarly, all of the following changes caused the trends of annual LOGBS incidence in both no and risk-based prevention areas compared with screening prevention areas to lose statistical significance: only including covariates with less than 10% of data imputed from another country (no prevention: 0.043, 95% CI -0.017 to 0.103; risk based prevention: 0.007, 95% CI -0.007 to 0.021); only using survey data (no prevention: 0.008, 95% CI -0.049 to 0.065; risk based prevention: 0.005, 95% CI -.0008 to 0.017); and using alternative geographical areas where more than one data source was available (no prevention: 0.012, 95% CI -0.050 to 0.074, risk-based prevention: 0.004, 95% CI -0.009 to 0.016). Only including data defined as 7 days onwards did not change the results and none of the sensitivity analyses changed the direction of the results.

13.4 Discussion

In this discussion, I will summarise the findings of this study and compare the findings with previous literature. In Chapter 14, I will discuss the strengths and the limitations as well as the research and policy implications related to this study.

13.4.1 Principal findings

In this study, I aimed to investigate whether GBS prevention strategies, particularly universal screening, have an impact on the trends of potential harmful outcomes of GBS screening programmes across countries. These harmful outcomes were incidences of LOGBS and early-onset *E. coli* and the percentages of EOGBS and neonatal GBS cases resistant to clindamycin and erythromycin. The findings suggest that the international trends of LOGBS incidence may indeed be related to GBS prevention strategies. LOGBS incidence increased under screening, however, contrary to expectations, it increased at a higher rate under no and risk-based prevention compared with screening. Based on the available evidence, the predicted values showed that LOGBS incidence increased from approximately 0.075 to 0.30 per 1,000 livebirths under screening prevention areas, 0.10 to 0.73 per 1,000 livebirths in risk-based prevention areas, and -1.45 to 0.74 per 1,000 livebirths in no prevention areas. The trends for the remaining harmful outcomes under risk-based, 'either' or no prevention were not different to universal screening. This would imply that there is no evidence that LOGBS, early-onset *E. coli* and antibiotic resistance would increase by adopting a GBS screening programme. Instead, LOGBS incidence would increase more under a risk-based or no prevention strategy, compared with screening. However, aside from the LOGBS analysis, the sample size in the remaining analyses was not sufficiently large, therefore, this evidence is inconclusive.

The difference in the trends of LOGBS incidence between risk-based and no prevention areas compared with screening areas was unstable across the sensitivity analyses. While I chose the most methodologically robust available options in selecting the data and generating the variables for the model, there were serious limitations in the data collected, thus, unstable results have important implications. Seven of the 10 sensitivity analyses caused the LOGBS differences between screening and risk-based prevention areas to lose statistical significance, while six caused the difference between screening and no prevention areas to lose statistical significance. As the direction of the results did not change, screening still had a smaller increase in LOGBS incidence compared with risk-based and no prevention. However, whether the predicted trends can be expected under each of the GBS prevention strategies in different populations or settings is uncertain. Nevertheless, for the purposes of this study investigating

whether universal screening has a harmful impact of increasing LOGBS incidence, the implication that screening does not increase LOGBS incidence compared with other strategies, remains unchanged. The main model and the sensitivity analyses showed that LOGBS incidence under screening did not increase more than other strategies.

The findings of early-onset *E. coli* incidence and the percentages of EOGBS and neonatal GBS cases resistant to clindamycin and erythromycin are not clear. While the statistically non-significant results could be genuine and screening may not increase these outcomes compared with other prevention strategies, similar to Chapter 12, they could also be due to the lack of statistical power from the small sample sizes in these analyses. As identified in Chapter 9, 63 observations in each prevention strategy provided an 80% chance to detect a change of 0.1 per 1,000 livebirths. Accounting for the imbalances between the observations in each prevention group, for the *E. coli* analysis, the sample size I achieved was 19 per prevention group for the comparison of either *versus* screening prevention, 80 for risk-based *versus* screening prevention, and 48 for no *versus* screening prevention. The annual differences in early-onset *E. coli* between the prevention strategies were well below 0.1 per 1,000 livebirths, therefore, the sample size was not large enough for these differences to be statistically significant. For the analyses on GBS resistance, the sample sizes achieved ranged from 26 to 31 in each prevention group and the resulting statistical power was less than 50%.

The lack of statistical power is especially important for the analyses on early-onset *E. coli* incidence and the percentage of neonatal GBS cases resistant to erythromycin. The trends in these outcomes were considerably different for each of the prevention strategies yet there was no statistically significant difference. Compared with screening, which showed an upward trend in early-onset *E. coli* incidence, 'either prevention' areas showed a decreasing yearly trend. Similarly, the predicted values show that the percentage of neonatal GBS cases resistant to erythromycin increased at a much steeper rate in screening prevention areas from approximately 3% to 22.5% during the study period, whereas it changed from around 13% to 16% in no prevention areas, and remained steady in risk-based prevention areas at around 11% to 12%. This may imply that erythromycin resistance increases at higher rate under screening programmes than risk-based programmes or no prevention, even though this did not reach statistical significance.

Overall, there is evidence that universal screening does not lead to an unintended increase in LOGBS incidence compared with other prevention strategies. Furthermore, there was no evidence that universal GBS screening has an impact on the trends of annual early-onset *E. coli* incidence or GBS resistance compared with other strategies, however, these analyses were

statistically under-powered to confirm this. Of particular importance, trends in neonatal GBS resistance to erythromycin showed a considerably higher but statistically non-significant increase over time in screening prevention areas compared with risk-based areas. This might have been statistically significant if larger sample sizes were analysed.

13.4.2 Comparison with previous literature

In this section, I separately compare the study findings with existing literature for each outcome (LOGBS, early-onset *E. coli* and antibiotic resistance). The trends of LOGBS are not as well documented as EOGBS. In line with the study findings, in the US, multi-state population surveillance from the CDC reported stable rates of LOGBS ranging from 0.29 to 0.39 per 1,000 livebirths from 1990 to 2008.[408, 409] During this time, the GBS prevention strategy changed from no to 'either' to screening prevention. Most recently, in 2015 the LOGBS incidence was 0.34 per 1,000 livebirths.[57] Under GBS screening in Italy, LOGBS has remained steady at 0.32 per 1,000 livebirths with no statistically significant variation from 2003 to 2010.[410] By contrast, Bauserman *et al.* (2013) found that LOGBS incidence increased from 0.8 to 1.1 per 1,000 NICU admissions between 1997 to 2001 ('either prevention') and 2002 to 2010 (universal screening).[172] Also in agreement with the study findings, literature from countries that have adopted risk-based prevention have shown increases in LOGBS incidence over time.[411] For example, in the UK under risk-based prevention, LOGBS incidence has increased from 0.24 per 1,000 livebirths in 2000/01 to 0.37 per 1,000 livebirths in 2014/15.[5] Similarly, in the Netherlands under risk-based prevention, LOGBS incidence increased from 0.03 to 0.13 per 1,000 livebirths over time.[66] However, from the report it is not clear but likely that the surveillance was voluntary, thus, the increase could be attributable to increases in reporting. The findings of this study mirror the existing evidence: increases in LOGBS incidence under risk-based prevention were more evident in this study and more consistent in the literature, whereas increases in LOGBS incidence under screening were smaller in this study and vary in the literature.

In agreement with the study findings, Stoll *et al.* (2002) in the US found no difference in all-cause sepsis and early-onset *E. coli* sepsis between very low birth neonates whose mothers were treated with IAP and those who were not.[20] Likewise, although Schrag *et al.* (2006) found that IAP increased the odds of early-onset *E. coli* infection in unadjusted analyses, IAP did not remain associated with early-onset *E. coli* when other factors were accounted for. In Taiwan, Tsai *et al.* (2012) also found that early-onset *E. coli* was not associated with antibiotic use after adjusting for gestational age.[188] Analysing trends over time, Bauserman *et al.* (2013) found that early-onset *E. coli* remained stable from the period of 'either prevention' to the

period of screening at 1.4 per 1,000 admissions.[172] Likewise, in Australia and New Zealand, Daley *et al.* (2004) found that early-onset *E. coli* decreased from 1992 to 2001 during either or risk-based prevention, which was not statistically significant.[72] An older Australian study showed similar results.[190] Contrary to the findings of this study, Bizzarro *et al.* (2008) found that early-onset *E. coli* in very low birthweight neonates (<1500g) increased from 2.83 per 1,000 admissions (no prevention) to 7.12 per 1,000 admissions (risk-based prevention) to 10.22 per 1,000 admissions (screening).[187] Similarly, in the study by Stoll *et al*. (2002) the rate of early-onset *E. coli* infections increased from 3.2 to 6.8 per 1000 livebirths in low birthweight infants between 1998 to 2000 during 'either' prevention.[20] Despite the low statistical power in this study, the majority of the current evidence in the literature supports the finding that screening does not lead to an increase in early-onset *E. coli* in all neonates. However, the evidence is limited as these are observational before and after studies subject to bias.

In the literature, antibiotic resistance in countries offering IAP prevention programmes for neonatal GBS prevention have increased over time. It is important to note, that GBS remains almost universally susceptible to penicillin as mentioned in Chapters 2 and 7,[86] although there are recent reports from small studies in Ethiopia and in Italy with evidence of penicillin resistance in GBS isolated from pregnant women.[179-181] In the US in 2005, 0.2% of GBS isolates had reached the upper level of susceptibility for beta-lactams.[182, 18] Similarly, in Japan, 5% to 15% of GBS isolates were reported to have reduced penicillin susceptibility;[183] approximately half of these were susceptible under European breakpoints and came from populations where chronic antibiotic exposure is likely to be common (due to chronic respiratory disease). The clinical significance of increased minimum inhibitory concentrations close to breakpoint is also uncertain.

However, in the last 20 years, both clindamycin and erythromycin resistance have increased in countries with risk-based prevention and universal screening.[14, 81] In countries adopting universal screening, reported rates of resistance have been higher compared with countries with risk-based prevention, though no formal comparison has been performed in neonates. In the US, where universal screening is adopted, resistance to erythromycin was reported at 48% and resistance to clindamycin was reported at 27% in EOGBS cases in 2010.[57] By contrast, in the UK in 2010 where risk-based prevention is adopted, erythromycin was reported at 15% in EOGBS[81] and clindamycin resistance at 9% though this included all GBS cases.[184] However, in an Italian study of 75 neonatal GBS strains, erythromycin resistance was reported at only 12% and clindamycin resistance was 7% under screening.[412] For neonatal GBS isolates in Tanzania where there is no prevention strategy, sensitivity to erythromycin was reported at

approximately 81% and sensitivity to clindamycin was 88%.[413] This population included all invasive and colonising strains. Although the difference in erythromycin trends in this study were not statistically significant, the increases were much steeper in screening areas compared with the increases risk-based prevention areas for both EOGBS and neonatal GBS. Indeed, in a time-trend population study in the US from 1996 to 2003 where prevention strategies changed from 'either prevention' to universal screening, erythromycin resistance increased from 15.8% to 32.8% and clindamycin resistance increased from 10.5% to 15%, although this analysis included invasive strains from adult populations.[414] With respect to 'either prevention', in Australia, there are low resistance rates of 6.4% erythromycin and 4.2% of clindamycin resistance, which did not increase between 1982 to 2001 (before 'either prevention' guidelines) and 2002 to 2006 (after 'either prevention' guidelines).[185] From the literature, it is difficult to draw conclusions on the impact of universal GBS screening on GBS resistance to clindamycin and erythromycin. Generally, there are higher rates of clindamycin and erythromycin resistance in countries that adopt universal screening compared with countries that adopt risk-based prevention, 'either prevention' or have no prevention. However, whether these differences or increases in resistance rates between different strategies are clinically or statistically significant remains unanswered in the literature, and in this study, as a result of low statistical power.

13.5 Conclusions for this chapter

- The findings in this chapter show that universal GBS screening has no harmful impact on the trends of LOGBS incidence compared with risk-based prevention, 'either prevention' and no prevention.
- The trends of LOGBS incidence increased over time in all areas, and were higher in risk-based and no prevention areas compared with screening areas. However, these higher rates were unstable under different assumptions.
- There was also no evidence that early-onset *E. coli* incidence and the percentage of GBS cases resistant to clindamycin and erythromycin are higher under universal GBS screening and widespread IAP treatment compared with other strategies.
- Importantly, these analyses did not have a sufficient sample size, therefore, the results might be a result of low statistical power as opposed to no true difference.
- The evidence remains inconclusive and larger sample sizes are required to determine the impact universal GBS screening has on the trends of early-onset *E. coli* and GBS resistant to clindamycin and erythromycin across countries.

14. DISCUSSION

In this discussion, I will summarise the key findings, strengths and the limitations as well as the research and policy implications related to part III of this thesis overall.

14.1 Summary of the findings from research questions 4 to 6

The research aim of this part of the thesis was to investigate whether the international data on the benefits and harms of universal GBS screening, compared with other prevention strategies, can be adjusted for country-level differences, to inform the clinical effectiveness of universal GBS screening. I conducted two ecological trend analysis studies investigating the benefits of universal GBS screening on the trends of annual EOGBS incidence (research question 4) and early-onset sepsis incidence (research question 5), compared with other prevention strategies. Based on the available evidence, EOGBS incidence decreased by 0.5 to 0.6 per 1,000 livebirths over 27 years, whereas in risk-based prevention, EOGBS increased by 0.4 to 0.5 per 1,000 livebirths over that time. In general, there was no difference between the trends of EOGBS incidence between screening and 'either prevention', as the incidence also decreased in areas reporting 'either strategy'. Finally, the results comparing no prevention with screening prevention were conflicting and depended on the data included and the assumptions generated for the analysis. The results of the study on early-onset sepsis showed that the international trends of early-onset sepsis do not differ by prevention strategy. However, the sample size in this study was not large enough to make firm conclusions.

I conducted a third ecological trend analysis study investigating the harms of universal GBS screening on the trends of early-onset *E. coli* incidence, LOGBS incidence, and clindamycin and erythromycin resistance in early-onset and neonatal GBS disease across time, compared with other prevention strategies (research question 6). There was consistent evidence that universal GBS screening did not lead to increases in LOGBS incidence compared with other prevention strategies. There was also no evidence that universal GBS screening impacts the trends of annual early-onset *E. coli* incidence or GBS resistance. However, these analyses were also statistically under-powered to confirm this.

Although screening consistently showed a downward trend of EOGBS incidence across the analyses, risk-based prevention consistently showed an upward trend, and LOGBS consistently did not increase under screening compared with other prevention strategies, there are numerous limitations and uncertainties in these results, as discussed below.

14.2 Strengths and limitations

These studies were one of the first attempts to compare the benefits and harms of universal GBS screening across different geographical areas and different times with other prevention strategies. In these studies, I collected data covering all world regions for most variables, resulting in the beginning of an international database which maps out EOGBS incidence across 46 geographical areas, early-onset sepsis incidence across 24 areas, LOGBS incidence across 41 areas, early-onset *E. coli* across 25 geographical areas, and neonatal GBS resistance across 23 geographical areas. In addition, I adjusted for confounding variables when comparing prevention programmes, which is the case in only a few studies. I controlled for compositional differences across the geographical areas, including maternal risk factors for GBS. I also adjusted for methodological differences between the areas due to the limitations in secondary data collection. Therefore, I was able to reduce bias from these factors. To avoid bias from missing data, I utilised MICE imputation and achieved convergence for the compositional covariates across the years. Finally, I also attempted to account for the multi-level structure of the data in a multi-level growth curve model for EOGBS incidence.

However, there are a number of limitations in these study that need to be considered in relation to the findings. Firstly, across the studies only a maximum of 41 out of 194 countries were represented (minimum 16 countries). It is suggested that, for self-completion questionnaires, a rate of below 50% would be a low response rate.[350] This risk of selection bias raises concerns about the external validity of the sample I achieved in these studies, how representative they are, and how generalisable the findings are. The small sample size may also be the reason why the results are varied across some analyses; a larger sample size may prevent the instability. Collecting data on the outcomes was a difficult task as they were not readily available from national surveillance institutions or collated into one dataset, as for other conditions. Contacting busy institutions across countries and asking for the completion of survey questionnaires was labour intensive and, although at initial contact institutions were interested in participating, due to their busy schedules and other commitments, they were not able to provide any data. I did manage to collect data from areas of every world region for most of the outcomes. It was particularly difficult for early-onset sepsis, possibly as sepsis is diagnosed clinically in hospitals and is not necessarily collected by national laboratories, making population surveillance more complicated. However, non-response bias is an important issue when using survey questionnaires[350] and the number of countries from each world region was not balanced. In particular, there were few countries from Sub-Saharan Africa, North Africa and the Middle East. Furthermore, there was no data from the UK for early-onset sepsis and no data from Oceania for resistance rates. There was also a higher

representation of areas that were from high income countries. In summary, survey questionnaires have these limitations and I may not have obtained a random or representative sample, leading to selection bias. Similarly, the quality of the data I collected across countries varied and in some cases institutions reported zero cases of disease outcomes in their area. This might be a result of poor diagnostic facilities or procedures, surveillance being voluntary in that area, or because the population that the institution covered was too small to allow identification of a case. I did attempt to control for this by adjusting for regional and economic differences as well as the coverage of the data.

The lack of data was particularly important for the analyses of early-onset sepsis and *E. coli* incidences as well as the GBS resistance rates as I was unable to achieve the calculated sample size required to provide sufficient statistical power for the analysis. Having a low sample size and low statistical power reduces the likelihood of detecting a true effect of the interventions.[415] With fewer than 63 observations per prevention group, the probability of finding a true difference of 0.1 per 1,000 livebirths per year between the prevention groups was reduced from 80% to 50% or lower. Therefore, the small sample size increased the probability of incorrectly not rejecting the null hypothesis and concluding that there is no effect of one GBS prevention strategy compared with another, also known as a type II error.[416] The differences in early-onset sepsis and *E. coli* incidences found between the groups in these studies were in fact lower than 0.1 per 1,000 livebirths and the chance of these differences being statistically significant, with the sample size achieved, are even lower. Therefore, no differences in the trends under screening compared with other GBS prevention strategies may be a result of the low sample size and statistical power, as opposed to no true difference. Low sample size bias may also make the results unreliable and they may not be reproducible in future studies.

Similarly, the lack of a representative or random sample increases the risk of the findings reflecting regression to the mean bias. As explained in Chapter 11, regression to the mean can occur in a group of observations if an outcome is extreme on the first measurement, as it will become closer to the mean for subsequent measurements irrespective of exposures and interventions.[403, 404] This was particularly important for EOGBS incidence, as St. Augustine had an extreme incidence on the first measurement, which then decreased closer towards the mean in subsequent years. In addition, the multi-level unadjusted analysis in Chapter 11, showed that geographical areas with lower than average incidence at first year tended to increase the most over the observation period and that areas with higher than average incidence at baseline tended to show above average decreases. Therefore, it is possible that

the yearly changes that occurred over time were due to the regression to the mean bias, as opposed to a change as a result of any or no prevention strategy.

Related to small sample size, to compare EOGBS and neonatal GBS resistance rates, I converted the raw numbers of resistant cases into percentages of EOGBS and neonatal GBS cases resistant to the antibiotics. This made the resistances across geographical areas and years more readily comparable as their baseline sample sizes or totals were different. While this made the comparisons more meaningful, the drawback is that for some percentages, the denominator for resistances were fewer than five. Neonatal GBS is a rare condition and data from one centre are not large enough to have a sufficient number of EOGBS cases. For such observations in the data, the representation of resistance may be blurred as small numbers become exaggerated, i.e. an observation where there was 0% or 100% resistance yet this only represented one case. Nevertheless, the majority of the data did not have denominators as small, and analysing raw numbers would also not have been comparable, as 1 in 4 cases and 2 in 30 cases are also not equivalent. A potential follow-up of this study would be to analyse the raw numbers instead of the percentages so that the results can be compared.

Secondly, although I tried to account for the most important compositional factors that had a valid theoretical foundation to be included, there were a range of challenges and weaknesses in the adjustments made. In particular for the analysis on early-onset sepsis, I was unable to account for compositional covariates of interest such as caesarean sections, fertility rate, skilled attendance at delivery, average maternal age, multiple or twin births, *per capita* government expenditure on health and GBS serotype. I had to reduce the number of covariates of interest to avoid overfitting the regression model to the small amount of data. Overfitting a model may result in the poor prediction for additional subjects, in this case areas, that were not in the original model and could overestimate the R^2 of the model.[417] In the literature there have been varying rules of thumb on how many covariates can be analysed in accordance with the sample size. Some have suggested five observations per covariate,[418] some 10 observations,[419] and others have suggested the number lies between 15 to 20 observations,[420] To keep a balance between the number of covariates to include and overfitting the model, I included 19 variables (continuous and categorical variables) for 162 observations in the largest model. More recent research has demonstrated, that for linear regression models, a minimum of two observations per covariate is adequate to estimate regression coefficients, standard errors and confidence intervals.[417] If these further covariates were included in the analysis the results might have been different.

In the remaining analyses, while I attempted to collect data on maternal risk factors, there were some I was unable to collect data for and had to exclude from the analysis. For example, preterm premature rupture of membranes is an important risk factor but I was not able to collect enough data to include it. Likewise, although I accounted for the total number of caesarean section in the study, I did not have enough data to break this down into elective and emergency caesarean sections, which have different implications for EOGBS. An elective caesarean section would reduce the risk of GBS transmission whereas an emergency caesarean section could increase it.[86] Similarly, I did not manage to collect enough data to adjust for the percentage of women who were tested and treated within the prevention programmes of each area. Although I was interested in the impact of having a guideline in place (irrespective to how well it is implemented), the differences in how well it is implemented could absolutely influence the incidence of diseases and could account for the patterns in incidence. I was also unable to include information on the testing methods used in screening programmes, for example, the type of culture media used, the sites swabbed, the use of rapid testing in addition to culture as well as the clinical risk factors that are used to offer IAP. These could equally influence the number of women treated, the incidence of diseases, therefore, the trends in the diseases. In particular, whether IAP is offered to all preterm births or not, is crucial because the burden of GBS is higher in preterm than term births,[5] however, the majority of preterm births would not be eligible for screening at 35 to 37 weeks. Treating all preterm births could have a substantial impact in areas that did so as part of their screening and/or risk-based prevention. In addition, there could be a wide range of compositional variables which I may not have considered as they have not been mentioned in the literature, yet could be accounting for the results. Any of these factors, beyond those controlled for in the study, may account for the results I found.

Even for the compositional covariates that I did adjust for in the analyses, many were not available across the different areas, or years, and were limited to their secondary and cross-sectional nature. For many covariates, especially maternal risk factors, such as GBS colonisation, prolonged rupture of membranes and intrapartum fever, I used a one point estimate for each geographical area. Although this provided some adjustment for these factors across geographical areas, there may have been a fluctuation of these factors across the years, which could influence the trends in outcomes. Secondly, for prolonged rupture of membranes and intrapartum fever in particular, I imputed the majority of the data from other countries, as most institutions did not provide this information. Therefore, the rates across many countries were the same for each world region. This was the best available alternative to include these factors in the models, however, it may not represent the specific context or burden of risk factors for each country. The definition of the compositional covariates may also have differed

in cases where the institutions provided the data which could affect the results. I could not account for this in the analysis as that would have resulted in too large a number of covariates. These limitations are expected and commonly seen in secondary research.

I was also unable to adjust the multi-level analysis for the compositional covariates due to the missing data across the years and the inability to impute them accounting for the data structure. Running this model without imputation would have reduced the sample size to below 50% of that achieved. Should this study be re-run, I would use the most recent data values across each of the years for each of the compositional covariates (not perform any imputation) and perform the multi-level repeated measures model on all of the completed cases (as they would no longer have missing data) to assess how this would alter the results.

Thirdly, the predictor variable of GBS prevention strategy also contains some weakness. I set out to investigate the relationship between the trend in annual EOGBS incidence with the annual prevention strategy, which would allow me to account for the change in prevention strategy and the impact this had on the outcomes. I tried to collect the GBS prevention strategy by year, or when it changed across the years, however, I did not receive data on this for every geographical area. Furthermore, for institutions that provided national guidelines, it was difficult to link these guidelines to the year when the hospitals covered changed their guidelines. Consequently, I chose the least biased strategy, to use the most recent GBS strategy during the period that data were provided. There are limitations in using this method as the most recent strategy may have only been in use for a short while. For example, the recent GBS strategy may have only been in place for a year as opposed to majority of the period where EOGBS incidence data were provided. Similarly, the majority of observed trends in EOGBS incidence might have actually been during the period where the previous GBS prevention strategy was used and may not be entirely attributable to the recent strategy. For example, in the US, there was a drastic decline between the 1990s and 2002, when either a risk-based or screening guideline was adopted, followed by a lower decrease thereafter when a screening guideline was adopted. In this case, we know that the initially drastic decline was during either guideline and not during screening. However, in this analysis, the decrease would be attributed to screening, as this is the recent guideline. In this study, the following areas changed guideline more than once. Only the US changed from no prevention to ‘either prevention’ to screening, and only two areas (Buenos Aires and Sao Paolo) changed from no prevention to risk-based prevention to screening. The others moved directly from one strategy to another. In addition, during the period of ‘either prevention’ in the US as well as geographical areas in this study, it is not clear which prevention was used more widely across institutions.

Fourthly, there are weaknesses in the definitions of the outcomes. As discussed throughout this chapter, different areas defined outcomes differently and I made an attempt to account for this in the analysis with respect to the number of days. In the survey, I requested the definition in terms of positive/negative culture, however, it was not completed by many institutions. Therefore, the definitions in this respect may have differed across geographical areas in this study. In the areas that did provide information about definition, the majority stated that it was positive sterile culture. As discussed in Chapter 2, a related limitation, that must be kept in mind for the EOGBS analysis, is that, although culture-proven EOGBS is the standard outcome, it is likely to underestimate the true burden of the disease due to IAP given to women. This would, consequently, overestimate the impact of prevention interventions. Another limitation is that I considered the rate of EOGBS in all births not in term births alone. Term births (37 weeks onwards) are the group who are eligible for screening and for whom screening could have an impact, as the recommended time for testing is 35 to 37 weeks. The results comparing GBS prevention strategies may be different if trends are assessed in term births alone.

For antibiotic resistance, different methods can be used to test susceptibility/resistance and different guidelines can be used to interpret antibiotic sensitivities/resistances.[421, 422] Due to a lack of time and resources as well as an overwhelming questionnaire, the differences in the testing and interpreting methods between geographical areas were not accounted for. Different methods can produce different results and different guidelines define resistance differently.[421, 422] While attempts are in progress to harmonise the guidelines, consensus has not yet been reached and countries in Europe and North America use different guidelines.[422] The lack of these adjustments in this study put the findings at risk of bias as the differences in methods and guidelines could be contributing to the higher increases under screening prevention. Nevertheless, in one meta-analysis on antibiotic resistance in paediatric urinary tract infections caused by *E. coli,* there were no differences in resistance rates by antibiotic sensitivity guidelines reported or not in studies.[423] A broader drawback of this study investigating the harms from screening is that I was unable to account for the many potential harms that were identified in the systematic review in Chapter 7. In particular, the key harms of gut microbiota changes, cerebral palsy, penicillin resistance and maternal anaphylaxis could not be explored as they require a long follow-up and/or can be very rare and were therefore not feasible. While I performed the analysis on the best available outcomes and data that could be practically collected, there are other potential harms from GBS screening and this study is not comprehensive.

Another statistical limitation of the analyses except for EOGBS incidence was that I was unable to account for the hierarchical structure of the data in the analysis. The data for each year were nested in the geographical area from which they originated; however, I combined all of the data across different geographical areas for each year to present the annual trends. The outcomes over time within each geographical area may be correlated with each other, thus, they are not independent. An important assumption of linear regression techniques is that the observations are independent. A consequence of not accounting for the hierarchical structure is that I may have underestimated the standard errors of the regression coefficients, which usually results in the overestimation of the statistical significance.[424] When setting out to undertake this study, I did attempt to account for the hierarchical structure by performing a multi-level model, however, with so few completed observations it was not possible.

As a result of all of these limitations, the predicted values particularly for the incidences of early-onset sepsis, LOGBS, *E. coli* and the percentages antibiotic resistance started with negative values below zero. While this is technically or statistically plausible as in linear probability models predicted values can lie outside zero, these values are implausible for the range of the outcomes, and not be a representation of the truth.

Finally, there are wider weaknesses of epidemiological ecological studies. The purpose of this study was to investigate the effect of GBS prevention strategy on the universal GBS screening outcomes at the population or ecological level. Therefore, these findings must be interpreted at this level and care must be taken not to fall into the ecological fallacy. This occurs when one makes conclusions about individuals based on analysis of group data.[425] I did not collect data at the individual level, in particular, on the type of tests women received or whether they received tests or treatment at all, therefore, interpretations of the impact of GBS prevention strategies for individuals cannot be made. Likewise, epidemiological studies are observational in their nature and are, therefore, not as robust as RCTs. Due to the observational nature, a causal link cannot be determined between GBS prevention strategies and the outcomes explored. My intention was to find an alternative to RCTs but, as a result of the limitations listed above that are common to observational studies, survey questionnaires and secondary data collection methods, I could not account for many factors. These factors, within and beyond the prevention strategy, could have caused the results.

14.3 Research and policy implications

Despite the potential implications of these ecological trend analyses on the strategies to adopt for the prevention of EOGBS, there is substantial uncertainty around the effectiveness and harms of universal GBS screening compared with other, or no prevention strategies. This uncertainty stems from the instability observed in the analyses, insufficient sample sizes as well as a series of unavoidable limitations in the data collected. Therefore, it is imperative to be cautious as no firm conclusion can be drawn about the impact of GBS prevention strategies.

There was instability in the comparison of EOGBS incidence in universal screening with other prevention strategies (research question 4), particularly no prevention. Nevertheless, in all the analyses, screening showed a decreasing trend in EOGBS incidence. Furthermore, from the predicted values it appears that countries adopting a risk-based prevention strategy may be experiencing an increase in EOGBS of approximately 0.01 to 0.02 per 1,000 livebirths every year, as also shown in the UK[5] and Netherlands.[66] By switching to universal screening, these countries may be able to prevent EOGBS cases and reduce their incidence by 0.02 per 1,000 livebirths every year (as estimated from the predicted values). Again, it is important to bear the instability in the analyses and the limitations in mind, which mean these estimations are not certain. Furthermore, it is not clear how these changes relate to the mortality or long-term disability from EOGBS.

As discussed in Chapter 2, focusing on culture-proven EOGBS as the outcome is likely to underestimate the true burden of the GBS disease due to IAP given to women. As an alternative, when investigating the impact of universal GBS screening on the trends of early-onset sepsis incidence (research question 5), there was a low sample size and statistical power. Therefore, conclusions cannot be reached and the implications for the clinical practice of universal screening, or other prevention strategies, remain unknown. A larger sample size is needed to determine whether, or not, screening is useful in reducing early-onset sepsis. Should a larger sample reproduce the same results, the implication would be that universal GBS screening has no impact on early-onset sepsis. Therefore, the findings on the effectiveness of screening reducing EOGBS incidence may be over-estimated due to IAP in the blood. Equally, while universal screening may reduce EOGBS, it may increase other organisms causing infection in neonates, balancing out any effects, or it could be a result of changes occurring in the environment beyond GBS screening. Overall, it would mean that universal screening is not supported by the evidence. By contrast, if the statistically non-significant trends in this study reached statistical significance with a larger sample, they would show that, under screening early-onset sepsis increases over time, whereas under risk-based prevention it

remains relatively stable. This would imply that risk-based prevention may be a safer option than universal screening. However, for now these are hypotheses and the research question remains unanswered.

With respect to the harms of universal GBS screening (research question 6), the ecological trend analysis found that screening did not lead to an increase in LOGBS, and there was no evidence that it increased early-onset *E. coli* and clindamycin and erythromycin resistance in neonatal GBS isolates, compared with other GBS prevention strategies. The findings for LOGBS incidence per 1,000 livebirths were based on a sufficient sample size and the main model and all sensitivity analyses showed that incidence was not higher under screening compared with other prevention strategies. This implies that if countries choose to adopt universal screening, LOGBS would not increase over time as a result of the programme. The main model suggests that countries adopting risk-based prevention may be experiencing an increase in LOGBS incidence that is larger than the increase seen in screening. The predicted values showed that on average LOGBS under risk-based prevention would increase by around 0.024 per 1,000 livebirths per year. Similarly, LOGBS under no prevention would also increase by around 0.08 per 1,000 livebirths. By contrast, LOGBS under screening would increase at a lower rate around 0.009 per 1,000 livebirths per year. Whether these lower rates would occur by changing prevention strategies is uncertain and should be treated with caution, as the higher rate in risk-based and no prevention strategies compared with screening lost statistical significance in the sensitivity analyses. What is more certain is that LOGBS would not increase more highly if countries adopted screening compared with other or no prevention strategies.

Given the low statistical power in the analyses for the remaining harms, it is difficult to reach implications on whether universal GBS screening increases early-onset *E. coli* and GBS resistance. A larger sample size is needed to determine whether screening would impact these outcomes. Should a larger sample reproduce the same results, the implications would be that universal screening does not lead to a larger increase in early-onset *E. coli* and GBS resistance compared with other prevention strategies, and does not pose this harm. This might be more convincing for early-onset *E. coli* and clindamycin resistance that increased at similar rates for screening and risk-based prevention. For neonatal GBS cases resistant to erythromycin there were marked differences between screening and particularly risk-based prevention. According to the predicted values, neonatal GBS resistance would increase under screening by around 0.95% every year, whereas under risk-based prevention it would only change by 0.05% and under no prevention by 0.15%. These differences might be clinically significant and could also be statistically significant in a larger sample size. However, in terms of

informing national policy, local epidemiology of the resistance rates within a country and the predominant circulating clones might be most important.

Given the limitations of these studies, further research could build on the work that has already been covered here, with improvements made on the data. The instability in the EOGBS and LOGBS results might be due to the small sample size achieved. Although the sample size met the power requirements to detect an annual difference of 0.1 per 1,000 livebirths, the differences observed between the years was lower than this and may still be clinically significant. Therefore, increasing the sample size could lead to more stable results. Likewise, for the remaining studies, the sample size was not sufficient to determine the impact of universal GBS screening. A larger sample size is needed to assess early-onset sepsis and the harms from screening, one that is even larger than 63 observations per group, so that clinically meaningful differences that are below 0.1 per 1,000 livebirths per year between strategies can be detected. Funding and time constraints also meant that I was unable to obtain data on the screening strategy by year or the compositional variables by year. Likewise, I was unable to collect data for maternal risk factors for many geographical areas. If all of this information could be collected, adjustment could be more comprehensive and informative, and conclusive answers might be reached about the impact of universal GBS screening *versus* other prevention strategies.

Ultimately, RCT evidence with a long-term follow up is needed to inform the benefits and harms of universal GBS screening. The current data that are available have many weaknesses, making it difficult to combine and analyse together. It might possible to perform an RCT for GBS screening in another country with a higher incidence of EOGBS (such as countries across Africa and Latin America). The incidence in the UK is low, and so whilst an RCT is possible and would answer the question, it would require an a very large sample size. On the other hand, as mentioned in Chapter 2, the positive predictive value of the screening test would be very low and overtreatment high, even if a large enough sample size was reached. An alternative strategy to RCT evidence and ecological comparisons between countries, would be a study combining international datasets on the outcomes of screening, risk-based and no prevention data at the individual level. If this is done for as many countries as possible, a sufficient sample size could be achieved for a quasi-experimental study. I initially attempted this method for this thesis and sought databases containing such data in the US and UK, however, they were not available or permission was not granted. For the meantime, the screening criteria that there should be evidence proving the clinical effectiveness of screening, and evidence that the benefits should outweigh the harms, are not met. The results from these studies contain some instability and numerous limitations in the data, all of which contribute

to substantial uncertainty. Trends in EOGBS incidence might decrease under screening compared with risk-based prevention, however, whether this decrease is clinically significant and outweighs the harms, remains unanswered.

14.4 Conclusions for this chapter

- The ecological trend analysis studies highlighted that EOGBS incidence declined by approximately 0.02 per 1,000 livebirths per year under universal GBS screening, whereas it increased by approximately 0.01 to 0.02 per 1,000 livebirths per year under risk-based prevention.
- There was little evidence of a difference in the trends of EOGBS incidence between screening and 'either prevention'. Areas that currently do not have GBS prevention displayed conflicting findings, with some analyses showing an increase, and others a decrease, in EOGBS incidence.
- There was no harmful impact of universal GBS screening on the trends of LOGBS incidence compared with risk-based prevention, 'either prevention' or no prevention.
- There was no evidence of universal GBS screening having an impact on annual early-onset sepsis trends compared with other, or no prevention strategies; however, this study did not have a sufficient sample size.
- There was also no evidence that early-onset *E. coli* incidence and the percentage of GBS cases resistant to clindamycin and erythromycin increase under universal screening and widespread IAP treatment, compared with risk-based or no prevention. Importantly, these analyses also did not have a sufficient sample size.
- Therefore, the last two results might be a result of low statistical power as opposed to no true difference.
- All of the remaining results must also be treated with caution as they contain some instability across the analyses and numerous limitations in the data, all of which contribute to substantial uncertainty in the findings.
- The evidence on the benefits and harms of universal GBS screening compared with other, or no prevention strategies remains inconclusive. Larger sample sizes with better data may be able to provide more conclusive answers.

PART IV. DISCUSSION & CONCLUSION

15. DISCUSSION & CONCLUSION

15.1 Original research aims restated

EOGBS is a rare but important health condition affecting 0.57 per 1,000 livebirths with a case fatality rate of 5.2%[5] and 9% to 16% suffering from long-term disability.[8, 54, 7] To prevent the transmission of GBS colonisation from mother to neonate and the progression to EOGBS, currently in the UK, a risk-based strategy is adopted where women who appear with known GBS risk factors are offered intrapartum antibiotic prophylaxis. As EOGBS incidence has risen across time in the UK and more than 30% of cases do not have risk factors, many advocate introducing universal GBS screening. In 2012, the UK NSC reviewed and recommended against introducing a universal GBS screening programme, concluding that there was insufficient evidence to ensure that the benefits of screening would outweigh the harms.[23] The review identified the following three key gaps in the literature.

Firstly, there was lack of evidence on the natural history as to why around 36% of mothers transmit GBS colonisation to their neonates and why 1% to 3% develop EOGBS (NSC criteria 1). Secondly, there was a lack of evidence on the harms from IAP and expanding its use in a screening programme (NSC criteria 13). Thirdly, with no RCT evidence, there was much uncertainty on the effectiveness of GBS screening (NSC criteria 11). With large sample size requirements, RCTs might not be feasible and a method was required to assess the impact of GBS (and other screening programmes of rare conditions) in their absence. Beyond these gaps, there was also a lack of information on the wider context on how policy decisions for screening programmes, such as GBS, are made. In this thesis, I addressed these research gaps to examine whether the GBS screening programme meets the screening criteria when these gaps are addressed, and whether the UK should introduce the programme as a result.

15.2 Research aims addressed

In this section, I will first present the findings on the systems and policy-making processes for screening in general and then in relation to GBS (research question 1). I will combine the findings from the literature review, the systematic reviews and the ecological trend analyses to assess universal GBS screening against each of the key international screening criteria. I will present the thesis findings that addressed criteria on the natural history of the condition (research question 2), the effectiveness of universal GBS screening (research question 4 and

5) and whether the benefits of universal GBS screening and large scale IAP outweigh the harms (research question 3 and 6). To present a comprehensive assessment of GBS screening, I will also discuss the criteria on the epidemiology of the condition, the test accuracy of selective rectovaginal culture at 35 to 37 weeks and the effectiveness of IAP treatment, based on the literature review findings (Chapter 2).

15.2.1 Screening policy-making (research question 1)

Findings from the systematic review on the systems and policy-making processes for screening (research question 1, Chapter 5) across 14 countries showed that all countries had a national body responsible for screening recommendations. These organisations assessed scientific evidence for a screening programme against a list of screening criteria or a list of key questions and a framework to judge whether the benefits of screening outweigh the harms from the programme. However, once these national screening recommendations were formulated, they were not enforced in all countries. Screening recommendations became regulations that required national implementation only in some countries. In other countries, the recommendations were similar to best practice guidelines, and local and regional health authorities could decide all or some of the screening programmes for their citizens. Therefore, screening practices may vary within countries.

Interestingly, in 15 of the 17 countries, the screening recommendations for GBS were not developed by the organisations that were nationally responsible for screening. With the exception of Canada and the UK, in the majority of countries, recommendations were produced by professional medical societies. In the US, they have been developed by the CDC, which is a preventative health organisation, but not the USPSTF, which is responsible for screening recommendations. It is not known whether these organisations took the key screening principles into account, and this may have implications on whether the critical and likely unseen harms of GBS screening have been considered.

There was also divergence with respect to the specific screening criteria that were utilised across countries. One of the most important criteria was that different countries required different levels of scientific evidence. Unlike the UK, 10 of the countries did not specifically require RCT evidence to prove screening effectiveness in reducing morbidity and mortality. Canada, Sweden, and the US may fulfil this need with the use of GRADE or similar tools for quality appraisal and strength of recommendation. The difference in evidence approach may reflect the decision-making structures for screening within the countries. Requiring RCT evidence may be more appropriate in countries where national screening decisions are similar

to regulations, whereas GRADE methodology may be more appropriate in countries where the recommendations are guidelines that regional or local health authorities decide to follow. This difference could have implications on whether programmes are introduced or not, as evidence other than RCT contain biases that could overestimate the benefits of screening. Another striking criterion that was not utilised in any country was the requirement where, if quality assurance is not met, the screening programme should be stopped. Sweden stated in their manual, that there should be criteria of when to stop a programme but this was not reflected in their criteria statement and how this should be done was not mentioned.

The differences in the organisations making GBS screening recommendation as well as the differences in the criteria and evidence requirements might explain why some countries recommend and implement universal GBS screening while others do not. With screening decisions not requiring adherence and many national screening bodies not having a GBS screening recommendation, lower level authorities may develop their own policies.

15.2.2 EOGBS condition, epidemiology and natural history (criterion 1)

Natural history

From the literature review (Chapter 2), the best evidence reported that 21% of pregnant women (approximately 150,806 in a year) are colonised with GBS in the UK. Without treatment, the best available estimate is that 36.4% would transmit GBS to their neonates and 1% to 3% of colonised neonates would develop EOGBS. The systematic review investigating whether bacterial load or bacterial molecular markers are associated with GBS transmission from mother to neonate, or progression from neonatal colonisation to EOGBS disease, (research question 2, Chapter 6) suggested that the natural history of maternal GBS colonisation has not been extensively researched and is still in its infancy. In addition to bacterial load, only three bacterial markers have been investigated.

Of all the evidence in the systematic review, the most promising finding was the association between high bacterial load and GBS transmission or EOGBS. This association was evident across different definitions of bacterial load and across study settings. Women colonised with heavier GBS bacterial load were approximately two to three times more likely to have a neonate with GBS colonisation compared with mothers with lighter GBS bacterial load. Neonates colonised with heavier compared with lighter GBS bacterial load were also at higher risk of developing EOGBS. While the association between bacterial load and 1) vertical transmission of GBS colonisation, and 2) neonatal colonisation *versus* invasive EOGBS was

consistent, evidence on the association between bacterial load and transition from maternal GBS colonisation to EOGBS was not as clear, possibly due to the small number of EOGBS cases in each study.

With respect to serotype, the pooled results from the meta-analysis estimated that neonates colonised with serotype III were approximately 1.5 to two times more likely to develop EOGBS than neonates with serotype Ia and II, respectively. However, the sensitivity analysis identified that the results may not be stable, especially for serotype III *versus* serotype II. The results of the meta-analysis were heavily influenced by one study, and removing it led to statistically non-significant results. Besides a potential lack of power, as confounding variables were not adequately adjusted for, there could have been specific population characteristics in the study setting for Madzivhandila *et al.* (2011) that contributed to the statistically significant findings.[39] Another study found that reaction to C-protein was not associated with EOGBS compared with asymptomatic colonisation in neonates, and neither was antigen type when serotype was accounted for.

Despite the evidence on the potential value of these factors to predict GBS colonisation or EOGBS, the risk of bias across the evidence was high or moderate. No study was at low risk of bias for all domains and the overall relationships identified in this systematic review could be partly, or completely, a result of confounding factors. The majority of the evidence is also published before the year 2000 and may have limited applicability to today's context.

Epidemiology (Chapter 2)

With IAP treatment, under the risk-based strategy, recent surveillance reported that the overall incidence of EOGBS is 0.57 per 1,000 livebirths in the UK and case fatality was 5.2% in 2014/15. Although the rates vary, studies have reported that between 9% and 16% of EOGBS survivors suffer from long-term disability. The rate of GBS-related stillbirth in the UK was approximately 4.0 per 100,000 total births in 2014; about half occurred before 37 weeks of gestation. Approximately 1% of all stillbirths in the UK were mostly or partly attributed to GBS.

Regarding the risk factors associated with EOGBS, recent surveillance in 2014/15 reported that approximately 22% of EOGBS cases were in preterm deliveries. In England and Wales, EOGBS incidence was inversely associated with gestational age at birth, decreasing from 4.42 per 1,000 livebirths before 28 weeks of gestation to 0.41 per 1,000 livebirths after 37 weeks. Prematurity was also an independent risk factor for death. Risk factors based on NICE and

2012 RCOG guidelines were present in 41.3% and 35.4% of EOGBS cases, respectively, but only 44% of those with RCOG risk factors were treated with IAP. The percentage of neonates with EOGBS born at term to mothers without any RCOG or NICE risk factors was 63% to 67%. As these cases would not be detected by the risk-based prevention strategy and would be born after 37 weeks, this is the cohort that universal GBS screening would try to detect. Thirty-seven percent of EOGBS deaths had at least one RCOG risk factor for GBS; only one mother of the 27 EOGBS babies who died received IAP. There were 10 deaths in babies with EOGBS born after 35 weeks' gestation; 60% to 70% of them did not have any maternal risk factors based on NICE and RCOG risk factors, respectively. Similarly, of the nine EOGBS deaths in neonates born after 37 weeks, the number without any maternal risk factors was between 56% to 67% of EOGBS deaths. These are the deaths that would not be prevented by the risk-based strategy, which universal GBS screening would try to prevent.

Overall, EOGBS is an important health condition, however, the natural history from GBS maternal carriage to EOGBS disease remains poorly understood. The most consistent evidence was that heavy bacterial load was associated with EOGBS, however, the evidence on this and bacterial markers contains uncertainties due to the risk of bias. Therefore, this criterion is not met. Research is required to fill the evidence gap on why mothers transmit GBS and why neonates develop EOGBS disease.

15.2.3 Test accuracy (criterion 4)

The recommended screening test for detecting GBS is selective enriched culture of rectovaginal swabs at 35 to 37 weeks gestation; however, it is not accurate in predicting EOGBS. Based on the literature review (Chapter 2), I estimated two PPVs for the ability of maternal GBS colonisation at 35 to 37 weeks to detect EOGBS using two approaches, and found a rate of 0.2% and 0.4%. There are also no agreed PPVs for antenatal culture detecting GBS colonisation at birth with a range of values reported. PPVs in the literature review ranged from 67.4% to 89.1%. Most studies show that approximately 20% to 30% of pregnant women who test positive for GBS at 35 to 37 weeks test negative during labour. Another limitation of this test is that it excludes the majority of women with preterm birth who have worse outcomes from GBS. Therefore, this criterion is also not met.

A better test is urgently required, however a poor understanding of the natural history of GBS vertical transmission and progression to EOGBS inhibits the ability to identify one. Developments are emerging in rapid intrapartum testing with real-time PCR showing the most promising results. However, practical limitations of rapid testing prevent its widespread use,

including its cost, its complex administration, and its inability to determine antibiotic sensitivity to direct the choice of antibiotic for women allergic to penicillin.

15.2.4 Effectiveness of IAP treatment (criterion 9)

IAP is currently the recommended treatment for EOGBS prevention. As shown in the literature review (Chapter 2), a Cochrane meta-analysis concluded that IAP appears to be effective in reducing culture-proven and probable EOGBS by 83%, compared with no treatment. However, this was based on three small and old trials at high risk of bias, therefore, the extent to which IAP decreases EOGBS disease and related morbidity and mortality is uncertain. In addition, there was no evidence that IAP reduced the incidence of all-cause mortality, mortality from GBS infection or from infections other than GBS. Observational findings have also suggested that the timing and duration of IAP have an impact on its effectiveness, with rates of EOGBS and clinical sepsis higher in mothers who received IAP for less than 4 hours compared with those who received it for four or more hours. Patients who received substandard IAP of clindamycin due to reported penicillin-allergy also showed a reduced effectiveness of IAP. As a result of the uncertainty in the effectiveness of IAP, this criterion is also not met. Higher quality evidence is required to address this, although RCTs may not be feasible when IAP has become the recommended treatment.

15.2.5 Effectiveness of universal GBS screening (criteria 11)

The literature review (Chapter 2) showed that the evidence on the effectiveness of universal GBS screening is limited. There have been no RCTs assessing the effects of screening on EOGBS and in their absence, it is difficult to quantify the impact of adding universal screening to current practice. Instead, there are observational studies using historical controls and comparing EOGBS incidence in different periods of time in which different GBS prevention strategies were adopted. The control periods (no prevention and/or risk-based strategies) precede the universal screening periods. Risk of bias from this kind of approach is well documented as participants in the study and control group are not contemporaneous, data are collected retrospectively and confounding factors are not usually considered adequately. The majority of the studies as well as pooling of the studies show that universal screening reduces the risk of EOGBS compared with no prevention and risk-based prevention, however, there have been some inconsistencies (Chapter 2). Finally, as most studies have not controlled for confounding variables, results may be country-specific.

In the absence of RCT data, in Chapter 11, I attempted to find an alternative approach to assess the potential impact of universal screening on EOGBS (research question 4). The approach was to combine ecological data from different countries and compare the trends of EOGBS incidence under the different strategies, while controlling for confounding factors. This proved to be a difficult task due to the differences in the data across countries and the limitations of retrospective data collection. As a result, there was substantial uncertainty around the impact of universal GBS screening on the trends of annual EOGBS incidence compared with other, and no GBS prevention strategies.

Bearing this caution in mind, the majority of the analyses showed that there was a decrease in in annual EOGBS incidence in areas that adopted screening whereas there was an increase in annual EOGBS incidence in areas that adopted risk-based prevention. In areas that adopted screening, the predicted values during the study period showed that EOGBS incidence decreased from around 0.8 to 0.9 per 1,000 livebirths in 1990 to 0.2 to 0.3 in 2015 in the adjusted linear regression, and from around 0.66 per 1,000 livebirths in 1990 to 0.19 in 2015 in the multi-level unadjusted regression. On average, EOGBS incidence decreased at around 0.02 per 1,000 livebirths yearly under screening. By contrast, in areas adopting risk-based prevention, the predicted values during the study period showed that EOGBS incidence increased from 0.1 per 1,000 livebirths in 1990 to 0.50 in 2015 in the adjusted linear regression and from 0.3 per 1,000 livebirths to 0.75 in the unadjusted multi-level regression. On average, EOGBS incidence increased by around 0.01 to 0.02 per 1,000 livebirths under risk-based prevention. Trends in EOGBS incidence in areas that adopted 'either prevention' strategy decreased by around 0.02 per 1,000 livebirths every year, and when compared with the trends in areas that adopted screening, there was no statistical significance in the majority of the analyses.

Results in areas with no prevention were complicated by greater uncertainty across the analyses as different assumptions and models revealed conflicting trends in EOGBS incidence. In particular, there was a strong influence of data from St. Augustine, which had extremely high EOGBS incidence that dramatically decreased. When St. Augustine was included in the analysis, there was no statistically significant difference in the trends of EOGBS incidence between areas that adopted screening and areas with no prevention. In the unadjusted multi-level analysis, EOGBS incidence decreased from around 0.84 per 1,000 livebirths in 1990 to 0.74 in 2015 during the study period, while in the adjusted linear regression EOGBS incidence decreased from 0.94 to 0.91 per 1,000 livebirths. By contrast, when these data were excluded from the analyses, trends of EOGBS incidence increased from 0.44 in 1990 to 1.11 per 1,000 livebirths in 2015 in the adjusted linear regression and from -

0.48 to 1.0 per 1,000 livebirths in the unadjusted multi-level analysis, which were statistically significant. Therefore, in countries with extremely high EOGBS incidence, there may be reasons in their context, beyond the GBS prevention strategy, that cause the high and drastic change in EOGBS incidence.

While these trends provide some useful exploratory information, it remains difficult to quantify the potential impact of screening, as there were many limitations in this study (see Section 15.3 for a more thorough account). A lack of a random sample with an extreme EOGBS incidence from St. Augustine at first observation that reduced closer to the mean in subsequent observations, may reflect regression to the mean for results including these data. In addition, the prevention strategy analysed was the most recently adopted prevention strategy and the reduction across time might be attributable previous strategies. The results were also on all livebirths, including preterm births who would not be eligible for screening. Finally, there were limitations in the confounding variables accounted for in this study as some were not available annually and some were imputed from other countries. Therefore, there is a considerable risk of bias in this study.

Most studies in the literature focus on culture-proven EOGBS (which is the standard outcome), however, any changes may reflect a decreased likelihood of neonatal cultures being positive due to IAP use, with the culture negative cases of EOGBS being undetected. Findings on early-onset sepsis have been more inconsistent than that on EOGBS, making the impact of universal screening difficult to assess (Chapter 2). Using the same approach, in Chapter 12, I combined ecological data from different countries and compared the trends of early-onset sepsis incidence under the different GBS prevention strategies, while controlling for confounding factors (research question 5). These results showed that there was no difference in the early-onset sepsis incidence by GBS prevention strategy. However, the sample size in this study was not large enough to make any reasonable conclusions. Alternatively, studies from the literature review have shown similar results that early-onset sepsis incidence does not decrease under screening. Early-onset sepsis incidence may not reduce in screening programmes (even if EOGBS incidence reduces) because of the selection pressure from IAP changing the profile of the organisms causing sepsis. Widespread IAP might decrease EOGBS yet increase gram-negative organisms causing infection, resulting in no overall reduction (Chapter 2).

Overall, this methodological approach could not be an alternative to RCT evidence, due to the lack of adequate data on GBS related variables across countries. As a result of the limitations in the available data, there is a substantial risk of bias in this study, and it remains difficult to

assess the impact of implementing universal GBS screening. Furthermore, the impact of universal screening on EOGBS related mortality and long-term disability was not estimated in this study due to the small sample size. Therefore, the effectiveness of universal GBS screening is not known and the criterion remains unmet.

15.2.6 Benefits of screening programme outweigh the harms (criteria 13)

As indicated above, it is not possible to quantify the benefits of screening, therefore, it is not possible to assess whether the benefits would outweigh the harms. Consequently, this criterion is unmet. Nevertheless, an examination of the potential harms is a standard part of the assessment of any screening proposal. As approximately 150,800 pregnant women would be eligible for IAP, of whom approximately at least 99% will gain no health benefit, it is particularly important in this context. Therefore, I attempted to quantify the harms side of the screening equation and in this section, I will summarise the findings on the harms of universal GBS screening, particularly from expanding IAP.

Findings from the systematic review (research question 3, Chapter 7) and the ecological study (Chapter 13) show that the harms from universal GBS screening and IAP expansion are unclear and cannot be quantified. The systematic review (Chapter 7) showed that the occurrence of harmful outcomes from IAP and their clinical importance has not been well explored. The systematic review resulted in a wide range of adverse events reported in 17 observational studies and 13 RCTs, including gut microbiota, antibiotic resistance, maternal thrush, bowel problems, cerebral palsy, functional impairment and neonatal infection. However, there was little high-quality evidence to determine the frequency of adverse events from IAP for neonatal GBS disease prevention. The studies were small and at high risk of risk bias. More importantly, there was a substantial evidence gap around the long-term effects of IAP.

There was only one study that assessed the long-term impact on the effects of IAP in women, on their children at age seven. This RCT showed an increased risk of serious consequences, such as cerebral palsy as well as bowel problems and functional impairment. However, the applicability of these findings is uncertain, as the drugs investigated were erythromycin or amoxicillin-clavulanate given for 10 days or until birth to a population in preterm labour. The drug recommendation for GBS IAP treatment is penicillin or clindamycin, given for shorter durations, at or near, term labour (Chapter 2). In this trial, the effect size was also small, and with multiple statistical comparisons on the same population, the probability of a chance result

is increased. Furthermore, the plausible biological mechanisms through which IAP can cause the development of cerebral palsy are not known.

On the other hand, studies with improved applicability that explicitly included IAP for GBS prevention consistently found that IAP could alter gut microbiota. However, all of these studies were observational and populations in these studies were not followed to clinical outcomes. Therefore, whether microbiota alterations from IAP are associated with any short or long-term health problems is not known.

There was also evidence that IAP may increase the risk of neonatal infections compared with no treatment, however, the evidence was at high risk of bias and inconsistent across studies. One study reported that IAP can increase the proportion of late-onset bacterial infections in infants, however, the indication was not stated and when the analysis was restricted to penicillin, the results were no longer statistically significant. In the ecological study on the harms from screening (Chapter 13), I investigated the impact of different prevention strategies on LOGBS incidence. While LOGBS incidence increased in areas adopting screening, the magnitude of this increase was lower than in areas adopting risk-based or no prevention. According to the predicted values, while LOGBS increased by around 0.02 per 1,000 livebirths on average every year under risk-based prevention and by around 0.08 per 1,000 livebirths on average under no prevention, it increased by around 0.009 per 1,000 livebirths on average under screening. While the statistical significance of the results varied in the sensitivity analysis, the direction did not change. Therefore, there was no evidence from this study that there is harm of increasing LOGBS incidence from introducing a screening programme. However, the limitations of the ecological study on the effectiveness of screening (in Section 15.2.5 above) apply here as well. While these were the best available data, there were limitations in the predictor variable of prevention strategy and the confounding variables, resulting in a substantial risk of bias in this study.

In addition to late-onset infections, one study in the systematic review found no difference in early-onset *E. coli* sepsis between neonates whose mothers were treated with IAP compared with those who were not (Chapter 7). In the ecological study (Chapter 13), I investigated the impact of different prevention strategies on early-onset *E. coli* incidence. In this study, there was no evidence of any differences in the trends of early-onset *E. coli* by prevention strategy. However, this study had a small sample size and did not have sufficient statistical power for confirmatory results. Therefore, similar to the results from the study on early-onset sepsis, these results may not be representative and a larger sample size may show different findings. The majority of the current evidence in the literature supports the findings in this study that

screening does not lead to an increase in early-onset *E. coli*, however, this evidence is limited as these studies are observational before and after studies and thus subject to bias (Chapter 2). It is not clear whether universal GBS screening would impact early-onset *E. coli* incidence or not.

The final key finding from the systematic review (Chapter 7) was increased antibiotic resistance in infants whose mothers were treated with IAP compared with those who were not. In one RCT there was evidence of azithromycin resistance in *S. aureus* and *S. pneumoniae* strains, however this was from azithromycin treatment. Two observational studies showed a higher proportion of ampicillin resistant organisms in infants whose mothers were treated compared with untreated mothers. Two further studies did not find differences in the cephalosporin or ampicillin resistance and amoxicillin resistance between treated and untreated groups. However, this observational evidence was at high or unclear risk of bias due to confounding variables and not all of the evidence was not related to IAP treatment for GBS.

In the ecological study (Chapter 13), the differences between the percentages of EOGBS and neonatal GBS cases resistant to clindamycin and erythromycin were not statistically significant by GBS prevention strategy. However, similar to early-onset *E. coli* incidence, the sample size achieved in this study lacked statistical power, therefore, it is difficult to reach conclusions as to whether adopting a universal GBS screening would have an impact on resistances rates or not. Larger sample sizes may find different results, especially for neonatal GBS resistance to erythromycin, where areas adopting screening increased more steeply from approximately 3% to 22.5% whereas areas adopting risk-based prevention had a steady rate and areas with no prevention changed by around 3%. Literature on the topic generally shows that rates of resistance are higher in countries adopting screening compared with countries that have adopted risk-based prevention strategies. However, there have been no formal comparisons on neonatal resistance across different strategies. Based on the literature and this study, conclusions about the impact of antibiotic resistance from universal GBS screening are not known. Nevertheless, GBS does remains almost universally susceptible to penicillin,[14] although there are recent small and poor quality studies in Ethiopia and in Italy with evidence of penicillin resistance in GBS isolated from pregnant women [179-181] and in the US, GBS isolates have shown reduced susceptibility to beta-lactams while in Japan, GBS isolates are showing reduced susceptibility to penicillin.[182, 18]

Overall, the harms from universal GBS screening and large scale IAP cannot be quantified. However, the scale of the overtreatment and range of plausible harms require a better understanding before IAP can be safely expanded. In the era of antibiotic resistance, such a

widespread IAP strategy may also be challenging in relation to the Department of Health's antibiotic resistance strategy to reduce unnecessary use of antibiotics.[426]

15.3 Thesis strengths and limitations

Throughout this thesis, I have discussed the strengths and limitations for each chapter's methodology and findings. Here, I will discuss the strengths and limitations for the thesis as a whole.

One of the strengths of this thesis is that the research aims and objectives were based on an extensive literature review and attempted to address a critical evidence gap, not only for GBS screening, but for screening as a whole. The systematic review on the policies and screening systems across countries was the first study of its kind; no previous literature had mapped the screening criteria or other processes used across countries. In addition, there are many nominated diseases for screening programmes that are rare and RCTs may never be available, thus, alternative methods to assess the effectiveness of screening are required. Specific to universal GBS screening, I addressed the key gaps in the evidence surrounding GBS that had not been previously researched. In the systematic reviews and the ecological studies, a range of experts were involved in the project to review the work and advise on microbiology, infectious disease, obstetrics and gynaecology, screening, statistics and epidemiology. This ensured the project had clinical and methodological validity. Finally, I performed sensitivity analyses to test the stability of any statistically significant findings throughout this thesis.

The searches for the systematic reviews were extensive with no date limit in order to cast a wide net and capture as much of the data as possible. To quality assure the search and ensure I found all documents, subject area experts reviewed the strategies and included articles. To assure the quality of all the review processes, each was duplicated and checked by a second reviewer.

The ecological studies were the first attempts to compare the temporal relationship of the benefits and harms of universal GBS screening across different GBS prevention strategies in different geographical areas. In addition to culture-proven EOGBS, to assess the benefits of screening I also assessed early-onset sepsis due to the limitations in assessing culture-proven EOGBS. I also assessed the key harms that were feasible to collect data for, including antibiotic resistance and neonatal infections other than EOGBS. I undertook an exhaustive

data collection process in an attempt to obtain the best available data on the GBS benefits and harms across countries. The findings from the studies were also adjusted for some of the key confounding variables including preterm births and low birthweights, as well as the methodological differences between the areas due to the limitations in retrospective data collection. Finally, by imputing data using statistical processes, I was able to perform the analyses using all of the collected data. Therefore, I attempted to reduce as much bias as possible from the methodological limitations, confounding variables and missing data.

As with all research projects, there are limitations that must be considered. While I attempted to obtain as much data as possible, for both the systematic reviews and the ecological studies, some data may be missing. In the systematic review on screening policy-making, using GBS may have limited the number of countries found from low and middle-income countries. Likewise, in the systematic reviews on the natural history of GBS and on the adverse events from IAP, I excluded articles not in English, case reports and case series. This could have increased the amount of data included from the literature, as there are laboratory studies and patient reports on small numbers of cases. Nevertheless, I excluded these studies as they have very little conclusive implications for patient outcomes and clinical practice, as the exposed groups with disease cannot be compared with control groups.

Similarly, in the ecological studies, although I undertook an exhaustive data collection processes, only 42 of 194 countries were included. In particular, there were few countries from Sub-Saharan Africa, North Africa and the Middle East that are less likely to have GBS prevention programmes. There was also a higher representation of areas that were from high income countries that are more likely to have prevention programmes. These limitations raise the risk of selection bias and the external validity of the findings. In the ecological studies, I did attempt to account for selection bias by adjusting for regional and economic differences in the study. For the analysis on EOGBS incidence, a non-random sample may also have resulted in regression to the mean bias.

The lack of collected data was more problematic for early-onset sepsis, early-onset *E. coli* and antibiotic resistance where the sample sizes were not sufficient to ensure internal validity of the studies. The lack of power means the statistically non-significant results could be incorrect as the probability of incorrectly not rejecting the null hypothesis or the type II error is increased. For these studies, as well as LOGBS, I was also unable to account for the hierarchical structure of the data in the analysis due to the lack of data, which may underestimate the standard errors of the regression coefficients and overestimate statistical significance.

Another consequence of the small number of observations is that I could not account for all of the important confounding variables that had a valid theoretical foundation. In particular, I did not manage to collect enough data to adjust for factors within the GBS prevention programmes such as the percentage of women who were tested and treated, testing methods used, and clinical risk factors used to offer IAP especially offering IAP to all preterm births with no other risk factors. As term births are the group for whom screening would be provided and could have an impact, results separated for term births only would have been more informative. There also were limitations in the adjustments that were made due to retrospective data collection methods. Some confounding variables only had a one point cross-sectional estimate and fluctuations of these factors across the years could influence the trends in outcomes. Furthermore, I imputed the majority of the data for prolonged rupture of membranes and intrapartum fever from other countries, which may not represent the specific context or burden of risk factors for each country. The definition of the confounding variables may also have differed and I could not account for this in the analysis.

Similarly, the quality of the data I collected across countries for the ecological studies varied, and some surveillance was only voluntary or hospital data, which could underestimate or overestimate incidence rates. I attempted to control for this by adjusting for the coverage of the data. The quality of data could also have varied according to the diagnostic facilities and procedures, and I attempted to control for these through economic variables, though this may not have been sufficient.

Most importantly, due to the retrospective data collection, I was unable to collect data on the differences in the GBS prevention strategy across the years in each geographical area and instead, had to use the most recent GBS strategy during the period that data were provided. The recent strategy may not reflect the strategy in place during the majority of the study period, and the observed trends might have actually occurred during the period where another strategy was used. Finally, the definitions of outcomes also differed across the studies and were not identically defined across the institutions that provided data. Due to the retrospective and secondary data collection methods, these limitations are expected. Therefore, I attempted to adjust for the differences across countries and chose the most appropriate methods for the analysis. Furthermore, I conducted sensitivity analyses to assess the stability of results where they were statistically significant in the main model. As a result of the limitations in the available data across countries, the predicted means included negative values, which were outside of the plausible range for the outcomes and not be a representation of the truth.

An important limitation of the ecological studies is that they were observational in nature, therefore, they suggest possible associations between the exposure (GBS prevention strategy) and the outcomes, however, the causality cannot be established (as would be possible with an RCT). Secondly, while the purpose of the ecological studies was to investigate the effect of the GBS prevention strategy on the population or ecological level, care must be taken not to fall into the ecological fallacy and make conclusions about the impact of universal GBS screening and IAP treatment for individuals.

Using the best available data and methodology, I addressed the gaps in the evidence on universal GBS screening. I am confident that the findings from the systematic review are robust and comprehensively demonstrate what is available. However, the ecological studies had significant amounts of missing data and issues with both internal and external validity. As a result, I was unable to accurately account for confounding variables and time structure, making it difficult to draw conclusions from the results.

15.4 Implications for policy and practice in the UK

This thesis investigated the research gaps in the assessment of universal GBS screening identified in the 2012 UK NSC review, namely the natural history of GBS, the effectiveness of screening and the adverse events from screening and expanding IAP. This was in an effort to inform whether the UK should introduce a GBS screening programme. As discussed in the summary of the thesis findings, none of the key research gaps have been addressed in the studies in the systematic reviews and the ecological studies in this thesis. As a result, there is insufficient evidence to ensure that the benefits of universal GBS screening using mainly enriched selective culture at 35 to 37 weeks would outweigh the harms. There were many weaknesses in the ecological studies as identified in the section above, which means it was not an adequate alternative to RCT data.

Keeping the limitations in mind, the results of the ecological studies imply that in the UK, where the EOGBS incidence is currently 0.57 per 1,000 livebirths under risk-based prevention, introducing universal screening would reduce EOGBS incidence by 0.1 per 1,000 livebirths by five years to 0.47 per 1,000 livebirths. Currently, there are approximately 443 cases of EOGBS in the UK (0.57/1000 in 776,352 neonates[101] born in the UK). Based on the predicted values, the expected impact of screening would be a decrease of around 16 cases every year, and by five years the number of EOGBS would be reduced to around 365 cases.

However, from the results it is not clear how these changes relate to the mortality or long-term disability from EOGBS.

With respect to the harms from screening, there are few policy implications that can be drawn. The number of harms that can be expected if countries change from adopting risk-based prevention to universal GBS screening remains unknown. Whether there would be an impact of universal GBS screening on gut microbiota changes, antibiotic resistance, early-onset *E. coli* and other neonatal infections, or have any long-term health effects, remains unclear. The ecological study implied that LOGBS incidence may increase at a slower rate if countries switch to universal GBS screening. In the UK, LOGBS incidence is currently 0.37 per 1,000 livebirths and this equates to 287 cases every year. If universal screening is introduced, LOGBS would still continue to increase. It is estimated that the following year LOGBS incidence would be 0.379 per 1,000 livebirths and 294 cases. By five years, the incidence of LOGBS would be 0.415 per 1,000 livebirths or 322 cases. Based on the surveillance in the UK, this is the rate that LOGBS incidence has been increasing under risk-based prevention, from 0.11 per 1,000 livebirths in 1991 to 0.29 per 1,000 livebirths in 2010,[81] or 0.24 per 1,000 livebirths in 2000/01 to 0.37 per 1,000 livebirths 2014/15.[5] According to the results, LOGBS in the UK would continue at around this rate and will not increase greater than it already is.

Finally, little is still known on the reasons why some GBS colonised women transmit GBS to their neonates or why some neonates develop EOGBS. With range of potential harms associated with screening and widespread IAP, bacterial factors might provide an opportunity for future prevention strategies to target patients with only the hypervirulent strains of GBS. Bacterial load and markers could provide innovative opportunities for more efficient prevention strategies, limiting the risk of harmful outcomes from widespread IAP. Bacterial load is the most promising of the factors, as despite the different measurements it was consistently associated with GBS transmission and EOGBS.

To conclude, GBS infection is an important health condition and its persistence combined with the currently poor options for screening tests and the harms from IAP stress the need for a better understanding of GBS and more effective prevention. Universal antenatal GBS culture screening so far does not meet the key UK NSC criteria needed to introduce screening programmes. We do not fully understand the natural history of why some mothers, but not others, transmit GBS to their neonates, or which neonates will develop EOGBS or suffer harm from EOGBS. Selective culture at 35 to 37 weeks gestation is not an accurate predictor of EOGBS. The proposed screening programme would offer all term pregnant women the antenatal GBS culture test, and at least 99% of screen-positive and treated mothers (and their

babies) would be over-treated. Finally, the effectiveness of IAP is also somewhat uncertain and the evidence to support a benefit of universal screening over risk-based prevention is inconsistent and confined to observational studies using historical controls. Combining these observational data across countries and adjusting for confounding variables did not prove to be adequate method to inform the benefits and harms from screening. Given the current evidence, we do not know the balance of the benefits and the harms of introducing universal GBS screening, therefore, it should not be introduced in the UK. Bacterial load, serotype, sequence type, and the more specific isolate characterisation that is feasible with the advent of genome sequencing, could potentially be involved in guiding future prevention interventions.

15.5 Implications for research

If we are to better inform the debate around the introduction of universal GBS screening, further research is needed that explores the balance of benefits and harms of screening. To measure this requires RCT evidence on the impact of universal screening on EOGBS, early-onset sepsis and the list of potential harms, with economic modelling to evaluate the associated costs. Indeed, the UK Department of Health and the Health Technology Assessment has recently decided to commission such an RCT in the UK.[427] The RCT would require a large sample size and need to have a sufficiently long follow-up to explore the harms of expanding IAP, which may be missed otherwise. However, it is estimated that 99% of women who test positive for GBS in the third trimester would not go on to have a neonate with EOGBS. The PPV of such a screening programme would be very low and overtreatment high.

The current universal screening strategy may be inadequate, and to improve the balance of benefits and harms for future proposed screening programmes, more research is needed to understand the natural history of GBS. Identifying patients with only the hypervirulent strains of GBS could help to reduce the number of women treated with antibiotics who are at low risk of having neonates with EOGBS. Although this research is required and is worth exploring, it is important to note that it may be unable to identify detectable factors above the current known risk factors that could be operationalised to change practice on who receives prophylaxis. This work should encompass research to reliably predict which mothers with GBS during labour will transmit GBS to the neonate (approximately 36.4% of GBS positive women in labour will transmit to the neonate) and which mothers will have a neonate that develops EOGBS. The characteristics may include clinical or demographic risk factors in the

mother, biochemical or molecular markers or bacterial load. This could also include an assessment of the predictive value on a combination of risk-factors and whether a risk-model could be used to identify women at highest risk. The work could also explore research to reliably predict which neonates with GBS colonisation will progress to EOGBS disease (even without IAP only up to 3% of neonates with GBS colonisation might progress to EOGBS disease). However, it may be difficult to identify neonates with GBS colonisation who will progress to EOGBS in a timely and highly accurate manner to rule out the approximately 99% of neonates with colonisation who do not go on to develop disease. There may be infant characteristics that give some prediction, although they would have to offer strong NPV to justify not treating positive infants.

Finally, test accuracy research is also needed to reliably detect GBS colonisation and bacterial load during labour (approximately 27% of GBS positive women at 35 to 37 weeks were negative during labour, and 5% of GBS negative women at 35 to 37 weeks were positive during labour). Although the latest in-labour tests have some practical issues, there may be a feasible option to more accurately measure who is colonised in labour and how heavily as well as developments in the rapid testing of antibiotic resistance. Tests must be timely to ensure that women are in the labour ward long enough to be tested and treated with IAP (optimally at least 4 hours before delivery), and even if a test is rapid enough, there may be issues around the practicality of offering tests to women who have chosen home births or midwife-led care.

In addition to universal GBS screening, there is a need to explore the risk factors used in risk-based prevention strategies to identify more EOGBS cases and how effective risk-based prevention strategies are according to the factors included. As only 44% of eligible EOGBS cases were not treated in the UK, risk-based prevention has not been properly tried or tested. Therefore, the reasons for low adherence should be investigated along with the effectiveness under higher adherence levels. It may also be interesting to compare the impact of including preterm birth as a risk factor to offer IAP compared with not including it on EOGBS incidence in both risk-based and screening programmes.

This thesis has addressed issues relating to the expansion of GBS prevention to include universal GBS screening and the beneficial and harmful impact this would have as a result. From the findings of this thesis, it is clear that we must tread cautiously as there are significant questions yet to be answered in deciding whether to implement a universal antenatal GBS screening programme. However, more research is urgently required in this area especially for the UK where the burden of EOGBS incidence is increasing. An alternative approach was trialled to replace RCT data to make screening decisions when RCT evidence is not available.

For GBS, this approach did not answer the question as GBS is a complex bacterium with many factors that can influence screening and treatment, and these covariates were poorly collected in most countries during the study period. However, beyond GBS screening, there are other rare conditions where RCT data are not available and alternative approaches to RCT evidence are required to make screening decisions. This ecological approach may work for conditions that are less complicated or better documented. On the other hand, methodologists may wish to try and combine data at the level of patients from different countries to assess if using data in this way could help to inform screening decisions.

16. REFERENCES

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17. APPENDICES

Appendix 1. Search strategies for systematic review on screening systems and policy processes across countries (objective 1)

Medline

# ▲	Searches
1	exp Mass Screening/
2	exp Policy Making/ or Public Policy/ or Health Policy/
3	exp Guideline/
4	exp Decision Making/
5	exp "review"/
6	health planning/ or exp health planning guidelines/ or exp health planning technical assistance/ or regional health planning/
7	(decision making* or decision-making*).kw,ti.
8	National Health Programs/ or Government Programs/
9	"screen*".kw,ti.
10	1 or 9
11	exp Streptococcus agalactiae/
12	"group b streptococc*".kw,ti.
13	"streptococc* agalactiae".kw,ti.
14	11 or 12 or 13
15	(polic* or guideline* or program* or strateg* or decision making* or decision-making* or process* or procedure* or review* or plan* or recommend* or committee*).ab,kw,ti.
16	exp Government Agencies/
17	2 or 3 or 4 or 5 or 6 or 7 or 8 or 15 or 16
18	10 and 14 and 17
19	limit 18 to yr="1996 -Current"

Embase

# ▲	Searches
1	exp screening/
2	policy/
3	exp health care policy/
4	exp hospital policy/
5	exp practice guideline/
6	exp health program/
7	decision making/
8	process design/ or process development/ or process optimization/
9	procedures/
10	"review"/
11	hospital planning/ or patient care planning/ or planning/ or strategic planning/ or health care planning/
12	program development/
13	exp advisory committee/

☐	14	"screen*".ti,kw.
☐	15	1 or 14
☐	16	(polic* or guideline* or program* or strateg* or decision making* or decision-making* or process* or procedure* or review* or plan* or recommend* or committee*).ti,ab,kw.
☐	17	exp consensus development/
☐	18	2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 16 or 17
☐	19	exp Streptococcus agalactiae/
☐	20	"streptococc* agalactiae".ti,ab,kw.
☐	21	"group b streptococc*".ti,ab,kw.
☐	22	19 or 20 or 21
☐	23	15 and 18 and 22
☐	24	limit 23 to yr="1996 -Current"

ASSIA
screen* AND (streptococci* agalactiae OR group b streptococci*) AND (polic* OR guideline* OR strategy* OR program* OR decision making OR decisionmaking OR process* OR procedure* OR review* OR plan* OR recommend* OR committee*)
1996-2013

SSCI
Title=(screen*) AND Title=(group b streptococc* OR streptococc* agalactiae) AND Topic=(polic* or guideline* or program* or strateg* or decision making* or decision-making* or process* or procedure* or review* or plan* or recommend* or committee*)
Timespan=1996-2013. Databases=SCI-EXPANDED.

Appendix 2. Data extraction sheet for screening policy-making systems and processes (objective 1)

No.	Country/ Level	Author	Title	Year	Disease	Screening body and authority	Criteria and how it is used	Stakeholder Involvement	Evidence synthesis methodology	Decision-making process	Other
	Country/ is it regional, national, local										
	If research article review comparing countries - put comparison										
	If research article recommending processes specify										

Appendix 3. Search strategies for systematic review on bacterial load and markers associated with GBS vertical transmission and EOGBS (objective 2)

Medline:

# ▲	Searches
1	exp Streptococcus agalactiae/
2	(group b adj streptococc*).ab,ti,tw.
3	"streptococc* agalactiae".ab,ti,tw.
4	1 or 2 or 3
5	exp Pregnancy/
6	exp Parturition/
7	exp Labor, Obstetric/
8	exp Delivery, Obstetric/
9	exp Pregnancy Complications, Infectious/
10	exp Infant/
11	(newborn* or new-born*).ab,ti,tw.
12	"infant*".ab,ti,tw.
13	"neonat*".ab,ti,tw.
14	(babies or baby).ab,ti,tw.
15	(antepartum* or ante-partum*).ab,ti,tw.
16	(intrapartum* or intra-partum*).ab,ti,tw.
17	(prenatal* or pre-natal*).ab,ti,tw.
18	(antenatal* or ante-natal*).ab,ti,tw.
19	"birth*".ab,ti,tw.
20	"pregnan*".ab,ti,tw.
21	"matern*".ab,ti,tw.
22	exp Maternal Health Services/
23	exp Obstetric Labor Complications/
24	(labor or labour).ab,ti,tw.
25	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26	exp bacterial load/
27	exp Genetic Markers/
28	"bacteria* load*".ab,ti,tw.
29	"bacteria* count*".ab,ti,tw.
30	Biomarkers/
31	Virulence/
32	Molecular Epidemiology/
33	((heav* or light* or low* or moderat* or intens*) and (colonis* or coloniz* or carriage)).ab,ti,tw.
34	((gene* or molecular* or dna or biological or immunological or chromosome) adj3 (marker* or biomarker*)).ab,ti,tw.
35	pathogenicity.ab,ti,tw.
36	26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35
37	4 and 25 and 36
38	limit 37 to (english language and humans)

Medline In-process:

# ▲	Searches
1	(group b adj streptococc*).ab,ti,tw.
2	"streptococc* agalactiae".ab,ti,tw.
3	(newborn* or new-born*).ab,ti,tw.
4	"infant*".ab,ti,tw.
5	"neonat*".ab,ti,tw.
6	(babies or baby).ab,ti,tw.
7	(antepartum* or ante-partum*).ab,ti,tw.
8	(intrapartum* or intra-partum*).ab,ti,tw.
9	(prenatal* or pre-natal*).ab,ti,tw.
10	(antenatal* or ante-natal*).ab,ti,tw.
11	"birth*".ab,ti,tw.
12	"pregnan*".ab,ti,tw.
13	"matern*".ab,ti,tw.
14	(labor or labour).ab,ti,tw.

15	"bacteria* load*".ab,ti,tw.
16	"bacteria* count*".ab,ti,tw.
17	((heav* or light* or low* or moderat* or intens*) and (colonis* or coloniz* or carriage)).ab,ti,tw.
18	((gene* or molecular* or dna or biological or immunological or chromosome) adj3 (marker* or biomarker*)).ab,ti,tw.
19	pathogenicity.ab,ti,tw.
20	1 or 2
21	3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
22	15 or 16 or 17 or 18 or 19
23	20 and 21 and 22
24	limit 23 to english language

Embase:

# ▲	**Searches**
1	exp Streptococcus agalactiae/
2	(group b adj streptococc*).ti,ab,tw.
3	"Streptococc* agalactiae".ti,ab,tw.
4	1 or 2 or 3
5	exp pregnancy/
6	exp birth/
7	exp labor/
8	exp prenatal period/
9	exp delivery/
10	exp pregnant woman/
11	exp newborn/
12	exp infant/
13	exp maternal care/
14	"matern*".ti,ab,tw.
15	(newborn* or new-born*).ti,ab,tw.
16	"infant*".ti,ab,tw.
17	"neonat*".ti,ab,tw.
18	(babies or baby).ti,ab,tw.
19	(antenatal* or ante-natal*).ti,ab,tw.
20	(antepartum* or ante-partum*).ti,ab,tw.
21	(intrapartum* or intra-partum*).ti,ab,tw.
22	(prenatal* or pre-natal*).ti,ab,tw.
23	"birth*".ti,ab,tw.
24	"pregnan*".ti,ab,tw.
25	exp labor complication/
26	(labor or labour).ti,ab,tw.
27	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26
28	exp bacterial load/
29	exp molecular marker/
30	exp genetic marker/
31	"bacteria* load*".ti,ab,tw.
32	"bacteria* count*".ti,ab,tw.
33	biological marker/
34	virulence/
35	molecular epidemiology/
36	pathogenicity/
37	((gene* or molecular* or dna or biological or immunological or chromosome) adj3 (marker* or biomarker*)).ti,ab,tw.
38	((heav* or light* or low* or moderat* or intens*) and (colonis* or coloniz* or carriage)).ti,ab,tw.
39	28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38
40	4 and 27 and 39
41	limit 40 to (human and english language)

Cochrane:


Edit #1 (group b near/5 streptococc*):ti,ab,kw
in Other Reviews, Trials and Technology Assessments
Edit #2 (streptococc* agalactiae):ti,ab,kw
in Other Reviews, Trials and Technology Assessments
#3 MeSH descriptor: [Streptococcus agalactiae] explode all trees
Edit #4 #1 or #2 or #3
#5 MeSH descriptor: [Pregnancy] explode all trees
#6 MeSH descriptor: [Parturition] explode all trees
#7 MeSH descriptor: [Labor, Obstetric] explode all trees
#8 MeSH descriptor: [Delivery, Obstetric] explode all trees
#9 MeSH descriptor: [Pregnancy Complications, Infectious] explode all trees
#10 MeSH descriptor: [Obstetric Labor Complications] explode all trees
#11 MeSH descriptor: [Maternal Health Services] explode all trees
Edit #12 matern*:ti,ab,kw
in Other Reviews, Trials and Technology Assessments
Edit #13 labour* or labor*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
Edit #14 antenatal* or ante-natal*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
Edit #15 prenatal* or pre-natal*:ti,ab,kw
in Other Reviews, Trials and Technology Assessments
Edit #16 intrapartum* or intra-partum*:ti,ab,kw
in Other Reviews, Trials and Technology Assessments
Edit #17 antepartum* or ante-partum*:ti,ab,kw
in Other Reviews, Trials and Technology Assessments
Edit #18 birth*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
Edit #18 birth*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
#19 MeSH descriptor: [Infant] explode all trees
Edit #20 newborn* or new-born*:ti,ab,kw
in Other Reviews, Trials and Technology Assessments
Edit #21 baby or babies:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
Edit #22 neonat*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
Edit #23 infant*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
Edit #24 pregnan*:ti,ab,kw
Edit #25 #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24
#26 MeSH descriptor: [Bacterial Load] explode all trees
#27 MeSH descriptor: [Genetic Markers] explode all trees
Edit #28 bacteria* load*:ti,ab,kw
Edit #29 bacteria* count:ti,ab,kw
#30 MeSH descriptor: [Biomarkers] this term only
#31 MeSH descriptor: [Virulence] this term only
#32 MeSH descriptor: [Molecular Epidemiology] this term only
Edit #33 pathogenicity:ti,ab,kw
Edit #34 ((gene* or molecular* or dna or biological or immunological or chromosone) near/3 (marker* or biomarker*)):ti,ab,kw
Edit #35 (heav* or light* or low* or moderat* or intens*) and (colonis* or coloniz* or carriage):ti,ab,kw
Edit #36 #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35
#37 #4 and #25 and #36
in Other Reviews, Trials, Technology Assessments and Economic Evaluations

Web of Science:

Set	Save History / Create Alert Open Saved History
# 26	(#25 NOT #19) *AND* **LANGUAGE:** (English) *Indexes=SCI-EXPANDED Timespan=All years*
# 25	#24 AND #20 AND #3 *Indexes=SCI-EXPANDED Timespan=All years*
# 24	#23 OR #22 OR #21 OR #17 OR #16 OR #15 *Indexes=SCI-EXPANDED Timespan=All years*
# 23	TS=(virulence) OR TI=(virulence) *Indexes=SCI-EXPANDED Timespan=All years*
# 22	TS=(pathogenicity) OR TI=(pathogenicity) *Indexes=SCI-EXPANDED Timespan=All years*
# 21	TS=((gene* or molecular* or dna or biological or immunological or chromosome) NEAR/3 (marker* or biomarker*)) OR TI=((gene* or molecular* or dna or biological or immunological or chromosome) NEAR/3 (marker* or biomarker*)) *Indexes=SCI-EXPANDED Timespan=All years*
# 20	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #18 *Indexes=SCI-EXPANDED Timespan=All year*
# 19	TS=(animal*) OR TI=(animal*) *Indexes=SCI-EXPANDED Timespan=All years*
# 18	TS=(matern*) OR TI=(matern*) *Indexes=SCI-EXPANDED Timespan=All years*
# 17	TS=(heav* or light* or low* or moderat* or intens*) AND TS=(colonis* or coloniz* or carriage) OR TI=(heav* or light* or low* or moderat* or intens*) AND TI=(colonis* or coloniz* or carriage) *Indexes=SCI-EXPANDED Timespan=All years*
# 16	TS=(bacteria* count*) OR TI=(bacteria* count*) *Indexes=SCI-EXPANDED Timespan=All years*
# 15	TS=(bacteria* load*) OR TI=(bacteria* load*) *Indexes=SCI-EXPANDED Timespan=All years*
# 14	TS=(infant*) OR TI=(infant*) *Indexes=SCI-EXPANDED Timespan=All years*
# 13	TS=(neonat*) OR TI=(neonat*) *Indexes=SCI-EXPANDED Timespan=All years*
# 12	TS=(baby or babies) OR TI=(baby or babies) *Indexes=SCI-EXPANDED Timespan=All years*
# 11	TS=(newborn* or new-born* or new born*) OR TI=(newborn* or new-born* or new born*) *Indexes=SCI-EXPANDED Timespan=All years*
# 10	TS=(antepartum* or ante-partum* or ante partum*) OR TI=(antepartum* or ante-partum* or ante partum*) *Indexes=SCI-EXPANDED Timespan=All years*
# 9	TS=(intrapartum* or intra-partum* or intra partum*) OR TI=(intrapartum* or intra-partum* or intra partum*) *Indexes=SCI-EXPANDED Timespan=All years*
# 8	TS=(prenatal* or pre-natal* or pre natal*) OR TI=(prenatal* or pre-natal* or pre natal*) *Indexes=SCI-EXPANDED Timespan=All years*
# 7	TS=(antenatal* or ante-natal* or ante natal*) OR TI=(antenatal* or ante-natal* or ante natal*) *Indexes=SCI-EXPANDED Timespan=All years*
# 6	TS=(labour or labor) OR TI=(labour or labor) *Indexes=SCI-EXPANDED Timespan=All years*
# 5	**TOPIC:** (birth*) *OR* **TITLE:** (birth*) *Indexes=SCI-EXPANDED Timespan=All years*
# 4	**TOPIC:** (pregnan*) *OR* **TITLE:** (pregnan*) *Indexes=SCI-EXPANDED Timespan=All years*
# 3	#2 OR #1 *Indexes=SCI-EXPANDED Timespan=All years*
# 2	TS=(b near/5 streptococc*) OR TI=(b near/5 streptococc*) *Indexes=SCI-EXPANDED Timespan=All years*
# 1	TS=("streptococc* agalactiae") OR TI=("streptococc* agalactiae") *Indexes=SCI-EXPANDED Timespan=All years*

Appendix 4. Data extraction sheet for systematic review on bacterial load and markers associated with GBS vertical transmission and EOGBS (objective 2)

Review Details					
Reviewer					
Study details					
Study ID Number					
First author surname					
Year of publication					
Country					
Number of centers					
Study design					
Study setting					
Total study duration *(including length of follow up if applicable)*					
Funding *(government/private/manufacturer/other - specify)*					
Aim of the study					
Methods of the study					
Recruitment dates					
Inclusion criteria					
Exclusion criteria					
Participants, Exposures and Outcomes definitions					
General definition of the sample:					
Definition and diagnostic methods for GBS maternal colonisation		*(e.g. site of swab, time, culture media)*			
Definition and diagnostic methods for GBS neonatal colonisation		*(e.g. site of swab, time, and culture media)*			
Definitions and diagnostic methods for EOGBS neonatal disease		*(e.g. site of swab, time, symptoms)*			
Exposure 1		*(Specify general definition of bacterial loads/molecular markers)*			
		Exposed group 1	**Exposed group 2**	**Non-exposed group**	**Total**
Definition of each group					
Sample size at baseline (total n)					
Sample size (analysed n)					
Lost to follow-up/withdrawals (n)					
Baseline characteristics	Mean (range or SD) age (years)				
	Mean (range or SD) gestational age (weeks)				
	Female children (n [%])				
	Mean birthweight (range or SD)				
	Race/ethnicity (n [%])				
	Co-morbidity (n [%])				
	Overall (n/N, [% or rate]) maternal OR neonatal GBS colonisation rate (specify)				
	Overall EOGBS rate (n/N, [rate per 1000])				
	Overall (n/N, [% or rate]) transmission or transition (specify mother to neonatal				

	colonisation OR mother to EOGBS disease OR neonatal colonisation to neonatal EOGBS disease)				
	Any treatments received (n [%]) Specify treatment (e.g. IAP)				
	Late onset GBS (n [%])				
	Other				
Exposure 2 *(Specify general definition of bacterial loads/molecular markers)*					
		Exposed group 1	**Exposed group 2**	**Non-exposed group**	**Total**
Definition of each group					
Sample size at baseline (total n)					
Sample size (analysed n)					
Lost to follow-up/withdrawals (n)					
Baseline characteristics	Mean (range or SD) age (years)				
	Mean (range or SD) gestational age (weeks)				
	Female children (n [%])				
	Race/ethnicity (n [%])				
	Co-morbidity (n [%])				
	Maternal GBS colonisation rate (if applicable)				
	Any treatments received (n [%]) Specify treatment (e.g. IAP)				
	Late onset GBS (n [%])				
	Other				
Exposure 3 *(Specify general definition of bacterial loads/molecular markers)*					
		Exposed group 1	**Exposed group 2**	**Non-exposed group**	**Total**
Definitions					
Sample size at baseline (total n)					
Sample size (analysed n)					
Lost to follow-up/withdrawals (n)					
Baseline characteristics	Mean (range or SD) age (years)				
	Mean (range or SD) gestational age (weeks)				
	Female children (n [%])				
	Race/ethnicity (n [%])				
	Co-morbidity (n [%])				
	Maternal GBS colonisation rate (if applicable)				
	Any treatments received (n [%])				

	Specify treatment (e.g. IAP)				
	Late onset GBS (n [%])				
	Other				
Add information for more exposures as necessary					
Outcomes					
GBS outcomes assessed *(GBS neonatal colonisation, early-onset GBS neonatal disease)*					
Other outcomes *(specify)*					

Results

Outcome *(Specify)*	**Exposure 1** *(Specify)*				**OR, RR, mean difference (95% CI)**		**Covariates adjusted for**
	Non-exposed *(Specify)*	**Exposed group 1** *(Specify)*	**Exposed group 2** *(Specify)*	**Total**	**Crude**	**Adjusted**	
Occurred							
Did not occur							
Total							

Outcome *(Specify)*	**Exposure 2** *(Specify)*				**OR, RR, mean difference (95% CI)**		**Covariates adjusted for**
	Non-exposed *(Specify)*	**Exposed group 1** *(Specify)*	**Exposed group 2** *(Specify)*	**Total**	**Crude**	**Adjusted**	
Occurred							
Did not occur							
Total							

Outcome *(Specify)*	**Exposure 3** *(Specify)*				**OR, RR, mean difference (95% CI)**		**Covariates adjusted for**
	Non-exposed	**Exposed group 1**	**Exposed group 2***)*	**Total**	**Crude**	**Adjusted**	
Occurred							
Did not occur							
Total							

Add more 2x2 tables for more exposures as necessary

Authors' conclusion:

Reviewer Notes:

Abbreviations: GBS=group B *Streptococcus*; EOGBS=early-onset GBS; OR=Odds ratio; RR=Risk ratio; 95% CI=95 percent confidence interval; SD=standard deviation; n=number

Appendix 5. Full-text studies excluded from systematic review on bacterial load and markers associated with GBS vertical transmission and EOGB, with reason (objective 2)

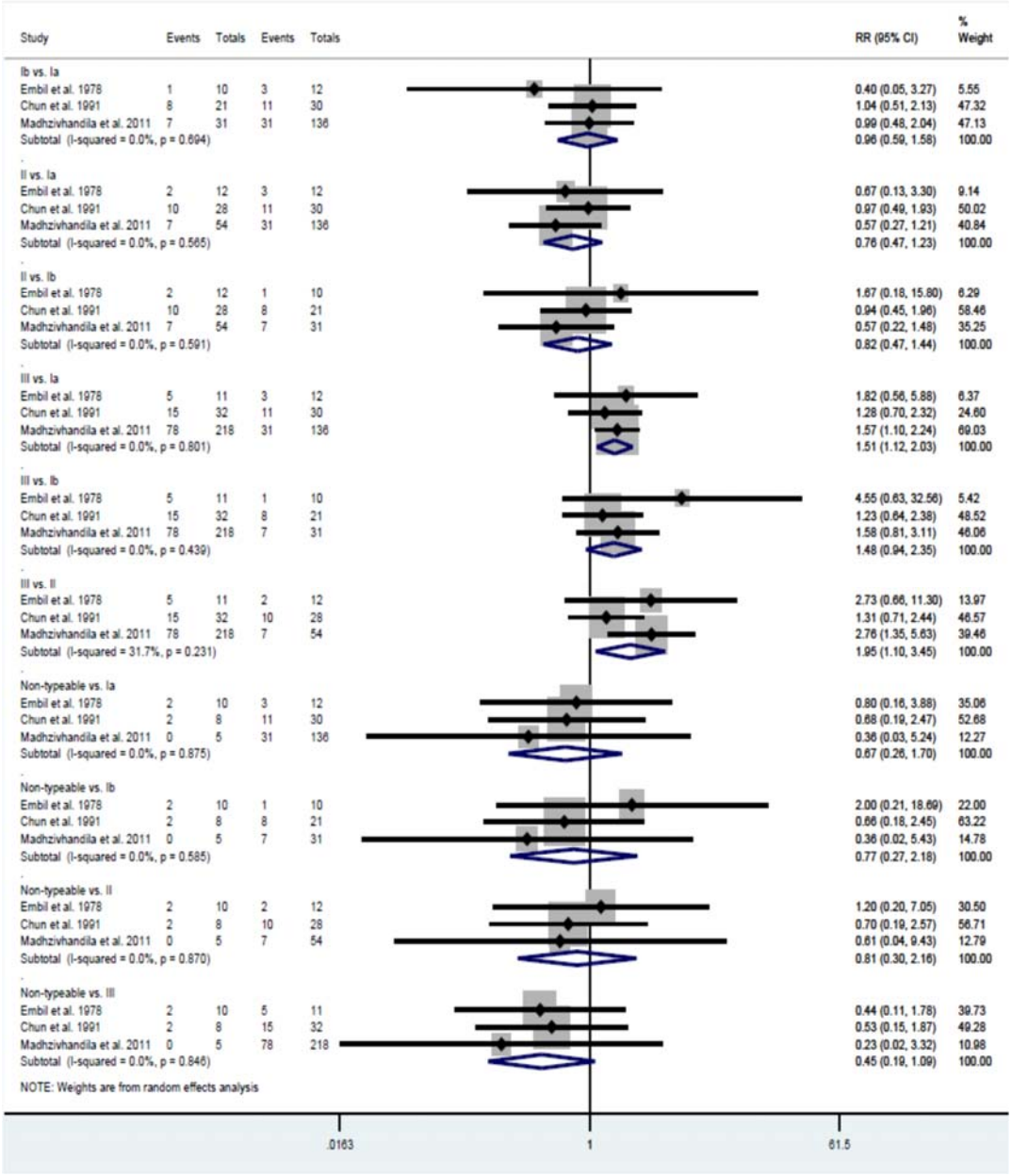
Reference	Reason
Alhhazmi, A., et al. (2016). "Epidemiology of Invasive Group B Streptococcal Disease in Alberta, Canada, from 2003 to 2013." Journal of Clinical Microbiology 54(7): 1774-1781.	Not on transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Almeida A, Villain A, Joubrel C, et al. Whole-Genome Comparison Uncovers Genomic Mutations between Group B Streptococci Sampled from Infected Newborns and Their Mothers. Journal of Bacteriology 2015; 197(20): 3354-66.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Ayoub EM, Swingle H. Pathogenic mechanisms in neonatal GBS infection. Antibiot Chemother 1985; 35: 128-41.	Review
Berardi A, Rossi C, Creti R, et al. Group B Streptococcal Colonization in 160 Mother-Baby Pairs: A Prospective Cohort Study. Journal of Pediatrics 2013; 163(4): 1099-+.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Berardi A, Rossi C, Guidotti I, et al. Factors associated with intrapartum transmission of group B Streptococcus. Pediatric Infectious Disease Journal 2014; 33(12): 1211-5.	Unable to distinguish data from those who received IAP and those who did not
Berner R, Bender A, Rensing C, Forster J, Brandis M. Low prevalence of the immunoglobulin-A-binding beta antigen of the C protein among Streptococcus agalactiae isolates causing neonatal sepsis.[Erratum appears in Eur J Clin Microbiol Infect Dis 2000 Jan;19(1):75]. Eur J Clin Microbiol Infect Dis. 1999;18(8):545-50.	Unable to distinguish data from those who received IAP and those who did not
Bidet P, Brahimi N, Chalas C, Aujard Y, Bingen E. Molecular characterization of serotype III group B-streptococcus isolates causing neonatal meningitis. Journal of Infectious Diseases 2003; 188(8): 1132-7.	Unable to distinguish data from early-onset cases to other cases
Bisharat N, Jones N, Marchaim D, et al. Population structure of group B streptococcus from a low-incidence region for invasive neonatal disease. Microbiology-(UK) 2005; 151: 1875-81.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Brigtsen AK, Jacobsen AF, Dedi L, Melby KK, Fugelseth D, Whitelaw A. Maternal Colonization with Group B Streptococcus Is Associated with an Increased Rate of Infants Transferred to the Neonatal Intensive Care Unit. Neonatology 2015; 108(3): 157-63.	Not on transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease) and no bacterial load factor or bacterial molecular marker
Campisi, E., et al. (2016). "Serotype IV Streptococcus agalactiae ST-452 has arisen from large genomic recombination events between CC23 and the hypervirulent CC17 lineages." Scientific Reports 6.	More than 10% of the participants had late-onset GBS
Chan GJ, Modak JK, Mahmud AA, Baqui AH, Black RE, Saha SK. Maternal and neonatal colonization in Bangladesh: prevalences, etiologies and risk factors. Journal of Perinatology 2013; 33(12): 971-6.	No bacterial load factor or bacterial molecular marker
Chatellier S, Huet H, Kenzi S, Rosenau A, Geslin P, Quentin R. Genetic diversity of rRNA operons of unrelated Streptococcus agalactiae strains isolated from cerebrospinal fluid of neonates suffering from meningitis. Journal of Clinical Microbiology 1996; 34(11): 2741-7.	More than 10% of the participants had late-onset GBS
Chatellier S, Ramanantsoa C, Harriau P, Rolland K, Rosenau A, Quentin R. Characterization of Streptococcus agalactiae strains by randomly amplified polymorphic DNA analysis. Journal of Clinical Microbiology 1997; 35(10): 2573-9.	More than 10% of the participants had late-onset GBS
Chaudhry BY, Akhtar N, Balouch AH. Vaginal carriage rate of group B Streptococcus in pregnant women and its transmission to neonates. J Ayub Med Coll Abbottabad 2010; 22(4): 167-70.	No bacterial load factor or bacterial molecular marker
D'Urzo N, Martinelli M, Pezzicoli A, et al. Acidic pH Strongly Enhances In Vitro Biofilm Formation by a Subset of Hypervirulent ST-17 Streptococcus agalactiae Strains. Applied and Environmental Microbiology 2014; 80(7): 2176-85.	Unable to distinguish data from early-onset cases to other cases

Reference	Reason
Davies HD, Jones N, Whittam TS, Elsayed S, Bisharat N, Baker CJ. Multilocus sequence typing of serotype III group B streptococcus and correlation with pathogenic potential. Journal of Infectious Diseases 2004; 189(6): 1097-102.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
De Francesco MA, Gargiulo F, Negrini R, Gelmi M, Manca N. Different sequence strains of Streptococcus agalactiae elicit various levels of cytokine production. Immunol Invest 2008; 37(8): 741-51.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Dore N, Bennett D, Kaliszer M, Cafferkey M, Smyth CJ. Molecular epidemiology of group B streptococci in Ireland: associations between serotype, invasive status and presence of genes encoding putative virulence factors. Epidemiology and Infection 2003; 131(2): 823-33.	Unable to distinguish data from early-onset GBS cases to others
Emaneini, M., et al. (2016). "Characterization of virulence factors, antimicrobial resistance pattern and clonal complexes of group B streptococci isolated from neonates." Microbial Pathogenesis 99: 119-122.	Not on transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Eskandarian N, Ismail Z, Neela V, van Belkum A, Desa MN, Amin Nordin S. Antimicrobial susceptibility profiles, serotype distribution and virulence determinants among invasive, non-invasive and colonizing Streptococcus agalactiae (group B streptococcus) from Malaysian patients. Eur J Clin Microbiol Infect Dis 2015; 34(3): 579-84.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Fluegge K, Wons J, Spellerberg B, et al. Genetic differences between invasive and noninvasive neonatal group B streptococcal isolates. Pediatr Infect Dis J 2011;30(12):1027-31.	Only includes infants with early-onset disease.
Freer J. Preventing perinatal transmission of group B streptococcal disease. JAAPA : official journal of the American Academy of Physician Assistants 2004; 17(3): 47-50; quiz 1-2.	Review, and no bacterial load factor or bacterial molecular marker
Friis-Moller A, Busk HE, Korner B, et al. Infections and colonisations with haemolytic streptococci group B in a Danish neonatal intensive care unit. Dan Med Bull 1984; 31(6): 494-9.	No bacterial load factor or bacterial molecular marker
Hakansson S, Granlund-Edstedt M, Sellin M, Holm SE. Demonstration and characterization of buoyant-density subpopulations of group B Streptococcus type III. Journal of Infectious Diseases 1990; 161(4): 741-6.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Hakansson S, Holm SE, Wagner M. Density profile of group B streptococci, type III, and its possible relation to enhanced virulence. Journal of Clinical Microbiology 1987; 25(4): 714-8.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Harper IA. The importance of group B streptococci as human pathogens in the British Isles. Journal of clinical pathology 1971; 24(5): 438-41.	Case-report, and no bacterial load factor or bacterial molecular marker
Helmig R, Halaburt JT, Uldbjert N, Thomsen AC, Stenderup A. Increased cell adherence of group B streptococci from preterm infants with neonatal sepsis. Obstet Gynecol 1990; 76(5 Pt 1): 825-7.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Hervas JA, Gonzalez L, Gil J, Paoletti LC, Madoff LC, Benedi VJ. Neonatal group B streptococcal infection in Mallorca, Spain. Clinical Infectious Diseases. 1993;16(5):714-8.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Hooven, T. A., et al. (2016). "The essential genome of Streptococcus agalactiae." Bmc Genomics 17.	Not human/clinical study
Imperi M, Gherardi G, Berardi A, et al. Invasive neonatal GBS infections from an area-based surveillance study in Italy. Clin Microbiol Infect 2011; 17(12): 1834-9.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)

Reference	Reason
Islam, M. S., et al. (2016). "Prevalence, Serotype Distribution, and Mortality Risk Associated with Group B Streptococcus Colonization of Newborns in Rural Bangladesh." Pediatric Infectious Disease Journal.	Outcome is death and not EOGBS and has no bacterial load factor or bacterial molecular marker
Kirmani N, Hafiz S, Jafarey SN, Hassan TJ. Carriage of beta haemolytic streptococci (BHS) in pregnant women and acquisition by neonates. JPMA J Pak Med Assoc 1994; 44(11): 256-7.	No bacterial load factor or bacterial molecular marker
Lin FYC, Whiting A, Adderson E, et al. Phylogenetic lineages of invasive and colonizing strains of serotype III group B streptococci from neonates: A multicenter prospective study. Journal of Clinical Microbiology 2006; 44(4): 1257-61.	Unable to distinguish data from those who received IAP and those who did not
Lin FY, Troendle JF. Hypothesis: Neonatal respiratory distress may be related to asymptomatic colonization with group B streptococci. Pediatr Infect Dis J 2006;25(10):884-8.	In IAP context.
Lin F, Sintchenko V, Kong F, Gilbert GL, Coiera E. Commonly used molecular epidemiology markers of Streptococcus agalactiae do not appear to predict virulence. Pathology 2009; 41(6): 576-81.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Malik, A., et al. (2016). "Neonatal Nasopharyngeal Colonization with Group B Streptococcus and its Association with Clinical Sepsis." American Journal of Perinatology 33(8): 800-807.	No bacterial load factor or bacterial molecular marker and >10% were treated with IAP
Manning S, Ki M, Marrs CF, et al. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. Bmc Infectious Diseases 2006; 6.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Marchaim D, Hallak M, Gortzak-Uzan L, Peled N, Riesenberg K, Schlaeffer F. Cell wall proteins of group B Streptococcus and low incidence of neonatal disease in southern Israel. Journal of Reproductive Medicine 2003; 48(9): 697-702.	No bacterial load factor or bacterial molecular marker
Meehan M, Cunney R, Cafferkey M. Molecular epidemiology of group B streptococci in Ireland reveals a diverse population with evidence of capsular switching. Eur J Clin Microbiol Infect Dis 2014; 33(7): 1155-62.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Melchers WJG, Bakkers J, Toonen M, van Kuppeveld FJM, Trijbels M, Hoogkamp-Korstanje JAA. Genetic analysis of Streptococcus agalactiae strains isolated from neonates and their mothers. FEMS Immunol Med Microbiol 2003; 36(1-2): 111-3.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Milligan TW, Baker CJ, Straus DC, Mattingly SJ. Association of elevated levels of extracellular neuraminidase with clinical isolates of type III group B streptococci. Infect Immun 1978; 21(3): 738-46.	Unable to distinguish data from early-onset cases to other cases
Muller-Vranjes A, Puntaric D, Curzik D, et al. Prevalence and significance of vaginal group B streptococcus colonization in pregnant women from Osijek, Croatia. Coll Antropol 2011; 35(1): 21-6.	No bacterial load factor or bacterial molecular marker
Nakstad, B., et al. (2016). "Early detection of neonatal group B streptococcus sepsis and the possible diagnostic utility of IL-6, IL-8, and CD11b in a human umbilical cord blood in vitro model." Infection and Drug Resistance 9: 171-179.	Not human/clinical study
Palacios GC, Eskew EK, Solorzano F, Mattingly SJ. Identification of the high-virulence clone of group B streptococci in Mexican isolates by growth characteristics at 40 degrees C. Curr Microbiol 1999; 38(2): 126-31.	Unable to distinguish data from early-onset cases to other cases
Palacios GC, Gonzalez MN, Beltran M, Arredondo JL, Torres J, Solorzano F. High-virulence clone of group B streptococci unable to grow at high temperatures is present in serotypes other than type III. Curr Microbiol 2007; 54(1): 42-7.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Palmeiro JK, Dalla-Costa LM, Fracalanzza SE, et al. Phenotypic and genotypic characterization of group B streptococcal isolates in southern Brazil. Journal of Clinical Microbiology 2010; 48(12): 4397-403.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)

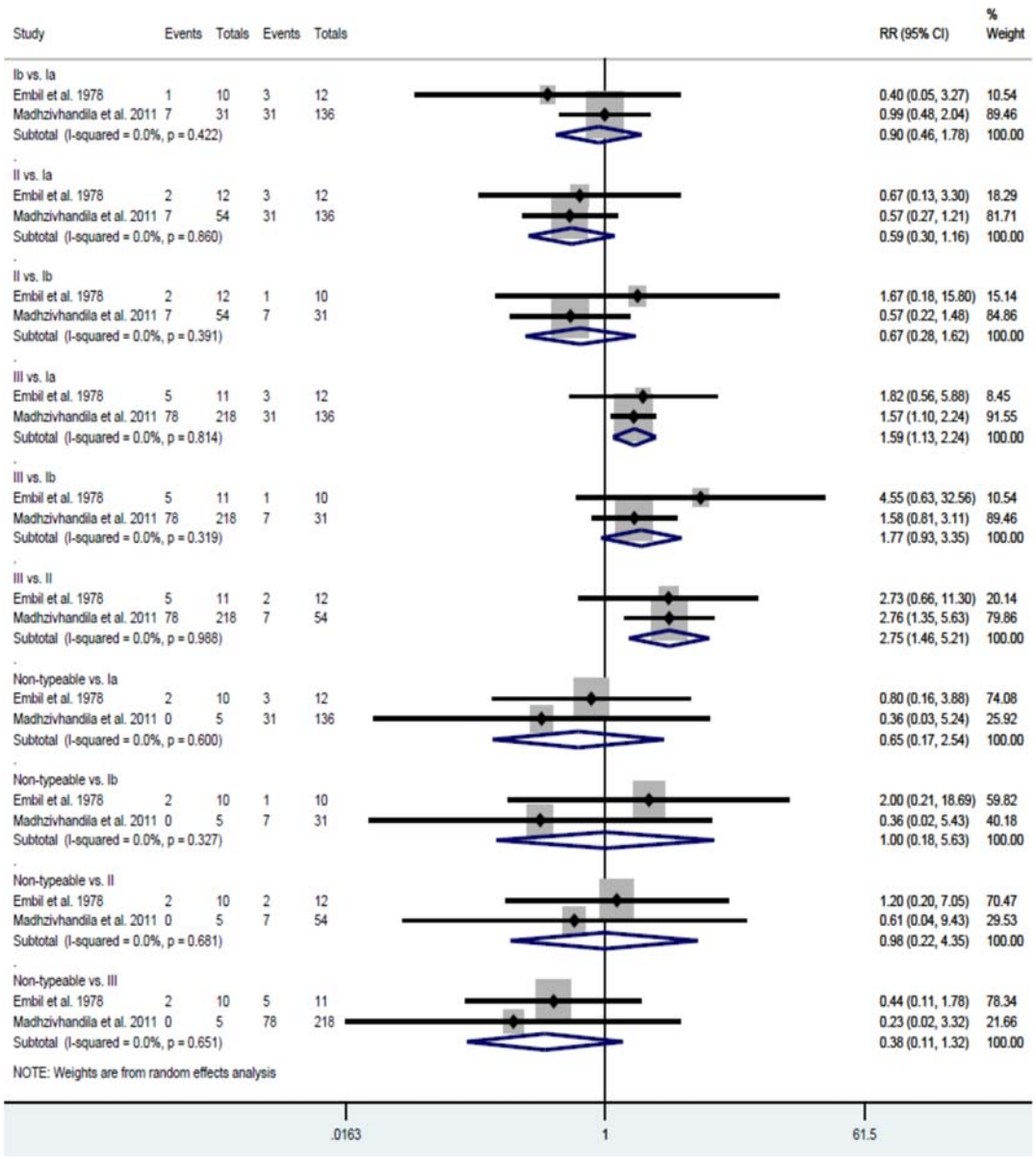
Reference	Reason
Parker, R. E., et al. (2016). "Association between genotypic diversity and biofilm production in group B Streptococcus." BMC Microbiology 16 (1) (no pagination)(86).	Not on transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Puopolo KM, Draper D, Wi S, et al. Estimating the Probability of Neonatal Early-Onset Infection on the Basis of Maternal Risk Factors. Pediatrics 2011; 128(5): E1155-E63.	No bacterial load factor or bacterial molecular marker
Regan JA, Klebanoff MA, Nugent RP, et al. Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group. Am J Obstet Gynecol 1996; 174(4): 1354-60.	Unable to distinguish data from those who received IAP and those who did not
Savonius, O., et al. (2016). "Swiftly decreasing cerebrospinal fluid cathelicidin concentration predicts improved outcome in childhood bacterial meningitis." Journal of Clinical Microbiology 54(6): 1648-1649.	More than 10% of the participants had late-onset GBS
Seale, A. C., et al. (2016). "Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya." Nature Microbiology 1(7): 16067.	Case series; no control group
Shabayek, S., et al. (2016). "A streptococcal NRAMP homologue is crucial for the survival of Streptococcus agalactiae under low pH conditions." Molecular Microbiology 100(4): 589-606.	Not on transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Sheppard, A. E., et al. (2016). "Capsular Typing Method for Streptococcus agalactiae Using Whole-Genome Sequence Data." Journal of Clinical Microbiology 54(5): 1388-1390.	Not on transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Siauw C, Kobsar A, Dornieden C, et al. Group B streptococcus isolates from septic patients and healthy carriers differentially activate platelet signaling cascades. Thromb Haemost 2006; 95(5): 836-49.	Unable to distinguish data from early-onset cases to other cases
Smith TC, Roehl SA, Pillai P, Li S, Marrs CF, Foxman B. Distribution of novel and previously investigated virulence genes in colonizing and invasive isolates of Streptococcus agalactiae. Epidemiology and Infection 2007; 135(6): 1046-54.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Teixeira LA, Figueiredo AM, Ferreira BT, et al. Sialic acid content and surface hydrophobicity of group B streptococci. Epidemiol Infect 1993; 110(1): 87-94.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Towers CV, Garite TJ, Friedman WW, Pircon RA, Nageotte MP. Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients. Am J Obstet Gynecol 1990; 163(3): 965-7.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
Valentin-Weigand P, Chhatwal GS. Correlation of epithelial cell invasiveness of group B streptococci with clinical source of isolation. Microb Pathog 1995; 19(2): 83-91.	Unable to distinguish data from early-onset cases to other cases
van der Mee-Marquet N, Domelier AS, Mereghetti L, et al. Prophagic DNA fragments in Streptococcus agalactiae strains and association with neonatal meningitis. Journal of Clinical Microbiology 2006; 44(3): 1049-58.	Bacterial load or bacterial molecular marker not related to transmission (from mother to baby) or transition (from early-onset colonisation to early-onset GBS disease)
van Elzakker E, Yahiaoui R, Visser C, et al. Epidemiology of and prenatal molecular distinction between invasive and colonizing group B streptococci in The Netherlands and Taiwan. Eur J Clin Microbiol Infect Dis 2009; 28(8): 921-8.	Unable to distinguish data from early-onset cases to other cases
Weindling AM, Hawkins JM, Coombes MA, Stringer J. Colonisation of babies and their families by group B streptococci. Br Med J (Clin Res Ed) 1981; 283(6305): 1503-5.	No bacterial load factor or bacterial molecular marker

Appendix 6. Summary forest plots of each pooled risk ratio by GBS serotype

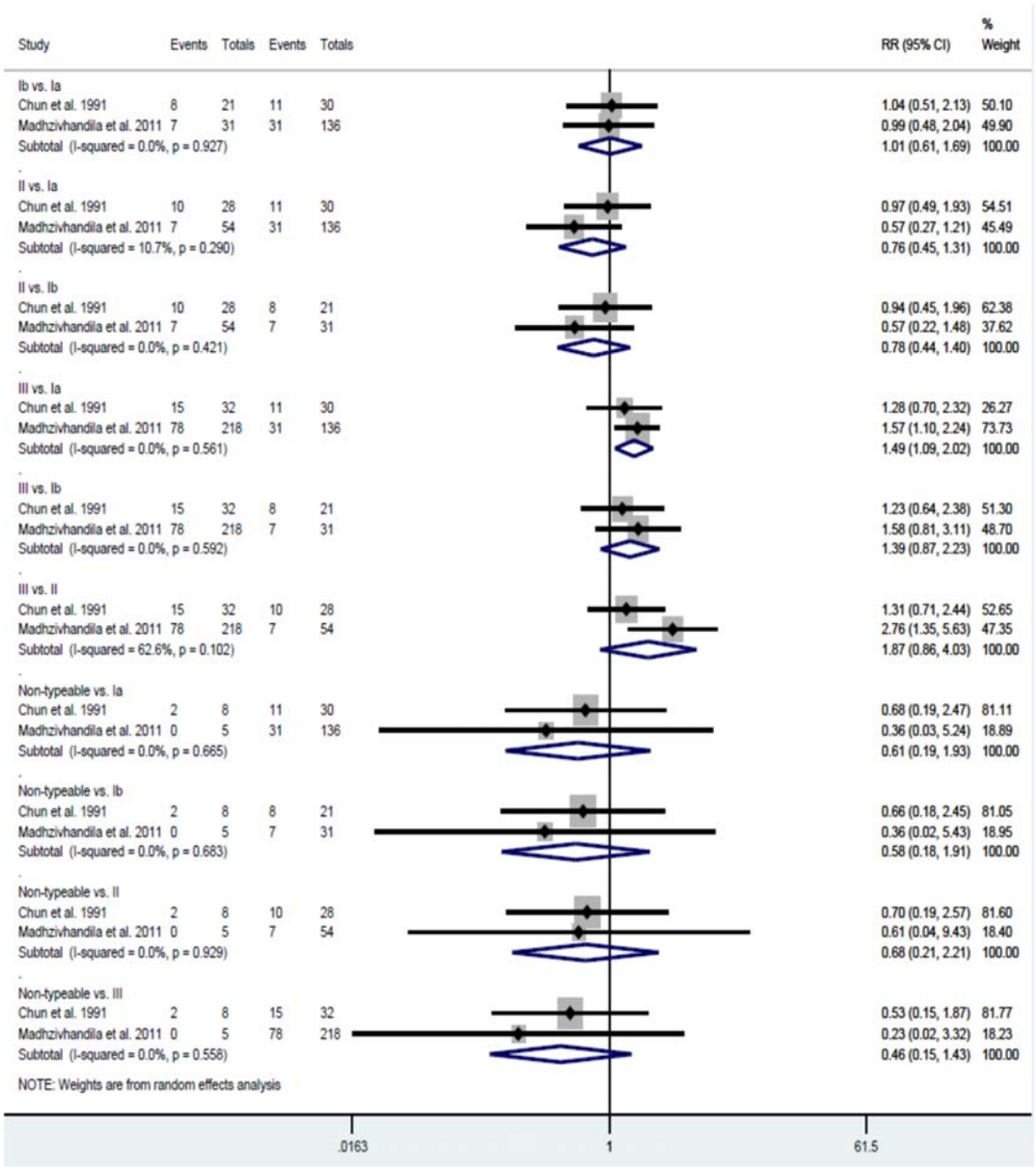


Appendix 7. Sensitivity analyses results for the meta-analysis of EOGBS by colonised serotype

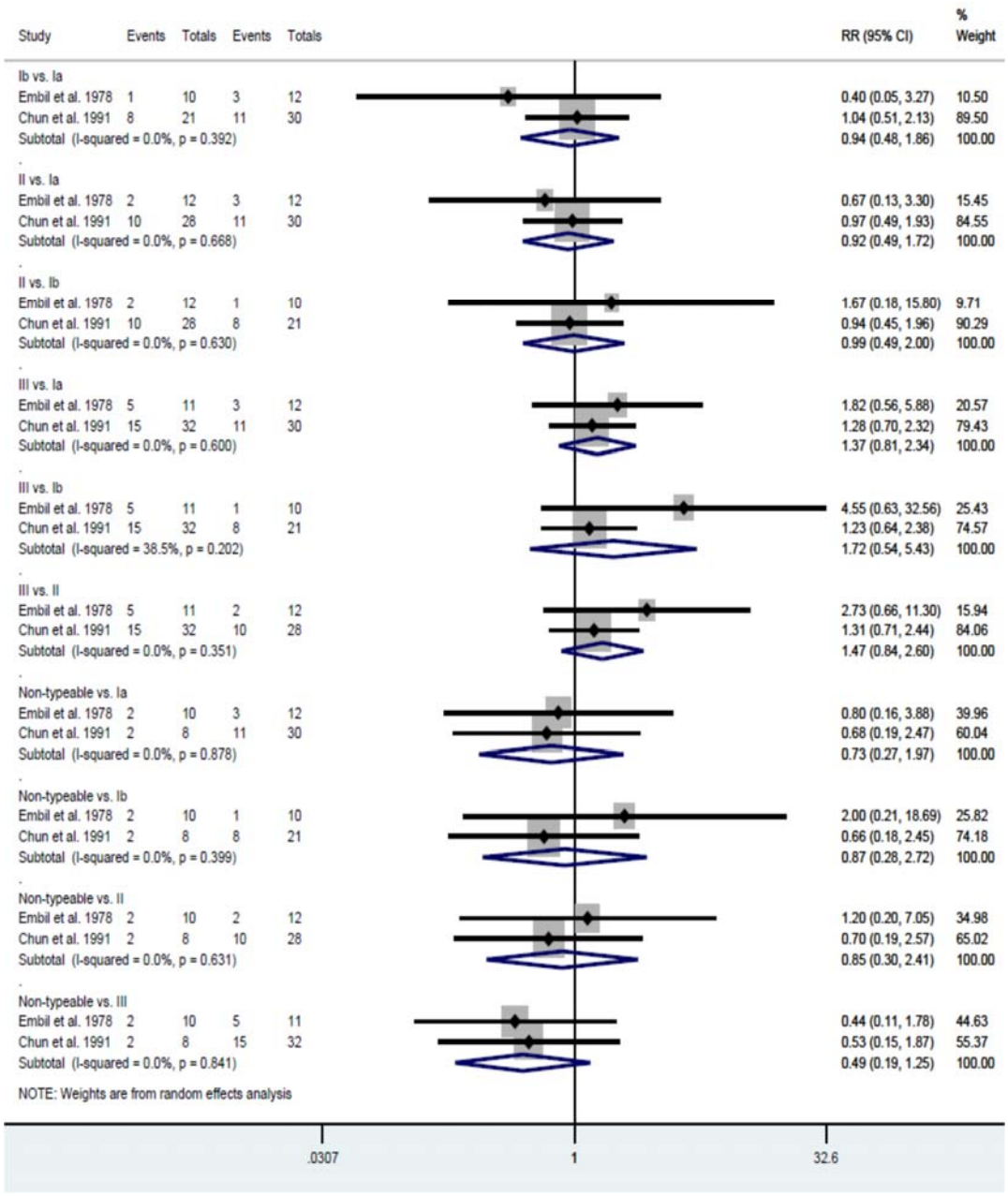
A. Cohort studies only



B. Not explicitly selective culture OR EOGBS definition explicitly sterile culture



C. Leaving out Madzivhandila et al. (2011)



Appendix 8. Search strategies for systematic review of adverse events from intrapartum antibiotic prophylaxis (objective 3)

Medline:

# ▲	Searches
1	exp Parturition/
2	exp Labor, Obstetric/
3	exp Delivery, Obstetric/
4	exp Obstetric Labor Complications/
5	exp Maternal Health Services/
6	(labour or labor).ab,ti,tw.
7	(intrapartum* or intra-partum*).ab,ti,tw.
8	"birth* ".ab,ti,tw.
9	"matern* ".ab,ti,tw.
10	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9
11	"prophyla*".ab,ti,tw.
12	exp Penicillins/
13	exp Erythromycin/
14	exp Clindamycin/
15	exp Cefazolin/
16	"penicillin*".ab,ti,tw.
17	"erythromycin*".ab,ti,tw.
18	"clindamycin*".ab,ti,tw.
19	"cefazolin*".ab,ti,tw.
20	"ampicillin*".ab,ti,tw.
21	"vancomycin*".ab,ti,tw.
22	exp Vancomycin/
23	12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
24	11 and 23
25	exp Antibiotic Prophylaxis/
26	exp Patient Harm/
27	exp Product Surveillance, Postmarketing/
28	exp Adverse Drug Reaction Reporting Systems/
29	exp Clinical Trials, Phase IV as Topic/
30	exp Poisoning/
31	exp Substance-Related Disorders/
32	exp "Drug-Related Side Effects and Adverse Reactions"/
33	exp abnormalities, drug induced/
34	exp Drug Monitoring/
35	exp Drug Hypersensitivity/
36	exp Postoperative Complications/
37	exp Intraoperative Complications/
38	(toxicity or complication* or noxious or tolerability).ab,ti,tw.
39	(safe or safety).ab,ti,tw.
40	"side effect*".ab,ti,tw.
41	((adverse or undesirable or harms* or serious or toxic) adj3 (effect* or reaction* or event* or outcome*)).ab,ti,tw.
42	(ae or to or po or co).fs.
43	exp Drug Resistance/
44	exp Microbiota/
45	exp Anxiety/co, de [Complications, Drug Effects]
46	exp Anaphylaxis/ci, co, de [Chemically Induced, Complications, Drug Effects]
47	exp Overweight/ci, co, de [Chemically Induced, Complications, Drug Effects]
48	exp Asthma/ci, co, de [Chemically Induced, Complications, Drug Effects]
49	exp Autistic Disorder/ci, co [Chemically Induced, Complications]
50	"autis*".ab,ti,tw.
51	"diabet*".ab,ti,tw.
52	"obes*".ab,ti,tw.
53	asthma.ab,ti,tw.
54	anxiety.ab,ti,tw.
55	(resistance or resistant).ab,ti,tw.
56	(microbiome or microbiota).ab,ti,tw.
57	"anaphyla*".ab,ti,tw.
58	(overweight or over-weight).ab,ti,tw.
59	exp Clostridium difficile/de [Drug Effects]
60	exp Diarrhea/ci, co, po [Chemically Induced, Complications, Poisoning]

61	("Clostridium difficile" or "c. diff" or "c. difficile").ab,ti,tw.
62	(Antibiotic-associated diarrhoea or Antibiotic-associated diarrhea or Antibiotic associated diarrhoea or Antibiotic associated diarrhea).ab,ti,tw.
63	exp Bacterial Infections/ci, co [Chemically Induced, Complications]
64	exp Sepsis/ci, co, to [Chemically Induced, Complications, Toxicity]
65	exp "Length of Stay"/
66	exp Skin Diseases/ci, co, to [Chemically Induced, Complications, Toxicity]
67	exp Respiratory Tract Diseases/ci, co, de [Chemically Induced, Complications, Drug Effects]
68	exp Cerebral Palsy/ci, co [Chemically Induced, Complications]
69	length of stay.ab,ti,tw.
70	(respiratory illness* or respiratory disease*).ab,ti,tw.
71	cerebral palsy.ab,ti,tw.
72	(Neonatal Necrotising Enterocolitis or Neonatal Necrotizing Enterocolitis or nec).ab,ti,tw.
73	exp Candidiasis/ci, co [Chemically Induced, Complications]
74	exp Enterocolitis, Necrotizing/ci, co [Chemically Induced, Complications]
75	(yeast infection* or Candidiasis).ab,ti,tw.
76	(suprainfection* or supra-infection*).ab,ti,tw.
77	exp Methicillin-Resistant Staphylococcus aureus/de [Drug Effects]
78	exp Vancomycin-Resistant Enterococci/de [Drug Effects]
79	exp Inflammatory Bowel Diseases/ci, co [Chemically Induced, Complications]
80	(Inflammatory bowel disease* or Crohn's disease* or Ulcerative colitis).ab,ti,tw.
81	exp "Growth and Development"/de [Drug Effects]
82	(Meticillin-resistant Staphylococcus aureus or Methicillin-resistant Staphylococcus aureus or Meticillin resistant Staphylococcus aureus or Methicillin resistant Staphylococcus aureus or MRSA).ab,ti,tw.
83	(skin disease* or dematologic* disease* or skin condition* or dematologic* condition*).ab,ti,tw.
84	(Vancomycin-resistant Enterococci or Vancomycin resistant Enterococci or VRE).ab,ti,tw.
85	(Extended Spectrum Beta-lactamase or Extended Spectrum Beta lactamase or ESBL).ab,ti,tw.
86	(Carbapenem-resistant Organism or Carbapenem resistant Organism or CRO).ab,ti,tw.
87	"antibiotic*".ab,ti,tw.
88	exp Diabetes Mellitus/ci [Chemically Induced]
89	(growth adj2 develop*).ab,ti,tw.
90	11 and 87
91	24 or 25 or 90
92	26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86 or 88 or 89
93	10 and 91 and 92
94	limit 93 to (english language and humans)

Medline In-process:

# ▲	**Searches**
1	(labour or labor).ab,ti,tw.
2	(intrapartum* or intra-partum*).ab,ti,tw.
3	"birth* ".ab,ti,tw.
4	"matern* ".ab,ti,tw.
5	"prophyla*".ab,ti,tw.
6	"penicillin*".ab,ti,tw.
7	"erythromycin*".ab,ti,tw.
8	"clindamycin*".ab,ti,tw.
9	"cefazolin*".ab,ti,tw.
10	"ampicillin*".ab,ti,tw.
11	"vancomycin*".ab,ti,tw.
12	(toxicity or complication* or noxious or tolerability).ab,ti,tw.
13	(safe or safety).ab,ti,tw.
14	"side effect*".ab,ti,tw.

15	((adverse or undesirable or harms* or serious or toxic) adj3 (effect* or reaction* or event* or outcome*)).ab,ti,tw.
16	"autis*".ab,ti,tw.
17	"diabet*".ab,ti,tw.
18	"obes*".ab,ti,tw.
19	asthma.ab,ti,tw.
20	anxiety.ab,ti,tw.
21	(resistance or resistant).ab,ti,tw.
22	(microbiome or microbiota).ab,ti,tw.
23	"anaphyla*".ab,ti,tw.
24	(overweight or over-weight).ab,ti,tw.
25	("Clostridium difficile" or "c. diff" or "c. difficile").ab,ti,tw.
26	(Antibiotic-associated diarrhoea or Antibiotic-associated diarrhea or Antibiotic associated diarrhoea or Antibiotic associated diarrhea).ab,ti,tw.
27	length of stay.ab,ti,tw.
28	(respiratory illness* or respiratory disease*).ab,ti,tw.
29	cerebral palsy.ab,ti,tw.
30	(Neonatal Necrotising Enterocolitis or Neonatal Necrotizing Enterocolitis or nec).ab,ti,tw.
31	(yeast infection* or Candidiasis).ab,ti,tw.
32	(suprainfection* or supra-infection*).ab,ti,tw.
33	(Inflammatory bowel disease* or Crohn's disease* or Ulcerative colitis).ab,ti,tw.
34	(Meticillin-resistant Staphylococcus aureus or Methicillin-resistant Staphylococcus aureus or Meticillin resistant Staphylococcus aureus or Methicillin resistant Staphylococcus aureus or MRSA).ab,ti,tw.
35	(skin disease* or dematologic* disease* or skin condition* or dematologic* condition*).ab,ti,tw.
36	(Vancomycin-resistant Enterococci or Vancomycin resistant Enterococci or VRE).ab,ti,tw.
37	(Extended Spectrum Beta-lactamase or Extended Spectrum Beta lactamase or ESBL).ab,ti,tw.
38	(Carbapenem-resistant Organism or Carbapenem resistant Organism or CRO).ab,ti,tw.
39	1 or 2 or 3 or 4
40	6 or 7 or 8 or 9 or 10 or 11
41	5 and 40
42	(sepsis or septicaemia).ab,ti,tw.
43	"bacteria* infection*".ab,ti,tw.
44	"antibiotic*".ab,ti,tw.
45	(growth adj2 develop*).ab,ti,tw.
46	5 and 44
47	41 or 46
48	12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 42 or 43 or 45
49	39 and 47 and 48
50	limit 49 to english language

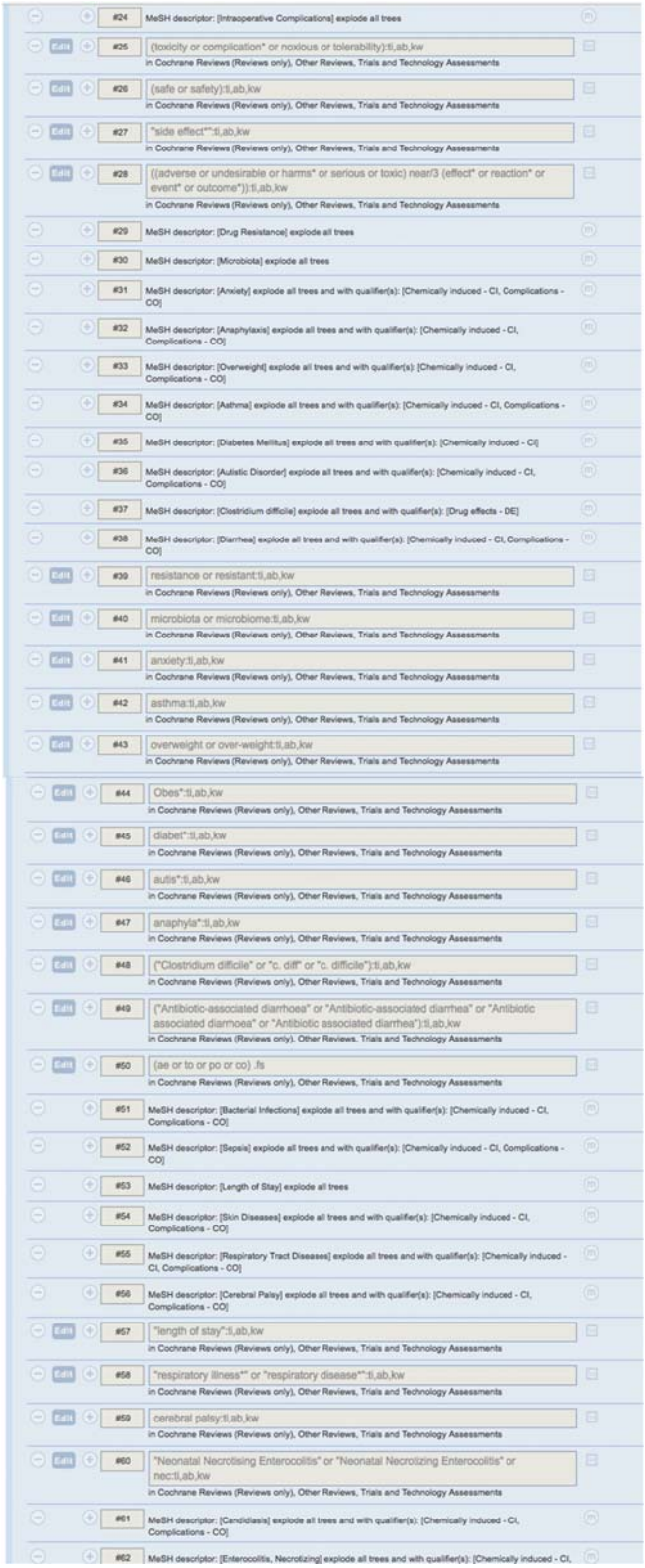
Embase:

# ▲	**Searches**
1	(intrapartum* or intra-partum*).ti,ab,tw.
2	"birth*".ti,ab,tw.
3	"matern*".ti,ab,tw.
4	exp birth/
5	exp labor/
6	exp delivery/
7	exp maternal care/
8	exp labor complication/
9	exp intrapartum care/
10	(labor or labour).ti,ab,tw.
11	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
12	"prophyla*".ti,ab,tw.
13	exp Penicillins/
14	exp Erythromycin/
15	exp Clindamycin/

16	exp Cefazolin/
17	"penicillin*".ti,ab,tw.
18	"erythromycin*".ti,ab,tw.
19	"clindamycin*".ti,ab,tw.
20	"cefazolin*".ti,ab,tw.
21	"ampicillin*".ti,ab,tw.
22	"vancomycin*".ti,ab,tw.
23	exp Vancomycin/
24	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25	12 and 24
26	exp Antibiotic Prophylaxis/
27	exp patient harm/
28	exp side effect/
29	exp adverse drug reaction/
30	exp drug toxicity/
31	exp intoxication/
32	exp drug safety/
33	exp drug monitoring/
34	exp drug hypersensitivity/
35	exp postmarketing surveillance/
36	exp drug surveillance program/
37	phase 4 clinical trial/
38	exp postoperative complication/
39	exp peroperative complication/
40	"side effect*".ti,ab,tw.
41	(ae or si or to or co).fs.
42	exp anaphylaxis/co, si [Complication, Side Effect]
43	exp microflora/
44	exp drug resistance/
45	exp anxiety/co [Complication]
46	exp obesity/co, si [Complication, Side Effect]
47	exp asthma/co, si [Complication, Side Effect]
48	exp diabetes mellitus/co, to, si [Complication, Drug Toxicity, Side Effect]
49	exp autism/co, si [Complication, Side Effect]
50	(resistance or resistant).ti,ab,tw.
51	"autis*".ti,ab,tw.
52	"diabet*".ti,ab,tw.
53	"obes*".ti,ab,tw.
54	asthma.ti,ab,tw.
55	anxiety.ti,ab,tw.
56	(microbiome or microbiota).ti,ab,tw.
57	"anaphyla*".ti,ab,tw.
58	(overweight or over-weight).ti,ab,tw.
59	((adverse or undesirable or harm* or serious or toxic) adj3 (effect* or reaction* or event* or outcome*)).ti,ab,tw.
60	(safe or safety).ti,ab,tw.
61	exp Peptoclostridium difficile/
62	("Clostridium difficile" or "c. diff" or "c. difficile").ti,ab,tw.
63	(Antibiotic-associated diarrhoea or Antibiotic-associated diarrhea or Antibiotic associated diarrhoea or Antibiotic associated diarrhea).ti,ab,tw.
64	exp bacterial infection/co, si [Complication, Side Effect]
65	exp sepsis/co, si [Complication, Side Effect]
66	exp "length of stay"/
67	exp skin disease/co, si [Complication, Side Effect]
68	exp respiratory tract disease/co, si [Complication, Side Effect]
69	exp cerebral palsy/co, si [Complication, Side Effect]
70	length of stay.ti,ab,tw.
71	(respiratory illness* or respiratory disease*).ti,ab,tw.
72	cerebral palsy.ti,ab,tw.
73	(Neonatal Necrotising Enterocolitis or Neonatal Necrotizing Enterocolitis or nec).ti,ab,tw.
74	exp necrotizing enterocolitis/co, si [Complication, Side Effect]
75	exp candidiasis/co, si [Complication, Side Effect]
76	(yeast infection* or Candidiasis).ti,ab,tw.
77	(suprainfection* or supra-infection*).ti,ab,tw.

78	(Meticillin-resistant Staphylococcus aureus or Methicillin-resistant Staphylococcus aureus or Meticillin resistant Staphylococcus aureus or Methicillin resistant Staphylococcus aureus or MRSA).ti,ab,tw.
79	exp vancomycin resistant Enterococcus/
80	(Extended Spectrum Beta-lactamase or Extended Spectrum Beta lactamase or ESBL).ti,ab,tw.
81	(Vancomycin-resistant Enterococci or Vancomycin resistant Enterococci or VRE).ti,ab,tw.
82	(Carbapenem-resistant Organism or Carbapenem resistant Organism or CRO).ti,ab,tw.
83	exp inflammatory bowel disease/co, si [Complication, Side Effect]
84	(Inflammatory bowel disease* or Crohn's disease* or Ulcerative colitis).ti,ab,tw.
85	exp "growth, development and aging"/
86	exp antibiotic associated diarrhea/
87	exp methicillin resistant Staphylococcus aureus/
88	(skin disease* or dematologic* disease* or skin condition* or dematologic* condition*).ti,ab,tw.
89	exp extended spectrum beta lactamase/
90	"antibiotic*".ti,ab,tw.
91	(growth adj2 develop*).ti,ab,tw.
92	12 and 90
93	25 or 26 or 92
94	27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86 or 87 or 88 or 89 or 91
95	11 and 93 and 94
96	limit 95 to (human and english language)

Cochrane:


#24 MeSH descriptor: [Intraoperative Complications] explode all trees
Edit #25 (toxicity or complication* or noxious or tolerability):ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #26 (safe or safety):ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #27 "side effect*":ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #28 ((adverse or undesirable or harms* or serious or toxic) near/3 (effect* or reaction* or event* or outcome*)):ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#29 MeSH descriptor: [Drug Resistance] explode all trees
#30 MeSH descriptor: [Microbiota] explode all trees
#31 MeSH descriptor: [Anxiety] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#32 MeSH descriptor: [Anaphylaxis] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#33 MeSH descriptor: [Overweight] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#34 MeSH descriptor: [Asthma] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#35 MeSH descriptor: [Diabetes Mellitus] explode all trees and with qualifier(s): [Chemically induced - CI]
#36 MeSH descriptor: [Autistic Disorder] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#37 MeSH descriptor: [Clostridium difficile] explode all trees and with qualifier(s): [Drug effects - DE]
#38 MeSH descriptor: [Diarrhea] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
Edit #39 resistance or resistant:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #40 microbiota or microbiome:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #41 anxiety:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #42 asthma:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #43 overweight or over-weight:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #44 Obes*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #45 diabet*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #46 autis*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #47 anaphyla*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #48 ("Clostridium difficile" or "c. diff" or "c. difficile"):ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #49 ("Antibiotic-associated diarrhoea" or "Antibiotic-associated diarrhea" or "Antibiotic associated diarrhoea" or "Antibiotic associated diarrhea"):ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #50 (ae or to or po or co) .fs
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#51 MeSH descriptor: [Bacterial Infections] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#52 MeSH descriptor: [Sepsis] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#53 MeSH descriptor: [Length of Stay] explode all trees
#54 MeSH descriptor: [Skin Diseases] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#55 MeSH descriptor: [Respiratory Tract Diseases] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#56 MeSH descriptor: [Cerebral Palsy] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
Edit #57 "length of stay":ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #58 "respiratory illness*" or "respiratory disease*":ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #59 cerebral palsy:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
Edit #60 "Neonatal Necrotising Enterocolitis" or "Neonatal Necrotizing Enterocolitis" or nec:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#61 MeSH descriptor: [Candidiasis] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#62 MeSH descriptor: [Enterocolitis, Necrotizing] explode all trees and with qualifier(s): [Chemically induced - CI,


#62 MeSH descriptor: [Enterocolitis, Necrotizing] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#63 "yeast infection*" or Candidiasis:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#64 suprainfection* or supra-infection:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#65 MeSH descriptor: [Methicillin-Resistant Staphylococcus aureus] explode all trees and with qualifier(s): [Drug effects - DE]
#66 MeSH descriptor: [Vancomycin-Resistant Enterococci] explode all trees and with qualifier(s): [Drug effects - DE]
#67 MeSH descriptor: [Inflammatory Bowel Diseases] explode all trees and with qualifier(s): [Chemically induced - CI, Complications - CO]
#68 "Inflammatory bowel disease*" or "Crohn's disease*" or "Ulcerative colitis":ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#69 MeSH descriptor: [Growth and Development] explode all trees and with qualifier(s): [Drug effects - DE]
#70 "Meticillin-resistant Staphylococcus aureus" or "Methicillin-resistant Staphylococcus aureus" or "Meticillin resistant Staphylococcus aureus" or "Methicillin resistant Staphylococcus aureus" or MRSA:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#71 growth near/2 develop*:ti,ab,kw
in Cochrane Reviews (Reviews and Protocols), Other Reviews, Trials and Technology Assessments
#72 "skin disease*" or "dematologic* disease*" or "skin condition*" or "dematologic* condition*":ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#73 "Vancomycin-resistant Enterococci" or "Vancomycin resistant Enterococci" or VRE:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#74 "Extended Spectrum Beta-lactamase" or "Extended Spectrum Beta lactamase" or ESBL:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#75 "Carbapenem-resistant Organism" or "Carbapenem resistant Organism" or CRO:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#76 #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27 or #28 or #29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37 or #38 or #39 or #40 or #41 or #42 or #43 or #44 or #45 or #46 or #47 or #48 or #49 or #50 or #51 or #52 or #53 or #54 or #55 or #56 or #57 or #58 or #59 or #60 or #61 or #62 or #63 or #64 or #65 or #66 or #67 or #68 or #69 or #70 or #71 or #72 or #73 or #74 or #75
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#77 MeSH descriptor: [Antibiotic Prophylaxis] explode all trees
#77 MeSH descriptor: [Antibiotic Prophylaxis] explode all trees
#78 antibiotic*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#79 prophyla*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#80 #78 and #79
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#81 MeSH descriptor: [Penicillins] explode all trees
#82 MeSH descriptor: [Erythromycin] explode all trees
#83 MeSH descriptor: [Clindamycin] explode all trees
#84 MeSH descriptor: [Cefazolin] explode all trees
#85 MeSH descriptor: [Vancomycin] explode all trees
#86 penicillin*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#87 Erythromycin*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#88 Clindamycin*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#89 Cefazolin*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#90 Vancomycin*:ti,ab,kw
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#91 ampicillin*
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#92 #81 or #82 or #83 or #84 or #85 or #86 or #87 or #88 or #89 or #90 or #91
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#93 #79 and #92
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#94 #77 or #80 or #93
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments
#95 #10 and #76 and #94
in Cochrane Reviews (Reviews only), Other Reviews, Trials and Technology Assessments

Web of Science:

Set	Save History / Create Alert Open Saved History
# 50	(#48 NOT #49) *AND* **LANGUAGE:** (English) *Indexes=SCI-EXPANDED Timespan=All years*
# 49	TS=(animal) OR TI=(animal) *Indexes=SCI-EXPANDED Timespan=All years*
# 48	#47 AND #46 AND #5 *Indexes=SCI-EXPANDED Timespan=All years*
# 47	#43 OR #42 OR #41 OR #40 OR #39 OR #38 OR #37 OR #36 OR #35 OR #34 OR #33 OR #32 OR #31 OR #30 OR #29 OR #28 OR #27 OR #26 OR #25 OR #24 OR #23 OR #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 *Indexes=SCI-EXPANDED Timespan=All years*
# 46	#45 OR #8 *Indexes=SCI-EXPANDED Timespan=All years*
# 45	#44 AND #7 *Indexes=SCI-EXPANDED Timespan=All years*
# 44	#14 OR #13 OR #12 OR #11 OR #10 OR #9 *Indexes=SCI-EXPANDED Timespan=All years*
# 43	TS=("bacteria* infection*") OR TI=("bacteria* infection*") *Indexes=SCI-EXPANDED Timespan=All years*
# 42	TS=("Carbapenem-resistant Organism" or "Carbapenem resistant Organism" or CRO) OR TI=("Carbapenem-resistant Organism" or "Carbapenem resistant Organism" or CRO) *Indexes=SCI-EXPANDED Timespan=All years*
# 41	TS=("Extended Spectrum Beta-lactamase" or "Extended Spectrum Beta lactamase" or ESBL) OR TI=("Extended Spectrum Beta-lactamase" or "Extended Spectrum Beta lactamase" or ESBL) *Indexes=SCI-EXPANDED Timespan=All years*
# 40	TS=("Vancomycin-resistant Enterococci" or "Vancomycin resistant Enterococci" or VRE) OR TI=("Vancomycin-resistant Enterococci" or "Vancomycin resistant Enterococci" or VRE) *Indexes=SCI-EXPANDED Timespan=All years*
# 39	TS=("skin disease*" or "dematologic* disease*" or "skin condition*" or "dematologic* condition*") OR TI=("skin disease*" or "dematologic* disease*" or "skin condition*" or "dematologic* condition*") *Indexes=SCI-EXPANDED Timespan=All years*
# 38	(TS=(growth NEAR/2 develop*) OR TI=(growth NEAR/2 develop*)) *AND* **LANGUAGE:** (English) *Indexes=SCI-EXPANDED Timespan=All years*
# 37	TS=("Meticillin-resistant Staphylococcus aureus" or "Methicillin-resistant Staphylococcus aureus" or "Meticillin resistant Staphylococcus aureus" or "Methicillin resistant Staphylococcus aureus" or MRSA) OR TI=("Meticillin-resistant Staphylococcus aureus" or "Methicillin-resistant Staphylococcus aureus" or "Meticillin resistant Staphylococcus aureus" or "Methicillin resistant Staphylococcus aureus" or MRSA) *Indexes=SCI-EXPANDED Timespan=All years*
# 36	TS=("Inflammatory bowel disease*" or "Crohn's disease*" or "Ulcerative colitis") OR TI=("Inflammatory bowel disease*" or "Crohn's disease*" or "Ulcerative colitis") *Indexes=SCI-EXPANDED Timespan=All years*
# 35	TS=(suprainfection* or supra-infection or supra infection) OR TI=(suprainfection* or supra-infection or supra infection) *Indexes=SCI-EXPANDED Timespan=All years*
# 34	TS=("yeast infection*" or Candidiasis) OR TI=("yeast infection*" or Candidiasis) *Indexes=SCI-EXPANDED Timespan=All years*
# 33	TS=("Neonatal Necrotising Enterocolitis" or "Neonatal Necrotizing Enterocolitis" or nec) OR TI=("Neonatal Necrotising Enterocolitis" or "Neonatal Necrotizing Enterocolitis" or nec) *Indexes=SCI-EXPANDED Timespan=All years*
# 32	TS=("cerebral palsy") OR TI=("cerebral palsy") *Indexes=SCI-EXPANDED Timespan=All years*
# 31	TS=("respiratory illness*" or "respiratory disease*") OR TI=("respiratory illness*" or "respiratory disease*") *Indexes=SCI-EXPANDED Timespan=All years*
# 30	TS=("length of stay") OR TI=("length of stay") *Indexes=SCI-EXPANDED Timespan=All years*
# 29	TS=("Antibiotic-associated diarrhoea" or "Antibiotic-associated diarrhea" or "Antibiotic associated diarrhoea" or "Antibiotic associated diarrhea") OR TI=("Antibiotic-associated diarrhoea" or "Antibiotic-associated diarrhea" or "Antibiotic associated diarrhoea" or "Antibiotic associated diarrhea") *Indexes=SCI-EXPANDED Timespan=All years*

# 28	TS=("Clostridium difficile" or "c. diff" or "c. difficile") OR TI=("Clostridium difficile" or "c. diff" or "c. difficile") *Indexes=SCI-EXPANDED Timespan=All years*
# 27	TS=(overweight or over-weight or over weight) OR TI=(overweight or over-weight or over weight) *Indexes=SCI-EXPANDED Timespan=All years*
# 26	TS=(anaphyla*) OR TI=(anaphyla*) *Indexes=SCI-EXPANDED Timespan=All years*
# 25	TS=(microbiome or microbiota) OR TI=(microbiome or microbiota) *Indexes=SCI-EXPANDED Timespan=All years*
# 24	TS=(resistance or resistant) OR TI=(resistance or resistant) *Indexes=SCI-EXPANDED Timespan=All years*
# 23	TS=(anxiety) OR TI=(anxiety) *Indexes=SCI-EXPANDED Timespan=All years*
# 22	TS=(asthma) OR TI=(asthma) *Indexes=SCI-EXPANDED Timespan=All years*
# 21	TS=(obes*) OR TI=(obes*) *Indexes=SCI-EXPANDED Timespan=All years*
# 20	TS=(diabet*) OR TI=(diabet*) *Indexes=SCI-EXPANDED Timespan=All years*
# 19	TS=(autis*) OR TI=(autis*) *Indexes=SCI-EXPANDED Timespan=All years*
# 18	TS=(((adverse or undesirable or harms* or serious or toxic) NEAR/3 (effect* or reaction* or event* or outcome*)) OR ((adverse or undesirable or harms* or serious or toxic) NEAR/3 (effect* or reaction* or event* or outcome*))) *Indexes=SCI-EXPANDED Timespan=All years*
# 17	TS=("side effect*") OR TI=("side effect*") *Indexes=SCI-EXPANDED Timespan=All years*
# 16	TS=(safe or safety) OR TI=(safe or safety) *Indexes=SCI-EXPANDED Timespan=All years*
# 15	TS=(toxicity or complication* or noxious or tolerability) OR TI=(toxicity or complication* or noxious or tolerability) *Indexes=SCI-EXPANDED Timespan=All years*
# 14	TS=(vancomycin*) OR TI=(vancomycin*) *Indexes=SCI-EXPANDED Timespan=All years*
# 13	TS=(ampicillin*) OR TI=(ampicillin*) *Indexes=SCI-EXPANDED Timespan=All years*
# 12	TS=(cefazolin*) OR TI=(cefazolin*) *Indexes=SCI-EXPANDED Timespan=All years*
# 11	TS=(clindamycin*) OR TI=(clindamycin*) *Indexes=SCI-EXPANDED Timespan=All years*
# 10	TS=(erythromycin*) OR TI=(erythromycin*) *Indexes=SCI-EXPANDED Timespan=All years*
# 9	TS=(penicillin*) OR TI=(penicillin*) *Indexes=SCI-EXPANDED Timespan=All years*
# 8	#7 AND #6 *Indexes=SCI-EXPANDED Timespan=All years*
# 7	TS=(prophyla*) OR TI=(prophyla*) *Indexes=SCI-EXPANDED Timespan=All years*
# 6	TS=(antibiotic*) OR TI=(antibiotic*) *Indexes=SCI-EXPANDED Timespan=All years*
# 5	#4 OR #3 OR #2 OR #1 *Indexes=SCI-EXPANDED Timespan=All years*
# 4	TS=(matern*) OR TI=(matern*) *Indexes=SCI-EXPANDED Timespan=All years*
# 3	TS=(birth*) OR TI=(birth*) *Indexes=SCI-EXPANDED Timespan=All years*
# 2	TS=(intrapartum* or intra-partum* or intra partum*) OR TI=(intrapartum* or intra-partum* or intra partum*) *Indexes=SCI-EXPANDED Timespan=All years*
# 1	TS=(labour or labor) OR TI=(labour or labor) *Indexes=SCI-EXPANDED Timespan=All years*

Appendix 9. Data extraction sheet for systematic review on adverse events from intrapartum antibiotic prophylaxis (objective 3)

Review Details						
Reviewer						
Study details						
Study ID Number						
First author surname						
Year of publication						
Country						
Study design						
Study setting						
Number of centers						
Total study duration	*(including length of follow up if applicable)*					
Funding	*(government/private/manufacturer/other - specify)*					
Aim of the study						
Methods of the study						
Recruitment dates						
Inclusion criteria						
Exclusion criteria						
Recruitment method (e.g. consecutive participants)						
Interventions and participants						
General definition of the sample:						
Intervention arm:			**Antibiotic prophylaxis**	**No treatment**	**Total**	
Dose of antibiotic						
Indication for antibiotic						
Antibiotic given						
Duration of antibiotic						
Sample size at baseline						
Sample size analysed						
Lost to follow-up/withdrawals						
Baseline characteristics	Mean (range or SD) age (years)					
	Mean (range or SD) gestational age (weeks)					
	Female children (n [%])					
	Race/ethnicity (n [%])					
	Elective Caesarean section (n [%])					
	Intrapartum fever (n [%])					
	Prolonged rupture of membranes (n [%])					
	Chorioamnionitis (n [%])					
	Co-morbidity (n [%]) specify what this included					
	History of allergy from antibiotic (n[%])					
	Co-intervention (n[%])specify what this included					
	Multiple births (n [%])					
	Mean (rage or SD) birth weight (g)					
	Smoking (n [%])					
	Other (specify)					
Outcomes						
Adverse event name		**Definition**		**Time point:**	**Measurement**	
Results						
Adverse event 1 *(specify)*	**Intervention (IAP) [n]**	**Control [n]**	**Total**	**OR, RR, mean difference (95%CI)**		**Covariates adjusted for**
				Crude	**Adjusted**	

Adverse event 1 *(specify)* Occurred						
Adverse event 1 *(specify)* Did not occur						
Total						
Adverse event 2 *(specify)*	**Intervention (IAP) [n]**	**Control [n]**	**Total [n]**	**OR, RR, mean difference (95%CI)** **Crude**	**Adjusted**	**Covariates adjusted for**
Adverse event 2 *(specify)* Occurred						
Adverse event 2 *(specify)* Did not occur						
Total						
Add more 2x2 tables and statistical results for more adverse events as necessary						
Authors' conclusion:						
Reviewer Notes:						
Abbreviations: 95% CI=95 percent confidence interval; SD=standard deviation; n=number						

Appendix 10. Full-text excluded from systematic on adverse events from intrapartum antibiotic prophylaxis, with reason (objective 3)

Reference	Reason
Aard LA, Saed F. Low-incidence cesarean section: 12-year experience. Mayo Clin Proc 1975; 50(7): 365-9.	Prophylaxis for Caesarean section
Accordino, F., et al. (2016). Risk factors for cerebral palsy in PPROM and preterm delivery with intact membranes[*]. Journal of Maternal-Fetal and Neonatal Medicine 29(23): 3854-3859.	More than 10% had symptoms in labour (chorioamnionitis)
Adeniran AS, Aboyeji AP, Fawole AA, Adesiyun OO, Saidu R. Role of Risk-Based Approach in the Prevention of Vertical Transmission of Neonatal Sepsis. Niger Postgrad Med J 2015; 22(2): 88-92.	More than 10% had symptoms in labour (intrapartum fever)
Andrews WW, Hauth JC, Cliver SP, Savage K, Goldenberg RL. Randomized clinical trial of extended spectrum antibiotic prophylaxis with coverage for Ureaplasma urealyticum to reduce post-cesarean delivery endometritis. Obstet Gynecol 2003; 101(6): 1183-9.	Prophylaxis for Caesarean section
Anonymous. Prophylactic antibiotics in caesarean section. Br Med J 1973; 2(5868): 675-6.	Consensus statement
Anonymous. Obesity in pregnancy. Obstet Gynecol 2015; 126(6): e112-e26.	Review
Anteby SO, Birkenfeld A, Weinstein D. Post cesarean section urinary tract infections, risk factors and prophylactic antibiotic treatment. Clin Exp Obstet Gynecol 1984; 11(4): 161-4.	Prophylaxis for Caesarean section
Apgar BS, Greenberg G, Yen G. Prevention of group B streptococcal disease in the newborn. Am Fam Physician 2005; 71(5): 903-10.	Review
Apuzzio JJ, Ganesh VV, Pelosi MA, Frisoli G. The effect of prophylactic antibiotics on risk factors for endomyometritis in adolescent patients undergoing cesarean section. Journal of adolescent health care : official publication of the Society for Adolescent Medicine, 1984.	Prophylaxis for Caesarean section
Ayangade O. Antibiotic prophylaxis in high-risk obstetrics. J Natl Med Assoc 1977; 69(11): 793-5.	Unable to identify timing of antibiotics
Ayangade O. Long vs short-course antibiotic prophylaxis in cesarean section: a comparative clinical study. J Natl Med Assoc 1979; 71(1): 71-3.	Prophylaxis for Caesarean section
Azad, M. B., et al. (2016). Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. BJOG: An International Journal of Obstetrics and Gynaecology 123(6): 983-993	More than 10% had symptoms in labour (prolonged rupture of membranes)
Battarino O, Battarino A. [Short-term antibiotic prophylaxis in cesarean section]. Minerva ginecologica, 1988.	Full-text not in English
Beattie PG, Rings TR, Hunter MF, Lake Y. Risk factors for wound infection following caesarean section. Aust N Z J Obstet Gynaecol 1994; 34(4): 398-402.	Prophylaxis for Caesarean section
Benigno BB, Ford LC, Lawrence WD, Ledger WJ, Ling FW, McNeeley SG. A double-blind, controlled comparison of piperacillin and cefoxitin in the prevention of postoperative infection in patients undergoing cesarean section. Surg Gynecol Obstet, 1986.	Prophylaxis for Caesarean section
Benjamin DK, Stoll BJ, Gantz MG, et al. Neonatal Candidiasis: Epidemiology, Risk Factors, and Clinical Judgment. Pediatrics 2010; 126(4): E865-E73.	Unable to distinguish intrapartum antibiotics with other timings
Berardi A, Rossi C, Creti R, et al. Group B Streptococcal colonization in 160 mother-baby pairs: A prospective cohort study. J Pediatr 2013; 163(4): 1099-104.e1.	No data on adverse events
Berardi, A., et al. (2016). "The burden of early-onset sepsis in Emilia-Romagna (Italy): a 4-year, population-based study." Journal of Maternal-Fetal and Neonatal Medicine 29(19): 3126-3131.	More than 10% had symptoms in labour (prolonged rupture of membranes)
Berkeley AS, Hirsch JC, Freedman KS, Ledger WJ. Cefotaxime for cesarean section prophylaxis in labor. Intravenous administration vs. lavage. Journal of Reproductive Medicine for the Obstetrician and Gynecologist 1990; 35(3): 214-8.	Prophylaxis for Caesarean section
Bibi M, Megdiche H, Ghanem H, et al. [Antibiotic prophylaxis in a priori cesarean sections without a high risk of infection. Experiences of a Tunisian maternity department]. Journal de gynécologie, obstétrique et biologie de la reproduction, 1994.	Full-text not in English
Birkenfeld A, Anteby SO. The effect of ampicillin and colistin on post-Caesarean section endometritis with identification of possible risk factors. Aust N Z J Obstet Gynaecol 1983; 23(4): 204-7.	Prophylaxis for Caesarean section

Reference	Reason
Block BS, Mercer LJ, Ismail MA, Moawad AH. Clostridium difficile-associated diarrhea follows perioperative prophylaxis with cefoxitin. Am J Obstet Gynecol 1985; 153(8): 835-8.	More than 10% prophylaxis for Caesarean section
Boothby R, Benrubi G, Ferrell E. Comparison of intravenous cefoxitin prophylaxis with intraoperative cefoxitin irrigation for the prevention of post-cesarean-section endometritis. Journal of Reproductive Medicine for the Obstetrician and Gynecologist 1984; 29(11): 830-2.	Prophylaxis for Caesarean section
Bourgeois FJ, Pinkerton JA, Andersen W, Thiagarajah S. Antibiotic irrigation prophylaxis in the high-risk cesarean section patient. Am J Obstet Gynecol 1985; 153(2): 197-201.	Prophylaxis for Caesarean section
Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. N Engl J Med 1986; 314(26): 1665-9.	More than 10% participants had symptoms in labour (maternal fever)
Bromiker R, Ernest N, Meir MB, et al. Correlation of bacterial type and antibiotic sensitivity with maternal antibiotic exposure in early-onset neonatal sepsis. Neonatology 2013; 103(1): 48-53.	More than 10% participants had symptoms in labour (maternal fever)
Brown J, Thompson M, Sinnya S, et al. Pre-incision antibiotic prophylaxis reduces the incidence of post-caesarean surgical site infection. J Hosp Infect 2013; 83(1): 68-70.	Prophylaxis for Caesarean section
Brozanski BS, Jones JG, Krohn MA, Sweet RL. Effect of a screening-based prevention policy on prevalence of early-onset group B streptococcal sepsis. Obstet Gynecol 2000; 95(4): 496-501.	No data on adverse events
Buchholz NP, Daly-Grandeau E, Huber-Buchholz MM. Urological complications associated with caesarean section. Eur J Obstet Gynecol Reprod Biol 1994; 56(3): 161-3.	Prophylaxis for Caesarean section
Busowski JD, Porter KB, Pendergraft S, O'Brien WF, Vodra J. Antibiotic prophylaxis for Cesarean delivery: a randomized trial of cefotetan, ampicillin-sulbactam and ciprofloxacin. Prenat Neonatal Med 2000; 5(6): 357-62.	Prophylaxis for Caesarean section
Carlson C, Duff P. Antibiotic prophylaxis for cesarean delivery: is an extended-spectrum agent necessary? Obstet Gynecol 1990; 76(3 Pt 1): 343-6.	Prophylaxis for Caesarean section
Carney E. Antibiotic prophylaxis in obstetrics and gynecology. J Med Assoc State Ala 1975; 44(9): 493-4, 9.	Review
Cassidy-Bushrow AE, Sitarik A, Levin AM, et al. Maternal group B Streptococcus and the infant gut microbiota. J Dev Orig Health Dis 2016; 7(1): 45-53.	More than 10% neonates received antibiotics after birth
Chan AC, Leung AK, Chin RK, Chang AM. Single dose prophylactic antibiotics in caesarean sections. Aust N Z J Obstet Gynaecol 1989; 29(2): 107-9.	Prophylaxis for Caesarean section
Chang PL, Newton ER. Predictors of antibiotic prophylactic failure in post-cesarean endometritis. Obstet Gynecol 1992; 80(1): 117-22.	Prophylaxis for Caesarean section
Chantharojwong P. An efficacy study of ampicillin versus cefazolin prophylaxis in patients undergoing cesarean section. J Med Assoc Thai 1993; 76(3): 165-70.	Prophylaxis for Caesarean section
Chimura T. The efficacy of ceftriaxone administered for prophylaxis of postoperative infection and infectious diseases in obstetrics and gynecology. J Chemother 1989; 1(4 Suppl): 1039-41.	Antibiotics administered after birth
Chittacharoen A, Manonai J, Suthutvoravut S, Phaupradit W. Single-dose amoxycillin-clavulanic acid vs. ampicillin prophylaxis in emergency cesarean section. International Journal of Gynecology and Obstetrics 1998; 62(3): 249-54.	Prophylaxis for Caesarean section
Conover WB, Moore TR. Comparison of irrigation and intravenous antibiotic prophylaxis at cesarean section. Obstet Gynecol 1984; 63(6): 787-91.	Prophylaxis for Caesarean section
Conturso R, Valsecchi A, De Lalla F. Evaluation of mezlocillin versus placebo as a prophylactic agent in cesarean section. Chemioterapia 1987; 6(2 Suppl): 611-3.	Prophylaxis for Caesarean section
Currier JS, Tosteson TD, Platt R. Cefazolin compared with cefoxitin for cesarean section prophylaxis: the use of a two-stage study design. J Clin Epidemiol 1993; 46(7): 625-30.	Prophylaxis for Caesarean section
Cyrkowicz A, Rytwińska E, Nytko J, Słowińska-Zabówka M. [Preparation for delivery in patients with missed labor considering low-dose heparin and prostaglandins]. Przegląd lekarski, 1996.	Full-text not in English
D'Angelo LJ, Sokol RJ. Short- versus long-course prophylactic antibiotic treatment in Cesarean section patients. Obstet Gynecol 1980; 55(5): 583-6.	Prophylaxis for Caesarean section
Daley AJ, Isaacs D. Ten-year study on the effect of intrapartum antibiotic prophylaxis on early onset group B streptococcal and Escherichia coli neonatal sepsis in Australasia. Pediatr Infect Dis J 2004; 23(7): 630-4.	Population level ecological study
Dashefsky B. Prophylaxis against neonatal group B streptococcal disease. Pediatr Infect Dis J 1990; 9(2): 147-9.	Letter

Reference	Reason
Davey P. Antimicrobial prophylaxis for caesarean section - the unanswered questions. Journal of Obstetrics and Gynaecology 1992; 12(SUPPL. 1): S21-S3.	Review
De Luca C, Buono N, Santillo V, et al. Screening and management of maternal colonization with Streptococcus agalactiae: an Italian cohort study. J Matern-Fetal Neonatal Med 2016; 29(6): 911-5.	No data on adverse events
Decavalas G, Maroulis G, Papaioannou C, Papapetropoulou M. Comparative study of ceftriaxone versus cefamandole for pre-operative prophylaxis of infections in patients undergoing cesarean section or vaginal hysterectomy. J Chemother 1989; 1(4 Suppl): 1048-50.	Prophylaxis for Caesarean section
Dlamini LD, Sekikubo M, Tumukunde J, et al. Antibiotic prophylaxis for caesarean section at a Ugandan hospital: a randomised clinical trial evaluating the effect of administration time on the incidence of postoperative infections. BMC Pregnancy & Childbirth 2015; 15: 91.	Prophylaxis for Caesarean section
Donnenfeld AE, Otis C, Weiner S. Antibiotic prophylaxis in cesarean section. Comparison of intrauterine lavage and intravenous administration. The Journal of reproductive medicine, 1986.	Prophylaxis for Caesarean section
Duff P, Park RC. Antibiotic prophylaxis for cesarean section in a military population. Mil Med 1980; 145(6): 377-81.	Prophylaxis for Caesarean section
Dumas AM, Girard R, Ayzac L, et al. Effect of intrapartum antibiotic prophylaxis against group B streptococcal infection on comparisons of rates of endometritis and urinary tract infection in multicenter surveillance. Infect Control Hosp Epidemiol 2008; 29(4): 327-32.	No data on adverse events
Easmon CSF, Hastings MJG, Deeley J. The effect of intrapartum chemoprophylaxis on the vertical transmission of group B streptococci. Br J Obstet Gynaecol 1983; 90(7): 633-5.	No data on adverse events
Ecker KL, Donohue PK, Kim KS, Shepard JA, Aucott SW. The impact of group B streptococcus prophylaxis on late-onset neonatal infections. J Perinatol 2013; 33(3): 206-11.	More than 10% participants had symptoms in labour (prolonged rupture of membranes)
Edwards RK, Clark P, Sistrom CL, Duff P. Intrapartum antibiotic prophylaxis 1: Relative effects of recommended antibiotics on gram-negative pathogens. Obstet Gynecol 2002; 100(3): 534-9.	More than 10% antibiotics received antibiotics before labour
Elliott JP, Flaherty JF. Comparison of lavage or intravenous antibiotics at cesarean section. Obstet Gynecol, 1986.	Prophylaxis for Caesarean section
Elliott JP, Freeman RK, Dorchester W. Short versus long course of prophylactic antibiotics. Am J Obstet Gynecol 1982; 143(7): 740-4.	More than 10% prophylaxis for Caesarean section
Elyan A, Mahran M, el-Maraghy M, Abou-Seeda M. Prophylactic intravenous metronidazole in cesarean section. Chemioterapia 1984; 3(1): 67-70.	Prophylaxis for Caesarean section
Engel K, Karschnia R. Bacterial flora changes resulting from antimicrobial treatment. Journal of Obstetrics and Gynaecology 1986; 6(SUPPL. 1): S6-S8.	More than 10% prophylaxis for Caesarean section
Engel K, Karschnia R, Rauch U, Amir B. Efficacy of a high dosage short course prophylactic treatment for postoperative infection complications in cesarean section - using a combination of mezlocillin and oxacillin (Optocillin). Chemioterapia 1982; 1(4 Suppl.): No. 326.	Prophylaxis for Caesarean section
Escobedo Lobatón JM, Rodríguez Hinojosa DE, Kistner Garza AM, Benavides de Anda L. [Prophylactic use of antibiotics in cesarean section]. Ginecología y obstetricia de México, 1991.	Full-text not in English
Faro S, Cox SM, Phillips L, Baker J. Influence of antibiotic prophylaxis on vaginal microflora. Journal of Obstetrics and Gynaecology 1986; 6(SUPPL. 1): S4-S6.	More than 10% received antibiotics for Caesarean section
Faro S, Martens MG, Hammill HA, Riddle G, Tortolero G. Antibiotic prophylaxis: is there a difference? Am J Obstet Gynecol 1990; 162(4): 900-7; discussion 7-9.	More than 10% prophylaxis for Caesarean section
Farret TCF, Dalle J, da Silva Monteiro V, Riche CVW, Antonello VS. Risk factors for surgical site infection following cesarean section in a Brazilian Women's Hospital: A case-control study. Braz J Infect Dis 2015; 19(2): 113-7.	Prophylaxis for Caesarean section
Fedele L, Acaia B, Marchini M, Baglioni A, Frigoli A, De Pascale A. Cefotetan and ceftriaxone for single-dose prophylaxis in cesarean section. J Chemother 1989; 1(4 Suppl): 1042-3.	Prophylaxis for Caesarean section
Fejgin MD, Markov S, Goshen S, Segal J, Arbel Y, Lang R. Antibiotic for cesarean section: the case for 'true' prophylaxis. Int J Gynaecol Obstet 1993; 43(3): 257-61.	Prophylaxis for Caesarean section
Felton DJ, Williams JD. Prophylactic ampicillin the the surgical induction of labour. J Obstet Gynaecol Br Commonw 1967; 74(6): 862-7.	Antibiotics administered before labour
Fonseca SNS, Sofia MH, Quintana S, Nogueira FDS, Levin AS. Successful control program to implement the appropriate antibiotic prophylaxis for cesarean section. Rev Inst Med Trop Sao Paulo 2008; 50(2): 79-82.	Prophylaxis for Caesarean section

Reference	Reason
Ford LC. Cost of antibiotic prophylaxis in cesarean section. Drug Intell Clin Pharm 1986; 20(7-8): 592-3.	Prophylaxis for Caesarean section
Ford LC, Tabsh K, Lebherz TB. Use of antibiotics for prophylaxis with caesarean section. Journal of Obstetrics and Gynaecology 1986; 6(SUPPL. 1): S68-S70.	Prophylaxis for Caesarean section
Francis C, Mumford M, Strand ML, Moore ES, Strand EA. Timing of prophylactic antibiotic at cesarean section: a double-blinded, randomized trial. J Perinatol 2013; 33(10): 759-62.	Prophylaxis for Caesarean section
Freeman GM. The efficacy of prophylactic antibiotics in high-risk patients undergoing cesarean section. J-Am-Osteopath-Assoc, 1982.	Prophylaxis for Caesarean section
Galask RP, Weiner C, Petzold CR. Comparison of single-dose cefmetazole and cefotetan prophylaxis in women undergoing primary caesarean section. J Antimicrob Chemother 1989; 23 Suppl D: 105-8.	Prophylaxis for Caesarean section
Gall SA. The efficacy of prophylactic antibiotics in cesarean section. Am J Obstet Gynecol, 1979.	Prophylaxis for Caesarean section
Gall SA, Hill GB. Single-dose versus multiple-dose piperacillin prophylaxis in primary cesarean operation. Am J Obstet Gynecol 1987; 157(2): 502-6.	S Prophylaxis for Caesarean section
Gerard P, Verghote-D'Hulst M, Bachy A, Duhaut G. Group B streptococcal colonization of pregnant women and their neonates. Epidemiological study and controlled trial of prophylactic treatment of the newborn. Acta Paediatr Scand, 1979.	Antibiotics administered after birth
Gerber B, Retzke F, Wilken H. [Effectiveness of perioperative preventive use of antibiotics with ampicillin/gentamycin or cefotiam in abdominal cesarean section]. Zentralblatt für Gynäkologie, 1989.	Full-text not in English
Gerstner G, Kofler E, Huber J. [Perioperative metronidazole-prophylaxis for cesarian section (author's transl]. Zeitschrift für Geburtshilfe und Perinatologie, 1980.	Full-text not in English
Ghuman M, Rohlandt D, Joshy G, Lawrenson R. Post-caesarean section surgical site infection: Rate and risk factors. N Z Med J 2011; 124(1339): 32-6.	Prophylaxis for Caesarean section
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Gibbs RS, Hunt JE, Schwarz RH. A follow-up study on prophylactic antibiotics in cesarean section. Am J Obstet Gynecol 1973; 117(3): 419-22.	Prophylaxis for Caesarean section
Gibbs RS, Weinstein AJ. Bacteriologic effects of prophylactic antibiotics in cesarean section. Am J Obstet Gynecol, 1976.	Prophylaxis for Caesarean section
Gidiri MF, Ziruma A. A randomized clinical trial evaluating prophylactic single-dose vs prolonged course of antibiotics for caesarean section in a high HIV-prevalence setting. J Obstet Gynaecol 2014; 34(2): 160-4.	Prophylaxis for Caesarean section
Giuliani B, Periti E, Mecacci F. Antimicrobial prophylaxis in obstetric and gynecological surgery. J Chemother 1999; 11(6): 577-80.	Surgical prophylaxis
Glasgow TS, Speakman M, Firth S, James B, Byington CL, Young PC. Clinical and economic outcomes for term infants associated with increasing administration of antibiotics to their mothers. Paediatr Perinat Epidemiol 2007; 21(4): 338-46.	Unclear when antibiotic was given
Gonen R, Samberg I, Levinski R. Effect of irrigation or intravenous antibiotic prophylaxis on infectious morbidity at cesarean section. Obstet Gynecol 1986; 67(4): 545-8.	Prophylaxis for Caesarean section
Gong SP, Guo HX, Zhou HZ, Chen L, Yu YH. Morbidity and risk factors for surgical site infection following cesarean section in Guangdong Province, China. J Obstet Gynaecol Res 2012; 38(3): 509-15.	Prophylaxis for Caesarean section
Gonik B, Shannon RL, Shawar R, Costner M, Seibel M. Why patients fail antibiotic prophylaxis at cesarean delivery: histologic evidence for incipient infection. Obstet Gynecol 1992; 79(2): 179-84.	Prophylaxis for Caesarean section
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Gordon SF, Russell J. A randomized controlled study comparing ceftizoxime, cefamandole, and cefoxitin in obstetric and gynecological surgery: A preliminary report. J Antimicrob Chemother 1982; 10(Suppl. C): 289-92.	Surgical prophylaxis
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Gronlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal flora after cesarean delivery. J Pediatr Gastroenterol Nutr 1999; 28(1): 19-25.	More than 10% participants had elective caesarean section
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Haesslein HC, Goodlin RC. Extraperitoneal cesarean section revisited. Obstet Gynecol 1980; 55(2): 181-3.	Prophylaxis for Caesarean section
Hager WD, Rapp RP, Billeter M, Bradley BB. Choice of antibiotic in nonelective cesarean section. Antimicrob Agents Chemother 1991; 35(9): 1782-4.	Prophylaxis for Caesarean section
Harger JH, English DH. Selection of patients for antibiotic prophylaxis in cesarean sections. Am J Obstet Gynecol 1981; 141(7): 752-8.	Prophylaxis for Caesarean section
Harries MJ, McIntyre SJ, Kingston TP. Co-amoxiclav-induced acute generalized exanthematous pustulosis confirmed by patch testing. Contact Dermatitis 2006; 55(6): 372.	Case report
Heilmann L, Tauber PF. [Short-term prevention with cefoxitin in cesarean section]. Geburtshilfe Frauenheilkd, 1984.	Full-text not in English
Iqbal R, Intsar A, Khurshid S, Manzoor T, Shehbaz S. Single dose antibiotic prophylaxis in emergency caesarean section. Pakistan Journal of Medical and Health Sciences 2012; 6(1): 77-80.	Prophylaxis for Caesarean section
Itskovitz J, Paldi E, Katz M. The effect of prophylactic antibiotics on febrile morbidity following cesarean section. Obstet Gynecol 1979; 53(2): 162-5.	Prophylaxis for Caesarean section
Jaffe R, Altaras M, Cohen I, Ben-Aderet N. Single-dose mezlocillin prophylaxis in emergency cesarean section. Clin Ther 1985; 7(4): 507-11.	Prophylaxis for Caesarean section
Jaffe R, Altaras M, Loebel R, Ben-Aderet N. Single- versus multiple-dose mezlocillin prophylaxis in emergency cesarean section. Chemotherapy 1986; 32(2): 173-7.	Prophylaxis for Caesarean section
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Kaimal AJ, Zlatnik MG, Cheng YW, et al. Effect of a change in policy regarding the timing of prophylactic antibiotics on the rate of postcesarean delivery surgical-site infections. Am J Obstet Gynecol 2008; 199(3): 310.e1-5.	Prophylaxis for Caesarean section
Kamilya G, Seal SL, Mukherji J, Roy H, Bhattacharyya SK, Hazra A. A Randomized controlled trial comparing two different antibiotic regimens for prophylaxis at cesarean section. Journal of Obstetrics and Gynecology of India 2012; 62(1): 35-8.	Prophylaxis for Caesarean section
Katz VL, Moos MK, Cefalo RC, Thorp Jr JM, Bowes Jr WA, Wells SD. Group B streptococci: Results of a protocol of antepartum screening and intrapartum treatment. Am J Obstet Gynecol 1994; 170(2): 521-6	>10% had premature rupture of membranes
Kayihura V, Osman NB, Bugalho A, Bergström S. Choice of antibiotics for infection prophylaxis in emergency cesarean sections in low-income countries: a cost-benefit study in Mozambique. Acta Obstet Gynecol Scand, 2003.	Prophylaxis for Caesarean section
Kittur ND, McMullen KM, Russo AJ, Ruhl L, Kay HH, Warren DK. Long-term effect of infection prevention practices and case mix on cesarean surgical site infections. Obstet Gynecol 2012; 120(2 Pt 1): 246-51.	Prophylaxis for Caesarean section
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Krasnodebski J, Stolecki M. [A single dose of antibiotic--as a prophylaxis during cesarean section]. Ginekol Pol, 1997.	Full-text not in English
Kreutner AK, Bene VE, Delamar D, Bodden JL, Loadholt CB. Perioperative cephalosporin prophylaxis in cesarean section: effect on endometritis in the high-risk patient. Am J Obstet Gynecol, 1979.	Prophylaxis for Caesarean section
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Kunze M, Ziegler A, Fluegge K, Hentschel R, Proempeler H, Berner R. Colonization, serotypes and transmission rates of group B streptococci in pregnant women and their infants born at a single University Center in Germany. J Perinat Med 2011; 39(4): 417-22.	No data on adverse events
Lemus Rocha R, García Gutiérrez LB, Basavilvazo Rodríguez MA, Cruz Avelar A, Peralta Pedrero ML, Hernández Valencia M. [Incidence of infected surgical wound	Full-text not in English

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and prophylaxis with cefotaxime in cesarean section]. Ginecología y obstetricia de México, 2005.	
Levine EM, Ghai V, Barton JJ, Strom CM. Intrapartum antibiotic prophylaxis increases the incidence of gram-negative neonatal sepsis. Infect Dis Obstet Gynecol 1999; 7(4): 210-3.	More than 10% had risk factors (preterm delivery, PROM, fever, prior GBS bacteriuria
Lewis DF, Otterson WN, Dunnihoo DR. Antibiotic prophylactic uterine lavage in cesarean section: a double-blind comparison of saline, ticarcillin, and cefoxitin irrigation in indigent patients. South Med J 1990; 83(3): 274-6.	Prophylaxis for Caesarean section
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Louie TJ, Binns BA, Baskett TF, Ross J, Koss J. Cefotaxime, cefazolin, or ampicillin prophylaxis of febrile morbidity in emergency cesarean sections. Clin Ther, 1982.	Prophylaxis for Caesarean section
Lyimo FM, Massinde AN, Kidenya BR, Konje ET, Mshana SE. Single dose of gentamicin in combination with metronidazole versus multiple doses for prevention of post-caesarean infection at Bugando Medical Centre in Mwanza, Tanzania: a randomized, equivalence, controlled trial. BMC Pregnancy & Childbirth 2013; 13: 123.	Prophylaxis for Caesarean section
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Matani, C., et al. (2016). "Streptococcus agalactiae: prevalence of antimicrobial resistance in vaginal and rectal swabs in Italian pregnant women." Infezioni in Medicina 24(3): 217-221.	No IAP information/ population level study
Mansueto GB, Tomaselli F. [Antibiotic prophylaxis in non-elective cesarean section with single-dose imipenem versus multiple-dose cefotaxime]. Rivista europea per le scienze mediche e farmacologiche = European review for medical and pharmacological sciences = Revue européenne pour les sciences médicales et pharmacologiques, 1989. =	Full-text not in English
Mathelier AC. A comparison of postoperative morbidity following prophylactic antibiotic administration by combined irrigation and intravenous route or by intravenous route alone during cesarean section. J Perinat Med 1992; 20(3): 177-82.	Prophylaxis for Caesarean section
Matorras R, Garcia-Perea A, Madero R, Usandizaga JA. Maternal colonization by group B streptococci and puerperal infection; analysis of intrapartum chemoprophylaxis. Eur J Obstet Gynecol Reprod Biol 1991; 38(3): 203-7.	More than 10% participants had elective caesarean section
Matorras R, Garcia-Perea A, Omenaca F, Diez-Enciso M, Madero R, Usandizaga JA. Intrapartum chemoprophylaxis of early-onset group B streptococcal disease. Eur J Obstet Gynecol Reprod Biol 1991; 40(1): 57-62.	More than 10% participants had elective caesarean section
May SM, Hartz MF, Joshi AY, Park MA. Intrapartum antibiotic exposure for group B Streptococcus treatment did not increase penicillin allergy in children. Annals of Allergy, Asthma and Immunology 2016; 116(2): 134-8.	More than 10% IAP given for Caesarean section surgery
McCowan L, Jackson P. The prophylactic use of metronidazole in caesarean section. N Z Med J 1980; 92(666): 153-5.	Prophylaxis for Caesarean section
McGregor JA, French JI, Makowski E. Single-dose cefotetan versus multidose cefoxitin for prophylaxis in cesarean section in high-risk patients. Am J Obstet Gynecol 1986; 154(4): 955-60.	Prophylaxis for Caesarean section
Mengist, A., et al. (2016). "Prevalence and antimicrobial susceptibility pattern of anorectal and vaginal group B Streptococci isolates among pregnant women in Jimma, Ethiopia." BMC Research Notes 9: 351.	No IAP information/ population level study
Melendez J, Claxton A, Erskine K. MRSA bacteraemia after caesarean section wound infection: when screening is missed and things go wrong. Arch Gynecol Obstet 2012; 285(3): 663-5.	Case report
Menson EN, Gilbert RE, Sharland MR. What is the effect of prepartum antimicrobials on neonatal infection? Curr Opin Infect Dis 2004; 17(3): 213-6.	Review
Meyer NL, Hosier KV, Scott K, Lipscomb GH. Cefazolin versus cefazolin plus metronidazole for antibiotic prophylaxis at cesarean section. South Med J, 2003.	Prophylaxis for Caesarean section
Mihailovic M, Hani A, Klainguti A, Soldini G. Antimicrobial prophylaxis in non-infected patients undergoing abdominal or vaginal hysterectomy or cesarean section. Comparative efficacy of a single preoperative dose of ceftriaxone and of multiple doses of combined amoxicillin plus metronidazole and of amoxicillin alone. J Chemother 1989; 1(4 Suppl): 1029-30.	Prophylaxis for Caesarean section or hysterectomy

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Mivumbi VN, Little SE, Rulisa S, Greenberg JA. Prophylactic ampicillin versus cefazolin for the prevention of post-cesarean infectious morbidity in Rwanda. Int J Gynaecol Obstet 2014; 124(3): 244-7.	Prophylaxis for Caesarean section
Moberg PJ, Schedvins K. Use of cefuroxime in preventing postcesarean infection in high-risk patients. Gynecol Obstet Invest 1989; 28(1): 19-22.	Prophylaxis for Caesarean section
Moodley J, Zeeman DJ. Prophylactic and antimicrobial therapy using lincomycin in patients undergoing emergency caesarean section. S Afr Med J 1981; 59(25): 911-3.	Prophylaxis for Caesarean section
Moro M, Andrews M. Prophylactic antibiotics in cesarean section. Obstet Gynecol 1974; 44(5): 688-92.	Prophylaxis for Caesarean section
Morrison JC, Coxwell WL, Kennedy BS, Schreier PC, Wiser WL, Fish SA. The use of prophylactic antibiotics in patients undergoing cesarean section. Surg Gynecol Obstet, 1973.	Prophylaxis for Caesarean section
Mothilal M, Thivya R, Anjalakshi C, Ramesh A, Damodharan N. Comparison of effectiveness of Azithromycin and Cefazolin in post caesarean section infection. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(SUPPL 3): 92-4.	Prophylaxis for Caesarean section
Newton ER, Prihoda TJ, Gibbs RS. A clinical and microbiologic analysis of risk factors for puerperal endometritis. Obstet Gynecol 1990; 75(3 Pt 1): 402-6.	Prophylaxis for Caesarean section
Newton ER, Wallace PA. Effects of prophylactic antibiotics on endometrial flora in women with postcesarean endometritis. Obstet Gynecol 1998; 92(2): 262-8.	Participants had endometritis at the beginning of study
Ng NK, Sivalingam N. The role of prophylactic antibiotics in caesarean section--a randomised trial. Med J Malaysia 1992; 47(4): 273-9.	Prophylaxis for Caesarean section
Ngoc NTN, Sloan NL, Thach TS, Liem LKB, Winikoff B. Incidence of postpartum infection after vaginal delivery in Viet Nam. J Heatlh Popul Nutr 2005; 23(2): 121-30.	No data on adverse events and antibiotics given after birth
Nice C, Feeney A, Godwin P, et al. A prospective audit of wound infection rates after caesarean section in five West Yorkshire hospitals. J Hosp Infect 1996; 33(1): 55-61.	Prophylaxis for Caesarean section
Nokiani FA, Akbari H, Rezaei M. Timing of prophylactic antibiotic administration in term cesarean section: A randomized clinical trial. Iranian Journal of Clinical Infectious Diseases 2009; 4(2): 71-6.	Prophylaxis for Caesarean section
O'Leary JA, Mullins JH, Andrinopoulos GC. Ampicillin vs. ampicillin-gentamicin prophylaxis in high-risk primary cesarean section. The Journal of reproductive medicine, 1986.	Prophylaxis for Caesarean section
Ogasawara KK, Goodwin TM. Efficacy of azithromycin in reducing lower genital Ureaplasma urealyticum colonization in women at risk for preterm delivery. The Journal of maternal-fetal medicine, 1999.	More than 10% had preterm premature rupture of membranes
Ogasawara KK, Murphy Goodwin T. The efficacy of prophylactic erythromycin in preventing vertical transmission of Ureaplasma urealyticum. Am J Perinatol 1997; 14(4): 233-7.	More than 10% preterm premature rupture of membranes
Ognissanti F, Bucciero A, Conturso R, et al. A comparison of mezlocillin and cefotetan in cesarean section prophylaxis: a prospective, randomized study. Preliminary results. J Chemother 1989; 1(4 Suppl): 1030-2.	Prophylaxis for Caesarean section
Oliva GC, Fratoni A, Papadia LS, Tartaglia E, Mancuso S. Antibiotic prophylaxis in emergency and elective cesarean section. J Chemother 1989; 1(4 Suppl): 1020-2.	Prophylaxis for Caesarean section
Owens SM, Brozanski BS, Meyn LA, Wiesenfeld HC. Antimicrobial Prophylaxis for Cesarean Delivery Before Skin Incision. Obstet Gynecol 2009; 114(3): 573-9.	Prophylaxis for Caesarean section
Padilla SL, Spence MR, Beauchamp PJ. Single-dose ampicillin for cesarean section prophylaxis. Obstet Gynecol 1983; 61(4): 463-6.	Prophylaxis for Caesarean section
Periti P, Mazzei T, Periti E. Prophylaxis in gynaecological and obstetric surgery: a comparative randomised multicentre study of single-dose cefotetan versus two doses of cefazolin. Chemioterapia : international journal of the Mediterranean Society of Chemotherapy, 1988.	Surgical prophylaxis
Persaud RR, Azad MB, Chari RS, et al. Perinatal antibiotic exposure of neonates in Canada and associated risk factors: a population-based study. J Matern-Fetal Neonatal Med 2015; 28(10): 1190-5.	No data on adverse events
Peterson CM, Medchill M, Gordon DS, Chard HL. Cesarean prophylaxis: a comparison of cefamandole and cefazolin by both intravenous and lavage routes, and risk factors associated with endometritis. Obstet Gynecol 1990; 75(2): 179-82.	Prophylaxis for Caesarean section
Phelan JP, Pruyn SC. Prophylactic antibiotics in cesarean section: a double-blind study of cefazolin. Am J Obstet Gynecol 1979; 133(5): 474-8.	Prophylaxis for Caesarean section

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Pitt C, Sanchez-Ramos L, Kaunitz AM. Adjunctive intravaginal metronidazole for the prevention of postcesarean endometritis: A randomized controlled trial. Obstet Gynecol 2001; 98(5): 745-50.	Prophylaxis for Caesarean section
Polk BF, Schoenbaum SC. Prophylactic antibiotics in obstetrics. Clin Obstet Gynecol 1979; 22(2): 379-84.	Review
Pothinam S, Chanpoo T, Lumbiganon P. Post-cesarean section puerperal morbidity. The incidence and risk factors at Srinagarind Hospital. J Med Assoc Thai 1992; 75(3): 173-7.	Prophylaxis for Caesarean section
Poulain P, Betremieux P, Donnio PY, Proudhon JF, Karege G, Giraud JR. Selective intrapartum anti-bioprophylaxy of group B streptococci infection of neonates: A prospective study in 2454 subsequent deliveries. Eur J Obstet Gynecol Reprod Biol 1997; 72(2): 137-40.	More than 10% had symptoms in labour
Puopolo KM, Madoff LC, Eichenwald EC. Early-onset group B streptococcal disease in the era of maternal screening. Pediatrics 2005; 115(5): 1240-6.	No data on adverse events
Rayburn W, Varner M, Galask R. Comparison of moxalactam and cefazolin as prophylactic antibiotics during cesarean section. Antimicrob Agents Chemother 1985; 27(3): 337-9.	Prophylaxis for Caesarean section
Raymond J, Lopez E, Bonacorsi S, et al. Evidence for transmission of escherichia coli from mother to child in late-onset neonatal infection. Pediatr Infect Dis J 2008; 27(2): 186-8.	Case report
Reggiori A, Ravera M, Cocozza E, Andreata M, Mukasa F. Randomized study of antibiotic prophylaxis for general and gynaecological surgery from a single centre in rural Africa. The British journal of surgery, 1996.	Surgical prophylaxis
Rehu M, Jahkola M. Prophylactic antibiotics in Caesarean section: effect of a short preoperative course of benzyl penicillin or clindamycin plus gentamicin on postoperative infectious morbidity. Ann Clin Res 1980; 12(2): 45-8.	Prophylaxis for Caesarean section
Renner RM, Renner A, Schmid S, et al. Efficacy of a strategy to prevent neonatal early-onset group B streptococcal (GBS) sepsis. J Perinat Med 2006; 34(1): 32-8.	No data on adverse events
Rentz AC, Samore MH, Stoddard GJ, Faix RG, Byington CL. Risk factors associated with ampicillin-resistant infection in newborns in the era of group B streptococcal prophylaxis. Arch Pediatr Adolesc Med 2004; 158(6): 556-60.	More than 10% symptomatic (chorioamnionitis and prolonged rupture of membranes)
Rijhsinghani A, Savopoulos SE, Walters JK, Huggins G, Hibbs JR. Ampicillin/sulbactam versus ampicillin alone for cesarean section prophylaxis: A randomized double-blind trial. Am J Perinatol 1995; 12(5): 322-4.	Prophylaxis for Caesarean section
Roex AJ, Van Loenen AC. Pharmacokinetics of three-dose cefoxitin prophylaxis in caesarean section. Pharm Weekbl Sci 1988; 10(6): 281-3.	Prophylaxis for Caesarean section
Roex AJM, Puyenbroek JI, Van Loenen AC, Arts NFT. Single- versus three-dose cefoxitin prophylaxis in caesarean section: A randomized clinical trial. Eur J Obstet Gynecol Reprod Biol 1987; 25(4): 293-8.	Prophylaxis for Caesarean section
Roth P, Schaal JP, Fromentin C, Guerrier T, Maillet R, Colette C. [Comparative study of 2 protocols for antibiotic therapy. Maternal-fetal non-specific bacterial infections during labor]. Journal de gynécologie, obstétrique et biologie de la reproduction, 1990.	Full-text not in English
Rothbard MJ, Mayer W, Wystepek A, Gordon M. Prophylactic antibiotics in cesarean section. Obstet Gynecol 1975; 45(4): 421-4.	Prophylaxis for Caesarean section
Rouse DJ, Hauth JC, Andrews WW, Mills BB, Maher JE. Chlorhexidine vaginal irrigation for the prevention of peripartal infection: A placebo-controlled randomized clinical trial. Am J Obstet Gynecol 1997; 176(3): 617-22.	Not systemic prophylaxis
Rudge MV, Atallah AN, Peracoli JC, Tristao Ada R, Mendonca Neto M. Randomized controlled trial on prevention of postcesarean infection using penicillin and cephalothin in Brazil. Acta Obstet Gynecol Scand 2006; 85(8): 945-8.	Prophylaxis for Caesarean section
Saad A, Finan R, Papas S, Anastabiades E. Evaluation of ceftizoxime in the prophylaxis of gynecological surgery. Revue Medicale Libanaise 2004; 16(1): 36-8.	Surgical prophylaxis and timing of antibiotics also unclear.
Sabir S. Infective morbidity following Caesarean section. Specialist 1996; 13(1): 29-32.	Prophylaxis for Caesarean section
Saezllorens X, Ahchu MS, Castano E, et al. Intrapartum Prophylaxis with Ceftriaxone Decreases Rates of Bacterial-Colonization and Early-Onset Infection in Newborns. Clin Infect Dis 1995; 21(4): 876-80.	More than 10% symptomatic (prolonged rupture of membranes)

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Saltzman DH, Eron LJ, Tuomala RE, Protomastro LJ, Sites JG. Single-dose antibiotic prophylaxis in high-risk patients undergoing cesarean section. A comparative trial. J Reprod Med 1986; 31(8): 709-12.	Prophylaxis for Caesarean section
Schrag SJ, Cutland CL, Zell ER, et al. Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of perinatal sepsis trial, Soweto, South Africa. Pediatr Infect Dis J 2012; 31(8): 821-6.	More than 10% symptomatic (prolonged rupture of membrane, foul smelling vaginal discharge)
Schrag SJ, Hadler JL, Arnold KE, Martell-Cleary P, Reingold A, Schuchat A. Risk factors for invasive, early-onset Escherichia coli infections in the era of widespread intrapartum antibiotic use. Pediatrics 2006; 118(2): 570-6.	More than 10% symptomatic (intrapartum fever, prolonged rupture of membrane)
Schuchat A, Zywicki SS, Dinsmoor MJ, et al. Risk factors and opportunities for prevention of early-onset neonatal sepsis: A multicenter case-control study. Pediatrics 2000; 105(1): 21-6.	More than 10% symptomatic (intrapartum fever, prolonged rupture of membrane)
Sengupta A, Kohli JK. Antibiotic prophylaxis in cesarean section causing anaphylaxis and intrauterine fetal death. J Obstet Gynaecol Res 2008; 34(2): 252-4.	Case report
Shrestha B, Marhatha R, Giri A, Jaisi S, Maskey U. Surgical site wound infection in relation to antibiotic prophylaxis given before skin incision and after cord clamping during cesarean delivery. Nepal Med Coll J 2014; 16(2-4): 148-51.	Prophylaxis for Caesarean section
Simchen E, Shapiro M, Michel J, Sacks TG. The successful use of antibiotic prophylaxis in selected high-risk surgical patients under non-trial, everyday conditions. J Hosp Infect 1980; 1(3): 211-20.	Surgical prophylaxis
Singleton ML. Group B strep prophylaxis: what are we creating? Midwifery Today Int Midwife 2007; (81): 18-20.	Editorial
Skjeldestad FE, Bjornholt JV, Gran JM, Erisken HM. The effect of antibiotic prophylaxis guidelines on surgical-site infections associated with cesarean delivery. International Journal of Gynecology and Obstetrics 2014; 128(2): 126-30.	Prophylaxis for Caesarean section
Smith AM, Cox CWFM. Necrotising fasciitis following caesarean section. Journal of Obstetrics and Gynaecology 1992; 12(4): 246-7.	Case report
Spandorfer SD, Graham E, Forouzan I. Postcesarean endometritis. Clinical risk factors predictive of positive blood cultures. J Reprod Med 1996; 41(11): 797-800.	Prophylaxis for Caesarean section
Spreafico P, Scian A, Epis A, Vassen L, Bonazzi C, Lovotti M. Cesarean section: antibiotic prophylaxis with ceftezole. Chemioterapia 1987; 6(2 Suppl): 613-6.	Prophylaxis for Caesarean section
Stage AH, Glover DD, Vaughan JE. Low-dose cephradine prophylaxis in obstetric and gynecologic surgery. J Reprod Med 1982; 27(3): 113-9.	Surgical prophylaxis
Stark MA, Ross MF, Kershner W, Searing K. Case Study of Intrapartum Antibiotic Prophylaxis and Subsequent Postpartum Beta-Lactam Anaphylaxis. Jognn 2015; 44(5): 610-7.	Case report
Stiver HG, Forward KR, Livingstone RA. Double blind placebo-controlled multicentre comparison of cefoxitin vs cefazolin prophylaxis against post-cesarean section infection. Clinical and Investigative Medicine 1982; 5(2-3): 34B.	Abstract
Stiver HG, Forward KR, Livingstone RA. Multicenter comparison of cefoxitin versus cefazolin for prevention of infectious morbidity after nonelective cesarean section. Am J Obstet Gynecol 1983; 145(2): 158-63.	Prophylaxis for Caesarean section
Stiver HG, Forward KR, Tyrrell DL, et al. Comparative cervical microflora shifts after cefoxitin or cefazolin prophylaxis against infection following cesarean section. Am J Obstet Gynecol 1984; 149(7): 718-21.	Prophylaxis for Caesarean section
Sullivan SA, Smith T, Chang E, Hulsey T, Vandorsten JP, Soper D. Administration of cefazolin prior to skin incision is superior to cefazolin at cord clamping in preventing postcesarean infectious morbidity: a randomized, controlled trial.[Erratum appears in Am J Obstet Gynecol. 2007 Sep;197(3):333]. Am J Obstet Gynecol 2007; 196(5): 455.e1-5.	Prophylaxis for Caesarean section
Suonio S, Saarikoski S, Vohlonen I, Kauhanen O. Risk factors for fever, endometritis and wound infection after abdominal delivery. Int J Gynaecol Obstet 1989; 29(2): 135-42.	Prophylaxis for Caesarean section
Szalontay AS. [Antibiotic prophylaxis in cesarean section]. Revista medico-chirurgicală a Societăţii de Medici şi Naturalişti din Iaşi, 1997.	Full-text not in English
Tassi PG, Tarantini M, Rampinelli F, et al. Piperacillin in antibiotic prophylaxis: a single-dose administration for cesarean section. J Chemother 1989; 1(4 Suppl): 1025-6.	Prophylaxis for Caesarean section

Reference	Reason
Tassi PG, Tarantini M, Cadenelli GP, Gastaldi A, Benedetti M. Ceftazidime in antibiotic prophylaxis for emergency cesarean section: a randomized prospective study. Int J Clin Pharmacol Ther Toxicol 1987; 25(10): 582-8.	Prophylaxis for Caesarean section
Teo SM, Mok D, Pham K, et al. The Infant Nasopharyngeal Microbiome Impacts Severity of Lower Respiratory Infection and Risk of Asthma Development. Cell Host Microbe 2015; 17(5): 704-15.	Unclear when antibiotics were given and delivery mode
Thigpen BD, Hood WA, Chauhan S, et al. Timing of prophylactic antibiotic administration in the uninfected laboring gravida: a randomized clinical trial. Am J Obstet Gynecol, 2005.	Prophylaxis for Caesarean section
Thurman AR, Anca Y, White CA, Soper DE. Post-cesarean delivery infectious morbidity: Focus on preoperative antibiotics and methicillin-resistant Staphylococcus aureus. Am J Infect Control 2010; 38(8): 612-6.	Prophylaxis for Caesarean section
To WW, Lau WN. A protocol of selective antibiotic prophylaxis for caesarean section based on risk factors. Aust N Z J Obstet Gynaecol 2001; 41(4): 402-6.	Prophylaxis for Caesarean section
Towers CV, Cart MH, Padilla G, Asrat T. Potential consequences of widespread antepartal use of ampicillin. Am J Obstet Gynecol 1998; 179(4): 879-83.	Unable to distinguish mothers treated in labour from mother treated in pregnancy as well
Tsai CH, Chen YY, Wang KG, Chen CY, Chen CP. Characteristics of early-onset neonatal sepsis caused by Escherichia coli. Taiwan J Obstet Gynecol 2012; 51(1): 26-30.	Unable to distinguish women who had emergency caesarean section from those that had elective caesarean section
Tully JL, Klapholz H, Baldini LM, Friedland GH. Perioperative use of cefoxitin in primary cesarean section. J Reprod Med 1983; 28(12): 827-32.	Prophylaxis for Caesarean section
Tuppurainen N, Hallman M. Prevention of neonatal group B streptococcal disease: intrapartum detection and chemoprophylaxis of heavily colonized parturients. Obstet Gynecol, 1989.	No data on adverse events
Turner MJ. Prophylactic antibiotics for caesarean section and hysterectomy. Journal of Obstetrics and Gynaecology 1994; 14(1): 54-5.	Editorial
Tzingounis V, Makris N, Zolotas J. Cefuroxime prophylaxis in caesarean section. Pharmatherapeutica 1982; 3(2): 140-2.	Prophylaxis for Caesarean section
van der Linden MC, van Erp EJ, Ruijs GJ, Holm JP. A prospective randomized study comparing amoxycillin/clavulanate with cefuroxime plus metronidazole for perioperative prophylaxis in gynaecological surgery. Eur J Obstet Gynecol Reprod Biol 1993; 50(2): 141-5.	Surgical prophylaxis
Van Scoy RE. Prophylactic antibiotic therapy: its use and abuse. Clin Obstet Gynecol 1976; 19(3): 721-33.	Review
Varner MW, Weiner CP, Petzold CR, Galask RP. Comparison of cefotetan and cefoxitin as prophylaxis in cesarean section. Am J Obstet Gynecol, 1986.	Prophylaxis for Caesarean section
von Mandach U, Huch R, Malinverni R, Huch A. Ceftriaxone (single dose) versus cefoxitin (multiple doses): success and failure of antibiotic prophylaxis in 1052 cesarean sections. J Perinat Med 1993; 21(5): 385-97.	Prophylaxis for Caesarean section
Wali A, Taj Z, Abbas Z. Chemoprophylaxis in caesarean sections. Journal of the College of Physicians and Surgeons Pakistan 2002; 12(2): 78-81.	Prophylaxis for Caesarean section
Wallace RL, Yonekura ML. The use of prophylactic antibiotics in patients undergoing emergency primary cesarean section. Am J Obstet Gynecol 1983; 147(5): 533-6.	Prophylaxis for Caesarean section
Wax JR, Hersey K, Philput C, et al. Single dose cefazolin prophylaxis for postcesarean infections: before vs. after cord clamping. J Matern Fetal Med 1997; 6(1): 61-5.	Prophylaxis for Caesarean section
Wegienka G, Havstad S, Zoratti EM, Kim H, Ownby DR, Johnson CC. Combined effects of prenatal medication use and delivery type are associated with eczema at age 2 years. Clin Exp Allergy 2015; 45(3): 660-8	Timing of antibiotics unclear and unable to distinguish between antibiotics and antifungals
Weinberg M, Fuentes JM, Ruiz AI, et al. Reducing infections among women undergoing cesarean section in Colombia by means of continuous quality improvement methods. Arch Intern Med 2001; 161(19): 2357-65.	More than 10% participants had elective caesarean section
Weissberg SM, Edwards NL, O'Leary JA. Prophylactic antibiotics in cesarean section. Obstet Gynecol 1971; 38(2): 290-3.	Prophylaxis for Caesarean section
Westen EHMN, Kolk PR, Van Velzen CL, et al. Single-dose compared with multiple day antibiotic prophylaxis for cesarean section in low-resource settings, a	Prophylaxis for Caesarean section

Reference	Reason
randomized controlled, noninferiority trial. Acta Obstet Gynecol Scand 2015; 94(1): 43-9.	
Wolfe HM, Gross TL, Sokol RJ, Bottoms SF, Thompson KL. Determinants of morbidity in obese women delivered by cesarean. Obstet Gynecol 1988; 71(5): 691-6.	Prophylaxis for Caesarean section
Wong R, Gee CL, Ledger WJ. Prophylactic use of cefazolin in monitored obstetric patients undergoing cesarean section. Obstet Gynecol 1978; 51(4): 407-11.	Prophylaxis for Caesarean section
Work BA, Jr. Role of preventive antibiotics in patients undergoing cesarean section. South Med J 1977; 70 Suppl 1: 44-5.	Prophylaxis for Caesarean section
Yip SK, Lau TK, Rogers MS. A study on prophylactic antibiotics in cesarean sections - Is it worthwhile? Acta Obstet Gynecol Scand 1997; 76(6): 547-9.	Prophylaxis for Caesarean section
Yonekura ML, Appleman M, Wallace R, Boucher M, Nakamura R. Predictive value of amniotic-membrane cultures for the development of postcesarean endometritis. Rev Infect Dis 1984; 6 Suppl 1: S157-64.	Prophylaxis for Caesarean section
Young BC, Hacker MR, Dodge LE, Golen TH. Timing of antibiotic administration and infectious morbidity following cesarean delivery: incorporating policy change into workflow. Arch Gynecol Obstet 2012; 285(5): 1219-24.	Prophylaxis for Caesarean section
Young R, Platt L, Ledger W. Prophylactic cefoxitin in cesarean section. Surg Gynecol Obstet 1983; 157(1): 11-4.	Prophylaxis for Caesarean section
Zhang J, Johnson CD, Hoffman M. Cervical cerclage in delayed interval delivery in a multifetal pregnancy: a review of seven case series. Eur J Obstet Gynecol Reprod Biol 2003; 108(2): 126-30.	Review of case series about cervical cerclage for multiple births

Appendix 11. Summary of the included studies in the systematic review on the adverse events from intrapartum antibiotic prophylaxis (objective 3)

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
Studies investigating outcome as adverse events of IAP								
Aloisio 2014[304] Italy	Cohort	52 infants 6-7 days old (26 treated 26 not treated)	GBS prophylaxis Intrapartum 2g ampicillin at least 4h before delivery, followed by 1g every 4h until delivery	Gut microbiota: *E. coli* *Bacteroides fragilis* *Bifidobacterium spp.* *Clostridium difficile* *Lactobacillus* spp.	 M: 8.18 (R: 4.09-12.70) M: 8.17 (R: 4.68-11.99) M: 5.85 (R: 3.24–7.79) M: 3.89 (R: 3.12–4.80) M: 6.69 (R: 5.40–8.93)	 M: 9.03 (R: 5.61-11.78) M: 8.53 (R: 5.22-11.16) M: 7.29 (R: 4.12–10.95) M: 3.70 (R: 2.85–5.46) M: 6.73 (R: 5.45–8.20)	 NS NS p=0.001 NS NS	 None
Aloisio 2016[305] Italy	Prospective cohort	20 infants 6-7 days old (10 treated 10 not treated)	GBS prophylaxis Intrapartum 2g ampicillin at least 4 h before delivery, followed by 1 g every 4 h until delivery	Gut microbiota composition: *Actinobacteria* *Bacteriodetes* *Proteobacteria* *Firmicutes*	 0.4% 16.0% 54.7% -	 3.8% 47.7% 15.5% -	 p< 0.05 p< 0.05 p< 0.05 -	 None None None -
				Gut microbiota composition: *Bifidobacteriaceae* genus Other microbial genus	 0.02%	 6.469%	 p< 0.05 non-significant	 None
				Gut microbiota: Sample richness and biodiversity	<u>Alpha diversity</u> The control group showed a more complex microbial profile compared with the IAP group who had a reduced level of richness and biodiversity: Chao1 and Shannon indexes: p=0.0081 and p=0.036, respectively; Bray-Curtis index: p > 0.05. <u>Beta diversity</u> Significant phylogenetic and relative abundance difference: unweighted UniFrac distance, p< 0.05. Principal coordinate			None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment Control	Summary measures (95%CI)	Factors adjusted for in the analysis
					analysis based on Weighted and Unweighted UniFrac distances at genus level shows segregation along axis1 for both UniFrac indices, indicating a separation in two clusters due to IAP treatment. Diversity at bacterial family level A more complex profile in control compared with IAP group in terms of biodiversity, with a more equal distribution at family/genus level. The IAP group had a lower number of bacterial families with some cases composed almost exclusively by *Enterobacteriaceae* family members (*Proteobacteria*) that can reach over 90% of relative abundance and a few samples are even characterised by the presence of *Streptococcaceae* family (with an average of 13%).		
Arboleya 2015[306] Spain	Cohort	27 preterm infants 2-90 days old (14 treated, 13 not treated)	Indication not known 1 mother received a single dose of penicillin, and 1 mother received 1 dose of ampicillin every 6 hours for 3 days. 12 mothers received ampicillin plus erythromycin [between 2 and 24 doses of each antibiotic)	Gut microbiota composition	Cluster analysis Day 2: Higher percentage of sequences from *Leuconostaceae* in controls. Day 10: Higher percentage of sequences from *Micrococcaceae* and *Propionibacteriaceae* in controls. Day 30: Higher relative amounts of *Comamonadaceae, Staphylococcaceae*, and unclassified *Bacilli* in controls. Higher *Bifidobacteriaceae*, *Streptococcaceae,* unclassified *Actinobacteria*, and unclassified *Lactobacillales* ($p< 0.05$) in controls. Lower percentage of *Enterobacteriaceae* in controls ($p< 0.05$) Day 90: Most differences disappeared except in *Ruminococcaceae* microbial group (differences unclear) Quantitative PCR: Day 2 and 10: No significant differences Day 30: Higher amounts of *Staphylococcaceae* in control. Lower amounts of *Enterobacteriaceae* and total bacteria in control. Day 90: higher amounts of *bifidobacteria* in control.		None
Arboleya 2016[307] Spain	Cohort (same cohort as	27 preterm infants	Indication not known	Gut microbiota composition	Day 1: no statistically significant differences on the bacterial phyla		None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
	above Arboleya 2015 study)	2-90 days old (14 treated, 13 not treated) (same cohort as above)	1 mother received a single dose of penicillin, and 1 mother received 1 dose of ampicillin every 6 hours for 3 days. 12 mothers received ampicillin plus erythromycin [between 2 and 24 doses of each antibiotic)		Day 30: higher relative frequency of *Actinobacteria* phylum ($p< 0.05$) and *Firmicutes* phylum ($p< 0.01$) in controls. Lower frequency of *Proteobacteria* phylum in controls. Higher levels of acetic ($p=0.075$) and total ($p=0.060$) short chain fatty acids in controls.			
Ashkenazi-Hoffnung 2011[308] Israel	Case-control	195 infants 7-90 days old (17 treated, 178 not treated)	GBS prophylaxis 94% ampicillin	Late-onset serious bacterial infections	8	63	OR per dose of IAP: 5.19 (0.01-93.11)	Infant age, maternal age birth weight, gestational age, type of delivery, and GBS status had no significant effect. Number of doses, time from antibiotic administration to delivery
				Ampicillin resistant late-onset serious bacterial infections	85% = 14.45/17 (Note: Numbers do not add up - 14 people would be 82% and 15	63% = 112/178	p=0.19	None – "Multivariate logistic regression did not identify any

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
					people would be 88%)			variable that was significantly associated with increased risk of resistance to ampicillin or FGCs"
				First-generation cephalosporin resistant late-onset serious bacterial infections	57% = 9.69/17 (Note: Numbers do not add up - 9 people would be 53% and 10 people would be 59%)	26% = 46/178	p=0.19	None – "Multivariate logistic regression did not identify any variable that was significantly associated with increased risk of resistance to ampicillin or FGCs"
				First-generation cephalosporin resistance in UTI only	75% (unable to calculate numbers)	23.5% (unable to calculate numbers)	p=0.04	None
				Ampicillin resistant *E. coli* only	100% (unable to calculate numbers)	54.5% (unable to calculate numbers)	p=0.14	None
				First-generation cephalosporin resistant *E. coli* only	60% (unable to calculate numbers)	22.7% (unable to calculate numbers)	p=0.21	None
				Gentamicin or third generation cephalosporin resistance	0	0	-	-
Balter 2003[309] US	Retrospective cohort	261 infants		5 minute APGAR score	Median: 8 IQR: 8-9	Median: 8 IQR: 8-9	-	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		(81 treated, 180 not treated)	GBS prophylaxis (59%) Other reasons (39%) Maternal fever (6%) Antibiotic not reported	Complete blood count	21	17	*RR: 2.75(1.53–4.92)*	None
				Blood culture drawn	10	10	*RR: 2.22 (0.96–5.13)*	None
				Urine culture *via* catheterisation	2	1	*RR: 4.44 (0.41-48.32)*	None
				Any urine culture	4	2	*RR: 4.44 (0.83-23.78)*	None
				Chest radiograph	3	8	*RR: 0.83 (0.23-3.06)*	None
				Infant given antibiotics within 7 days	6	8	*RR: 1.67 (0.60-4.65)*	None
				Infant given intravenous catheter	4	8	*RR: 1.11 (0.34-3.58)*	None
				Infant in NICU	3	7	*RR: 0.95 (0.25-3.59)*	None
				Mechanical ventilation	1	0	-	None
				Supplemental oxygen	5	9	*RR: 1.23 (0.43-3.57)*	None
				Hospitalisation ≥ 48 hours	14	12	*RR: 2.59 (1.26-5.35)*	None
				Hospitalisation > 72 hours	14	17	*RR: 1.83 (0.95-3.53)*	None
				Length of hospitalisation	56.8 hours median	47 hours median	p=0.02	None
Briody 2016[310] US	Retrospective cohort	165 intrapartum women (73 received 'appropriate' IAP, 92 received	GBS prophylaxis Appropriate IAP: Penicillin, Cefazolin Inappropriate IAP: Clindamycin,	Neonate placed on antibiotics	3	4	*RR: 0.94 (0.22-4.09)*	None
				Hospital stay > 2 days	25	22	*RR: 1.43 (0.88-2.32)*	None
				Hospital stay > 3 days	15	16	*RR: 1.18 (0.63-2.23)*	None
				5 minute APGAR score	M: 9 (R: 5-10)	M: 9 (R: 3-10)	p=0.24	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		'inappropriate' IAP)	Erythromycin, Vancomycin	Number of blood cultures performed	M: 2 (SD: 2.7)	M: 9 (SD: 9.9)	p=0.11	None
Corvaglia 2016[311] Italy	Prospective cohort	84 infants 7-30 days old (35 treated, 49 not treated)	GBS Prophylaxis Intravenous ampicillin every 4 hours until delivery (first dose 2 g, following doses 1 g each)	Gut Microbiota composition: *Bifidobacterium spp.* 7 days *Bifidobacterium spp.* 30 days *Lactobacillus spp.* 7 days *Lactobacillus spp.* 30 days *Bacteroides fragilis spp.* 7 days *Bacteroides fragilis spp.* 30 days	 Median: 6.01 (IQR: 5.51–6.98) Median: 8.41 (IQR: 7.71-8.80) Median: 5.56 (IQR: 4.94-6.14) Median: 5.29 (IQR: 4.68-6.01) Median: 7.71 (IQR: 5.80-9.33) Median: 7.36 (IQR 5.80-9.09)	 Median: 7.80 (IQR: 6.61–8.26] Median: 8.39 (IQR: 7.96-8.86) Median: 5.45 (IQR: 4.81-6.14) Median: 5.25 (IQR: 4.60-6.15) Median: 7.75 (IQR: 5.87-9.61) Median: 8.51 (IQR: 5.86-9.37)	 p=0.000 p=0.363 p=0.872 p=0.932 p > 0.05 p > 0.05	 Feeding
Cox 1996[312] US	Randomised controlled trial	78 intrapartum women (39 treated, 39 not treated)	Preterm labour 2g ampicillin and 1g sulbactam parenterally every 6 hours for 8 doses, followed by ampicillin-clavunate 250mg orally every 8 hours for 5 days.	Symptomatic vulvovaginitis caused by *Candida albicans*	27	Not stated	-	-
				Pseudo-membranous enterocolitis caused by *Clostridium difficile*	1	Not stated	-	-
Dinsmoor 2005[313] US	Retrospective cohort	435 mother-infant pairs 0-	136 for GBS Prophylaxis. Other mothers	Neonatal thrush	21	18	OR: 1.87 (0.97-3.63)	None
				Maternal thrush	22	17	OR: 2.1 (1.08-4.08)	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		1 month post-partum (173 treated, 262 not treated)	received antibiotics for other indications	Total candidiasis	26	20	OR: 2.14 (1.15-3.97)	None
Glasgow 2005[314] US	Case-control	182 infants 7-90 days old (62 treated, 120 not treated)	Indication not known Penicillin, ampicillin, or broad spectrum	Late-onset serious bacterial infection	37 Penicillin only: 10/23 Broad-spectrum: 29/39	53 80 61	OR: 1.96 (1.05–3.66) OR: 0.95 (0.37-2.44) OR: 4.95 (2.04–11.98)	Hospital of delivery, maternal chorioamnionitis and breastfeeding
				Ampicillin-resistant late-onset serious bacterial infections	24 Penicillin only: 4/9 Ampicillin only: 12/18 Other IAP: 8/10	13 33 25 29	OR: 5.7 (2.3–14.3) OR: 2.5 (0.6-10.6) OR: 6.2 (1.9-19.7) OR: 12.3 (2.3-65.5)	Hospital of delivery
				Ampicillin resistant UTI infections	Not reported	Not reported	OR: 4.3 (1.6–11.7)	Hospital of delivery
				Other serious bacterial infections (meningitis, omphalitis, and bacteraemia without UTI)	Not reported	Not reported	OR: 25 (1.8–346)	Hospital of delivery
Gordon 1995[315] US	Randomised controlled trial	117 intrapartum women (58 treated, 59 not treated)	Preterm labour Ceftizoxime for 5 days or 3 days	Bleeding abnormalities	0	-	-	-
				Clostridium difficile colitis	0	-	-	-
				Multi-resistant bacterial infections	0	-	-	-

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
Jaureguy 2004[316] France	Prospective cohort	50 infants 3 days old (25 treated, 25 not treated)	GBS Prophylaxis Intravenous 2g amoxicillin at the time of labour and then 1g every 4 h until delivery	Gut microbiota: Numbers colonised with:				
				Enterobacteria	13	16	p=0.58	
				Enterococci	15	17	p=0.73	
				Staphylococci	21	22	p=1.00	
				Bacteroides	13	7	p=0.15	
				Clostridium	3	10	p=0.04	
				Bifidobacterium	6	12	p=0.18	None
				Gut microbiota composition (log CFU/gram):				
				Enterobacteria	Median: 8.4 (R: 3.3–9.5)	Median: 9.2 (R: 3.3–9.8)	p=0.18	
				Enterococci	Median 8.3 (R: 3.6–10.3)	Median: 7.3 (R: 3.3–9.5)	p=0.78	
				Staphylococci	Median: 6.5 (R: 3.6–8.0)	Median: 7.0 (R: 4.0–9.3)	p=0.53	
				Bacteroides	Median: 8.0 (R: 6.3–10.3)	Median: 7.9 (R: 3.6–9.6)	p=0.12	
				Clostridium	Median: 5.3 (R: 4.3–5.8)	Median: 6.2 (R: 3.6–8.1)	p=0.01	
				Bifidobacterium	Median: 8.2 (R: 4.3–9.5)	Median: 8.5 (R: 6.9-10.3)	p=0.10	None
				Amoxicillin-resistant *Enterobacteria*	10	12	*RR: 0.83 (0.44-1.56)*	None
				Amoxicillin-resistant *E. coli*	6	11	*RR: 0.55 (0.24-1.25)*	None
Kampikaho 1993[317] Uganda	Quasi-randomised controlled trial	660 intrapartum women (330 treated, 330 not treated)	Post-partum infection prevention 1g streptomycin or 0.8MU penicillin	Side effects	0	-	-	-

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
Keettel 1949[319] US	Controlled trial	895 intrapartum women (465 treated, 430 not treated)	Post-partum infection prevention 300,000/600,000 units of penicillin at the indication of labour and then after 24-hour intervals.	Mild urticaria	7	-	-	-
				General urticaria	2 (8-12 days, 600,000 units)	-	-	-
				Local allergic manifestations	5 (900,000 units)	-	-	-
				Abscess formations at site of injections	0	-	-	-
				Discomfort following injections	Relatively uncommon and never severe or persistent	-	-	-
Keettel 1950[318] US	Controlled trial	773 intrapartum women (382 treated, 391 not treated)	Post-partum infection prevention 600,000 units of penicillin at the indication of labour, and then after 24-hour intervals.	General urticaria	1 (8 days)	-	-	-
				Local allergic manifestations	1	-	-	-
				Abscess formations at the site of injections	0	-	-	-
Kenyon 2008[320] UK	Factorial randomised trial	3173 children 0-7 years old (numbers differ for outcomes – see treatment column)	Spontaneous preterm labour 375 mg amoxicillin–clavulanate (n=763), 250 mg erythromycin (n=785), amoxicillin–clavulanate and erythromycin (n=796), double placebo (n=735)	Mild functional impairment	ERY and AMC: 181/769 ERY: 191/785 AMC: 168/763	151/735	OR: 1.00 (reference) OR: 1.24 (0.96–1.60) OR: 1.29 (1.00–1.65) OR: 1.10 (0.85–1.42)	Maternal baseline, social class, and other factors
				Moderate functional impairment	ERY and AMC: 91/769 ERY: 94/785 AMC: 85/763	77/735	OR: 1.00 (reference) OR: 1.22 (0.88–1.70) OR: 1.24 (0.89–1.72) OR: 1.09 (0.78–1.53)	Maternal baseline, social class, and other factors
				Severe functional impairment	ERY and AMC: 53/769 ERY: 48/785	47/735	OR: 1.00 (reference) OR: 1.17 (0.77–1.77) OR: 1.04 (0.68–1.59)	Maternal baseline, social class, and other factors

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
					AMC: 46/763		OR: 0.97 (0.63–1.49)	
				Any functional impairment	ERY and AMC: 325/769 ERY: 333/785 AMC: 299/763	275/735	OR: 1.00 (reference) OR: 1.22 (1.00–1.51) OR: 1.23 (1.00–1.51) OR: 1.08 (0.88–1.33)	Maternal baseline, social class, and other factors
				Three or more abnormal attributes	ERY and AMC: 72/769 ERY: 59/785 AMC: 75/763	74/735	OR: 1.00 (reference) OR: 0.92 (0.66–1.30) OR: 0.73 (0.51–1.04) OR: 0.97 (0.69–1.37)	Maternal baseline, social class, and other factors
				Cerebral palsy	ERY and AMC: 35/769 ERY: 18/785 AMC: 15/763	12/735	OR: 1.00 (reference) OR: 2.91 (1.50–5.65) OR: 1.42 (0.68–2.98) OR: 1.22 (0.57–2.62)	Maternal baseline, social class, and other factors
			Any erythromycin, 250mg (n=1554), no erythromycin (n=1498)	Functional impairment	None: 896 Mild: 372 Moderate: 185 Severe: 101 Any: 658 Three or more abnormal attributes: 131	None: 924 Mild: 319 Moderate: 162 Severe: 93 Any: 574 Three or more abnormal attributes: 149	OR: 1.00 (reference) OR: 1.20 (1.01–1.43) OR: 1.18 (0.94–1.48) OR: 1.12 (0.83–1.51) OR: 1.18 (1.02–1.37) OR: 0.83 (0.65–1.07)	Maternal baseline, social class, and other factors
				Behaviour	Emotional symptoms: 327 Conduct problems: 480 Hyperactivity: 424 Peer problems: 405 Prosocial behaviour: 122	Emotional symptoms: 330 Conduct problems: 420 Hyperactivity: 415 Peer problems: 391 Prosocial behaviour: 99	OR: 0.94 (0.79–1.12) OR: 1.15 (0.98–1.34) OR: 0.98 (0.84–1.15) OR: 1.00 (0.85–1.17) OR: 1.20 (0.91–1.59) OR: 1.03 (0.87–1.21) OR: 1.13 (0.95–1.35)	Maternal baseline, social class, and other factors

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
					Overall difficulties: 384 Impact on families: 334	Overall difficulties: 363 Impact on families: 292		
			Any erythromycin, 250mg (n=1611), no erythromycin (n=1562)	Cerebral palsy	53	27	OR: 1.93 (1.21–3.09)	Maternal baseline, social class, and other factors
				Seizures	149	116	OR: 1.27 (0.99–1.64)	Maternal baseline, social class, and other factors
				Seizures on prescribed medication	27	17	OR: 1.55 (0.84–2.85)	Maternal baseline, social class, and other factors
				Hydrocephalus with shunt	2	3	OR: 0.65 (0.11–3.87)	Maternal baseline, social class, and other factors
				ADHD from SDQ or parental report	120	116	OR: 1.0 (0.77–1.31)	Maternal baseline, social class, and other factors
				Other developmental problems	10	15	OR: 0.64 (0.29–1.44)	Maternal baseline, social class, and other factors
				Wheezing in last year	295	295	OR: 0.96 (0.81–1.15)	Maternal baseline, social class, and other factors
				Medication for chest problems in last year	262	280	OR: 0.89 (0.74–1.07)	Maternal baseline, social

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
								class, and other factors
				Admission to hospital in last year	243	202	OR: 1.20 (0.98–1.46)	Maternal baseline, social class, and other factors
				Admission for chest problems	32	38	OR: 0.81 (0.51–1.31)	Maternal baseline, social class, and other factors
				Diabetes	0	2	-	-
				All bowel disorders	64	38	OR: 1.66 (1.10–2.49)	Maternal baseline, social class, and other factors
			Any erythromycin, 250mg (n=2375), no erythromycin (n=2279)	Stillbirths	20	24	OR: 0.80 (0.44-1.45)	Maternal baseline, social class, and other factors
				Deaths in first year	61	41	OR: 1.44 (0.96–2.14)	Maternal baseline, social class, and other factors
				Deaths after first year	5	5	OR: 0.97 (0.28–3.34)	Maternal baseline, social class, and other factors
				Total deaths	86	70	OR: 1.19 (0.86–1.63)	Maternal baseline, social class, and other factors
			Any erythromycin, 250mg	Educational attainment (children failing to achieve	Reading: 377 Writing: 413 Maths: 239	Reading: 367 Writing: 413 Maths: 225	OR: 1.0 (0.96–1.04)	Maternal baseline, social

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
			(n=1641), no erythromycin (n=1598)	level 2 or higher in national curriculum tests)			OR: 1.0 (0.97–1.04) OR: 0.99 (0.96–1.03)	class, and other factors
			Any amoxicillin–clavulanate, 375 mg (n=1532), no amoxicillin–clavulanate (n=1520)	Functional impairment	None: 908 Mild: 349 Moderate: 176 Severe: 99 Any: 624 Three or more abnormal attributes: 147	None: 912 Mild: 342 Moderate: 171 Severe: 95 Any: 608 Three or more abnormal attributes: 133	OR: 1.00 (reference) OR: 1.02 (0.86–1.22) OR: 1.03 (0.82–1.30) OR: 1.05 (0.78–1.41) OR: 1.03 (0.89–1.19) OR: 1.11 (0.87–1.42)	Maternal baseline, social class, and other factors
				Behaviour	Emotional symptoms: 341 Conduct problems: 454 Hyperactivity: 418 Peer problems: 396 Prosocial behaviour: 112 Overall difficulties: 385 Impact on families: 312	Emotional symptoms: 316 Conduct problems: 446 Hyperactivity: 421 Peer problems: 400 Prosocial behaviour: 109 Overall difficulties: 362 Impact on families: 314	OR: 1.09 (0.92–1.30) OR: 1.01 (0.87–1.18) OR: 0.98 (0.84–1.15) OR: 0.98 (0.83–1.15) OR: 1.02 (0.78–1.34) OR: 1.07 (0.91–1.27) OR: 0.98 (0.82–1.17)	Maternal baseline, social class, and other factors
			Any amoxicillin–clavulanate, 375 mg (n=1587), no amoxicillin–clavulanate (n=1586)	Cerebral palsy	50	30	OR: 1.69 (1.07–2.67)	Maternal baseline, social class, and other factors
				Seizures	144	121	OR: 1.21 (0.94–1.56)	Maternal baseline, social class, and other factors
				Seizures on prescribed medication	22	22	OR: 1.0 (0.55–1.81)	Maternal baseline, social

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
								class, and other factors
				Hydrocephalus with shunt	4	1	OR: 4.01 (0.45–35.87)	Maternal baseline, social class, and other factors
				ADHD from SDQ or parental report	128	108	OR: 1.20 (0.92–1.57)	Maternal baseline, social class, and other factors
				Other developmental problems	8	17	OR: 0.47 (0.20–1.09)	Maternal baseline, social class, and other factors
				Wheezing in last year	291	299	OR: 0.97 (0.81–1.16)	Maternal baseline, social class, and other factors
				Medication for chest problems in last year	257	285	OR: 0.88 (0.73–1.06)	Maternal baseline, social class, and other factors
				Admission to hospital in last year	220	225	OR: 0.97 (0.80–1.19)	Maternal baseline, social class, and other factors
				Admission for chest problems	33	37	OR: 0.89 (0.55–1.43)	Maternal baseline, social class, and other factors
				Diabetes	2	0	-	-
				All bowel disorders	54	48	OR: 1.13 (0.76–1.68)	Maternal baseline, social

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
								class, and other factors
			Any amoxicillin–clavulanate, 375mg (n=2304), no amoxicillin–clavulanate (n=2350)	Stillbirths	20	24	OR: 0.85 (0.47–1.54)	Maternal baseline, social class, and other factors
				Deaths in first year	49	53	OR: 0.94 (0.63–1.39)	Maternal baseline, social class, and other factors
				Deaths after first year	6	4	OR: 1.53 (0.43–5.42)	Maternal baseline, social class, and other factors
				Total deaths	75	81	OR: 0.94 (0.68–1.30)	Maternal baseline, social class, and other factors
			Any amoxicillin–clavulanate, 375 mg (n=1608), no amoxicillin–clavulanate (n=1631)	Educational attainment (children failing to achieve level 2 or higher in national curriculum tests)	Reading: 366 Writing: 395 Maths: 230	Reading: 378 Writing: 431 Maths: 234	OR: 0.99 (0.95–1.03) OR: 0.99 (0.95–1.02) OR: 0.99 (0.95–1.03)	Maternal baseline, social class, and other factors
Keski-Nisula 2013[321] Finland	Prospective cohort	45 mother-infant pairs immediately after birth (17 treated, 28 not treated)	Intrapartum antibiotics according to hospital protocol including GBS, PROM, caesarean section, chorioamnionitis	Lactobacillus-dominant mixed flora transmission	1	13	OR: 0.08 (0.007–0.80)	Fetal sex, maternal smoking during pregnancy, meconium in amniotic fluid, duration of ruptured membranes

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
			Intravenous penicillin or amoxicillin in vaginal deliveries and intravenous second-generation cephalosporins in Caesarean deliveries					
Keuchkerian 2005[322] Uruguay	Randomised controlled trial	96 intrapartum women (47 treated, 49 not treated)	Preterm labour Amoxicillin 1000 mg sulbactam 500 mg IV every 8 h during the first 48 h and they continued to receive an oral intake of amoxicillin 250 mg sulbactam 250 mg every 8 h for 5 days	Palpitations, flushes, nausea and vomiting	2	0	-	-
				Asymptomatic bacteriuria	0	1	-	-
				Urinary infection	1	0	-	-
Lin 2006[296] US	Retrospective cohort	1594 infants (213 treated, 1378 not treated)	GBS prophylaxis Penicillin	Respiratory distress	44	95	RR: 2.62 (1.79–3.83)	Mother's race, mother's race not known, age <20 yr, primigravida, fever during labour, caesarean delivery, Medicaid/public

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
								assistance and positive prenatal culture for GBS, missing values of rupture of membranes and prenatal cultures the degree of colonisation, gestational age by week, race, insulin requirement during pregnancy, suspected infection during labour, intrauterine catheter, not known Pitocin use, not known prenatal GBS culture
				Discharge diagnosis of a respiratory disorder	12	39	*RR: 1.96 (1.04-3.69)*	None
Mazzola 2016[194] Italy	Prospective cohort	26 infants 7-30 days old (13 treated [7 breastfed and 6 mixed fed] 13 not treated [7 breastfed	GBS prophylaxis 2g ampicillin at least 4 h before delivery, followed by 1g to maximum 4g.	Gut microbiota composition	At the phylum level, in breastfed infants, at day 7 *Actinobacteria* were not detected in IAP infants and were present at 17% in control infants (p< 0.001) and there were significantly higher abundances of *Proteobacteria* in IAP infants than controls (p< 0.062). IAP infants were dominated by genera belonging to the *Enterobacteriaceae* family (p=0.044), particularly *Escherichia*, which accounted for 52% of the total relative			None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		and 6 mixed fed])			abundance, compared with 14% in the control group. *Bifidobacteria* were not detected in any of the IAP infants at day 7 but 16% of the relative abundance from control infants (p=0.001), and control infants also had higher levels of *Bacteroides* than IAP infants (20% vs. 7%), although not statistically significant (p=0.078). At day 30, *Bifidobacteria* numbers appeared to have recovered in the IAP group accounting for 6% of the relative abundance (p=0.025) in both groups; *Enterobacteriaceae* continued to dominate in IAP infants compared with control infants (44% vs.16%); Additionally, there was a significantly higher level of the *Veillonellaceae* family in control infants compared with IAP infants (p=0.035). *Veillonella* is affected by the antibiotic treatment, as it does not increase in the IAP group between 7 and 30 days, whereas a strong increase is shown within control samples at the same sampling times in control samples. At the phylum level, in mixed-fed infants, at day 7 there was a higher abundance of *Proteobacteria*, (37% vs. 17%) and *Firmicutes* (41% *versus* 29%) in infant and control infants. On the other hand, *Actinobacteria* (8% vs. 1%) and *Bacteroidetes* (36% vs. 21%) were highest in the control compared with IAP group. At day 7, IAP infants contained high abundances of organisms belonging to family *Enterobacteriaceae* (35% vs. 17%), and *Streptococcus* (32% vs. 10%) compared with the control group. Control infants had higher levels of *Bacteroides*, (32% vs. 13%), and *Bifidobacterium* (5% vs. 1%), compared with IAP infants. By day 30, *Actinobacteria* levels increased in the IAP infants to 7% and *Firmicutes* and *Proteobacteria* reduced to 30% and 28%. *Bacteroidetes* were the dominant phylum in both groups, representing 26% in control and 34% in IAP treated infants. At genus level, the microbiota composition was more uniform than that at day 7. Members of the *Enterobacteriaceae* family fall to 28% and *Streptococcus* was significantly reduced to 8%		

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment Control	Summary measures (95%CI)	Factors adjusted for in the analysis
					(p=0.042); The *Lachnospiraceae* family, absent at day 7 in IAP treated infants, was detected at 4% at day 30. *Bifidobacteria* significantly increased in IAP infants from 0% at day 7 to 6% at day 30, (p=0.013) and remain highest in control infants (19%).		
				Gut microbiota sample richness and biodiversity	<u>Alpha diversity</u> In breastfed infants, at day 7 there was a significantly lower diversity in breastfed IAP infants compared with breast fed control (Chao1 p=0.012), Simpson p=0.035, Shannon p=0.0082 and observed species p=0.021). By day 30 the Chao index and observed species increased in the IAP infants, although this was not a significant increase, and the Simpson and Shannon indices remained largely unchanged. In mixed-fed infants, at day 7 there were no significant differences in diversity although Chao1, Shannon and observed species indices were highest in the control infants. At day 30, alpha diversity was similar in both MF groups <u>Beta diversity</u> In breastfed infants, at day 7, principal coordinate analysis (PCoA) plots constructed using unweighted UniFrac distance matrices shows clear separation IAP samples from those of the Control infants. By day 30, no clear separation was observed, suggesting that microbial communities became more uniform over time. In mixed-fed infants, principal coordinate analysis showed no clustering of samples from either IAP or control groups at day 7 or 30.		None
				Absolute quantification of total bacteria and *bifidobacteria*	Total bacteria numbers were similar across the four groups, ranging between 9.38 to 9.71 at day 7 and 9.53 to 9.83 log CFU/g at day 30, with no significant differences between groups observed At day 7, *Bifidobacterium* spp numbers were observed to be significantly lower in IAP infants compared with control infants (breast fed IAP vs. control: 5.86 log CFU/g vs. 8.16		None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
					log CFU/g, p=0.005; mixed fed IAP vs. control: 5.81 log CFU/g vs. 7.19 log CFU/g, p=0.03). By day 30, a significant increase was observed in both IAP groups (breast fed: 7.72 log CFU/g, p=0.035 and mixed fed: 8.50 log CFU/g, p=0.036). Numbers remain higher in breast fed control infants compared with IAP infants (8.62 log CFU/g vs. 7.72 log CFU/g) but this no longer a significant difference. Numbers have significantly increased in mixed fed control infants to 8.55 log CFU/g (p=0.028) and are similar to that of IAP infants, 8.50 log CFU/g.			
McGregor 1986[323] US	Randomised controlled trial	58 intrapartum women (29 treated, 29 not treated)	Preterm labour 21 enteric-coated erythromycin tablets over 7 days	Withdrawal from study due to nausea/and or vomiting	1	1	*RR: 1.00 (0.07-15.24)*	-
Rajaei 2006[325] Iran	Randomised controlled trial	80 Intrapartum women (38 treated, 42 not treated)	Preterm labour 400 mg erythromycin every 6 h orally for 10 days.	Side effects: nausea, vomiting, hot flushes, decreased deep tendon reflexes, emotional disturbances or drug intolerance	-	-	No significant difference in side effects	-
Roca 2016[326] Gambia	Randomised controlled trial	829 intrapartum women and 843 infants (414 women and 419 infants treated, 415 women and 424 infants not treated)	Neonatal sepsis prevention A single dose of oral 2g azithromycin (4 tablets of 0.5g)	Adverse events/serious adverse events in newborns	0	-	-	-
				Moderate urticarial rash	1	-	-	-
				Newborn GBS nasopharyngeal samples resistant to Azithromycin	Day 2: 0 Day 3: 0 Day 6: 1 (0.3%) Day 14: 1 (0.3%) Day 28: 1 (0.3%)	Day 2: 0 Day 3: 2 (0.5%) Day 6: 2 (0.5%) Day 14: 1 (0.3) Day 28: 0	- - PR: 0.51 (0.05–5.62) PR: 1.02 (0.06–16.31) -	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
				Newborn *S. pneumoniae* nasopharyngeal samples resistant to Azithromycin	Day 2: 0 Day 3: 0 Day 6: 0 Day 14: 2 (0.5%) Day 28: 8 (2.2%)	Day 2: 0 Day 3: 0 Day 6: 2 (0.5%) Day 14: 4 (1.0%) Day 28: 8 (2.1%)	- - - PR: 0.51 (0.009–2.78) PR: 1.04 (0.40–2.75)	None
				Newborn *S. aureus* nasopharyngeal samples resistant to Azithromycin	Day 2: 3 (0.7%) Day 3: 41 (10.6%) Day 6: 48 (12.7%) Day 14: 57 (15.3%) Day 28: 60 (16.7%)	Day 2: 4 (1.0%) Day 3: 27 (6.8%) Day 6: 20 (5.2%) Day 14: 13 (3.4%) Day 28: 17 (4.5%)	PR: 0.77 (0.17–3.40) PR: 1.56 (0.98–2.48) PR: 2.46 (1.49–4.06) PR: 4.49 (2.50–8.06) PR: 3.68 (2.19–6.18)	None
				Newborn any bacteria nasopharyngeal samples resistant to Azithromycin	Day 2: 3 (0.7%) Day 3: 41 (10.6%) Day 6: 49 (13.0%) Day 14: 60 (16.1%) Day 28: 69 (19.2%)	Day 2: 4 (1.0%) Day 3: 29 (7.3%) Day 6: 24 (6.2%) Day 14: 17 (4.5%) Day 28: 25 (6.7%)	PR: 0.77 (0.17–3.40) PR: 1.45 (0.92–2.28) PR: 2.09 (1.31–3.34) PR: 3.61 (2.15–6.08) PR: 2.88 (1.86–4.44)	None
				Maternal GBS nasopharyngeal samples resistant to Azithromycin	Day 2: 0 Day 3: 0 Day 6: 0 Day 14: 0 Day 28: 1 (0.3%)	Day 2: 0 Day 3: 0 Day 6: 0 Day 14: 0 Day 28: 1 (0.3%)	- - - - PR: 1.01 (0.06–16.1)	None
				Maternal *S. pneumoniae* nasopharyngeal samples resistant to Azithromycin	Day 2: 6 (1.4%) Day 3: 3 (0.8%) Day 6: 3 (0.8%) Day 14: 7 (1.8%) Day 28: 7 (1.8%)	Day 2: 0 Day 3: 2 (0.5%) Day 6: 4 (1.0%) Day 14: 3 (0.8%) Day 28: 1 (0.3%)	- PR: 1.51 (0.25–8.97) PR: 0.75 (0.17–3.35) PR: 2.33 (0.61–8.96) PR: 7.09 (0.88–57.4)	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
				Maternal *S. aureus* nasopharyngeal samples resistant to Azithromycin	Day 2: 6 (1.4%) Day 3: 16 (4.0%) Day 6: 22 (5.6%) Day 14: 36 (9.2%) Day 28: 48 (12.6%)	Day 2: 11 (2.7%) Day 3: 7 (1.7%) Day 6: 14 (3.5%) Day 14: 12 (3.1%) Day 28: 11 (2.8%)	PR: 0.55 (0.20–1.46) PR: 2.30 (0.96–8.97) PR: 1.58 (0.82–3.04) PR: 3.00 (1.85–5.68) PR: 4.42 (2.33–8.38)	None
				Maternal any bacteria nasopharyngeal samples resistant to Azithromycin	Day 2: 12 (2.9%) Day 3: 19 (4.8%) Day 6: 25 (6.3%) Day 14: 4 (10.5%) Day 28: 56 (14.7%)	Day 2: 11 (2.7%) Day 3: 9 (2.2%) Day 6: 14 (3.5%) Day 14: 15 (3.8%) Day 28: 13 (3.4%)	PR: 1.09 (0.49–2.45) PR: 2.12 (0.97–4.63) PR: 1.48 (0.81–2.69) PR: 2.73 (1.54–4.86) PR: 4.36 (2.43–7.85)	None
				Maternal GBS vaginal samples resistant to Azithromycin	Day 2: 1 (0.2%) Day 8-10: 6 (1.5%)	Day 2: 2 (0.5%) Day 8-10: 1 (0.3%)	PR: 0.50 (0.05–5.51) PR: 6.06 (0.73–50.1)	None
				Maternal *S. pneumoniae* vaginal samples resistant to Azithromycin	Day 2: 0 Day 8-10: 0	Day 2: 0 Day 8-10: 0	- -	None
				Maternal *S. aureus* vaginal samples resistant to Azithromycin	Day 2: 0 Day 8-10: 27 (6.9%)	Day 2: 7 (1.7%) Day 8-10: 4 (1.0%)	- PR: 6.82 (2.41–19.3)	None
				Maternal any bacteria vaginal samples resistant to Azithromycin	Day 2: 1 (0.2%) Day 8-10: 32 (8.4%)	Day 2: 9 (2.2%) Day 8-10: 5 (1.3%)	PR: 0.11 (0.01–0.88) PR: 6.67 (2.63–16.9)	None
				Maternal GBS breast milk samples	Day 3: 1 (0.3%) Day 6: 1 (0.3%) Day 14: 1(0.3%)	Day 3: 2 (0.5%) Day 6: 2 (0.5%) Day 14: 1 (0.3%)	PR: 0.51 (0.05–5.56) PR: 0.50 (0.05–5.52)	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
				resistant to Azithromycin	Day 28: 0	Day 28: 0	PR: 1.00 (0.006–15.9) -	
				Maternal *S. pneumoniae* breast milk samples resistant to Azithromycin	Day 3: 0 Day 6: 0 Day 14: 0 Day 28: 0	Day 3: 0 Day 6: 0 Day 14: 0 Day 28: 0	- - - -	None
				Maternal *S. aureus* breast milk samples resistant to Azithromycin	Day 3: 19 (4.8%) Day 6: 20 (5.1%) Day 14: 22 (5.7%) Day 28: 14 (3.7%)	Day 3: 7 (1.8%) Day 6: 8 (2.0%) Day 14: 3 (0.8%) Day 28: 5 (1.3%)	PR: 2.75 (1.17–6.47) PR: 2.51 (1.12–5.64) PR: 7.31 (2.21–24.2) PR: 2.82 (1.03–7.76)	None
				Maternal any bacteria breast milk samples resistant to Azithromycin	Day 3: 20 (5.1%) Day 6: 20 (5.3%) Day 14: 23 (5.9%) Day 28: 14 (3.7%)	Day 3: 9 (2.3%) Day 6: 10 (2.5%) Day 14: 4 (1.0%) Day 28: 5 (1.3%)	PR: 2.25 (1.04–4.88) PR: 2.11 (1.01–4.42) PR: 5.74 (2.00–16.4) PR: 2.82 (1.03–7.76)	None
Salman 2015[327] Gambia	Sub-study of Roca et al.'s randomised controlled trial	40 infants from Roca et al.[326] (20 treated, 20 not treated)	Neonatal sepsis prevention A single dose of oral 2g azithromycin (4 tablets of 0.5g)		0	0	-	-
		419 newborns from Roca et al.[326] (all treated)		Infantile hypertrophic pyloric Stenosis (IHPS)	0	-	95% CI: 0-11.3/1,000 cases	-
Sinha 2003[328] US	Case-control study	228 infants 0-30 days old (114 cases of non-GBS infection and 114 controls,	GBS prophylaxis Penicillin G (41%= 7 people), ampicillin	Bloodstream infection	-	-	RR: 0.20 (0.011-3.6)	Sex and year of birth
				Pneumonia	-	-	RR: 2.5 (0.43-14.0)	Sex and year of birth
				Any infection syndrome	-	-	RR: 1.0 (0.38-2.9)	Sex and year of birth

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		17 infants were treated	(41%= 7 people), clindamycin (18%= 3 people)					
Stoll 2002[20] US	Retrospective cohort study	5447 intrapartum women (3554 treated, 1893 not treated)	Indication not known Ampicillin (49%), penicillin (14%), and erythromycin (13%)	Early-onset sepsis	63	21	OR: 1.1 (0.6–1.8)	Gestational age, the presence or absence of intrauterine growth restriction, birth weight, race or ethnic group, and sex
				E. coli Sepsis or death	-	-	No association was found for any maternal antibiotic (data were not shown).	None
		33 infants (28 treated, 5 not treated)	Indication not known Ampicillin	Ampicillin-resistant *E. coli*	26	1	p=0.01	None
		5447 intrapartum women	Indication not known IAP within 72 hours (3399), no IAP within 72 hours (2048) Indication not known Ampicillin IAP within 72 hours (2348), no ampicillin IAP within 72 hours (3099)	Early onset sepsis	58	26	OR: 1.0 (0.6–1.6)	Gestational age, the presence or absence of intrauterine growth restriction, birth weight, race or ethnic group, and sex
				E. coli sepsis	25	12	p=0.004	NS When gestational age and interval between

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
								membrane rupture and delivery adjusted for
Svare 1997[329] Denmark	Randomised controlled trial	110 intrapartum women (59 treated, 51 not treated)	Preterm labour Ampicillin 2g intravenously every six hours for 24 hours followed by pivampicillin 500 mg orally every eight hours for seven days, plus metronidazole 500 mg intravenously every eight hours for 24 hours followed by metronidazole 400 mg orally every eight hours for seven days	Side effects and allergic reactions (undefined)	4	1	*RR: 3.46 (0.40-29.95)*	None
Wohl 2015[330] US	Retrospective cohort study	492 children 2 years old (128 treated, 364 not treated)	Indication not known Penicillins (108), macrolides (16), aminoglycosides (3),	Diagnosing atopic dermatitis	Any IAP 37 IAP 0-4 hours: 9/28 IAP 4-12 hours: 11/53 IAP 12-24 hours: 7/26	100	RR: 1.03 (0.75–1.41) RR 1.17 (0.66–2.06) RR 0.76 (0.44–1.31) RR 0.98 (0.51–1.89) RR 1.99 (1.13–3.49)	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
			cephalosporins (1)		IAP >24 hours: 6/11			
Studies investigating outcomes as IAP benefits in randomised controlled trials that could also be affected by IAP as a harm								
Cox 1996[312] US	Randomised controlled trial	82 infants (40 treated, 42 not treated)	Preterm labour 2g ampicillin and 1g sulbactam parenterally every 6 hours for 8 doses, followed by ampicillin-clavunate 250mg orally every 8 hours for 5 days.	5 minute APGAR score < 7	1	1	*RR: 1.05 (0.07-16.23)*	None
				Neonatal ICU days	M: 19 (SEM: 0.2, R: 0-21)	M: 22 (SEM: 0.2, R: 0-27)	NS	None
				Respiratory distress ventilation	8	8	NS (unable to calculate RR as some missing)	None
				Necrotizing enterocolitis	0	1	NS (unable to calculate RR as some missing)	None
				Still birth	0	0	NS (unable to calculate RR as some missing)	None
				Neonatal death	1	0	NS (unable to calculate RR as some missing)	None
Gordon 1995[315] US	Randomised controlled trial	117 intrapartum women (58 treated, 59 not treated)	Preterm labour Ceftizoxime for 5 days or 3 days	Maternal infection	2	3	*RR: 0.68 (0.12-3.91)*	None
				Neonatal pneumonia	0	0	NS	None
				Neonatal sepsis	0	0	NS	None
				Neonatal positive cultures	2	2	*RR: 1.02 (0.15-6.98)*	None
Kampikaho 1993[317] Uganda	Quasi-randomised controlled trial	660 intrapartum women (167 streptomycin, 163 penicillin, 330 not treated)	Post-partum infection prevention 1g streptomycin (n=167) or 0.8MU penicillin (n=163)	Laboratory-confirmed post-partum infection	Streptomycin: 14/167 Penicillin: 15/163	51/330	1.00 (reference) Streptomycin RR: 0.54 (0.31-0.95) Penicillin RR: 0.60 (0.35-1.03)	None
Keettel 1949[319] US	Randomised controlled trial	895 intrapartum women (465	Post-partum infection prevention	Puerperium fever	66	89	*RR: 0.69 (0.51-0 92)*	None
				Puerperium Endometritis	13	40	*RR: 0.30 (0.16-0.55)*	None

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		treated, 430 not treated)	300,000/600,000 units of penicillin at the indication of labour and then after 24-hour intervals.	Puerperium Pyelitis	4	1	*RR: 3.70 (0.42-32.96)*	None
				Puerperium Mastitis	5	3	*RR: 1.54 (0.37-6.41)*	None
				Stillbirths	9	12	*RR: 0.69 (0.30-1.63)*	None
				Neonatal deaths	12	12	*RR: 0.92 (0.42-2.04)*	None
Keettel 1950[318] US	Controlled trial	773 intrapartum women (382 treated, 391 not treated)	Post-partum infection prevention 600,000 units of penicillin at the indication of labour, and then after 24-hour intervals.	Fever	29	61	*RR: 0.49 (0.32-0.74)*	None
				Stillbirth	5	3	*RR: 1.71 (0.41-7.09)*	None
				Neonatal death	4	2	*RR: 2.05 (0 38-11.11)*	None
Keuchkerian 2005[322] Uruguay	Randomised controlled trial	96 intrapartum women (47 treated, 49 not treated)	Preterm labour Amoxicillin 1000 mg sulbactam 500 mg IV every 8 h during the first 48 h and they continued to receive an oral intake of amoxicillin 250 mg sulbactam 250 mg every 8 h for 5 days	1 minute APGAR score < 7	3	2	RR: 1.57 (0 27-8.94)	None
				Respiratory distress syndrome	3	3	RR: 1.04 (0.22-4.91)	None
				Neonatal sepsis	0	0	-	-
				Fetal death	1	1	RR: 1.04 (0.07-16.19)	None
				Neonatal death	0	0	-	-
McGregor 1986[323] US	Randomised controlled trial	17 intrapartum women (8 treated, 9 not treated)	Preterm labour 21 enteric-coated erythromycin	Maternal days in hospital	M: 6.1 (SD: 4.7, R: 3-15)	M: 6.3 (SD: 6.2, R: 2-18)	NS	-
				Amniotic fluid infection	0	0	-	-

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
			tablets over 7 days	Maternal febrile morbidity	0	0	-	-
				Initial requirement of neonate intermediate or intensive care nursery	2	3	*RR: 0.75 (0.16-3.41)*	None
				Total days in intermediate or intensive care nursery	9	62	-	-
				Total days in any nursery	M: 3 (SD: 2.1)	M: 9.6 (SD: 13.5)	p=0.08	-
				Neonates treated with antibiotics	0	1	-	-
Nadiasaukiene 1996[324] Lithuania	Randomised controlled trial	102 mother-infant pairs (44 treated, 58 not treated)	Preterm labour 2 x 5g ampicillin four hours apart or 1 hour before delivery if labour proceeded quickly	1 minute APGAR score < 7	26	40	*RR: 0.86 (0.63-1.16)*	None
				Neonate did not survive first week	8	12	*RR: 0.88 (0.39-1.96)*	None
				Neonatal infection	4	38	*RR: 0.14 (0.05-0.36)*	None
				Histological chorioamnionitis	6	28	*RR: 0.28 (0.13-0.62)*	None
				Puerperal uterine infection	8	26	*RR: 0.41 (0.20-0.81)*	None
Rajaei 2006[325] Iran	Randomised controlled trial	80 Intrapartum women (38 treated, 42 not treated)	Preterm labour 400 mg erythromycin every 6 h orally for 10 days.	Admission to NICU	14	25	p<0.05 *RR: 0.62 (0.38-1.01) Risk difference: −22.68% (95% CIs: −44.02- −1.34), and p=0.043*	None
Roca 2016[326] Gambia	Randomised controlled trial	829 intrapartum women and 843 infants (414 women and 419 infants treated,	Neonatal sepsis prevention A single dose of oral 2g azithromycin (4 tablets of 0.5g)	Maternal deaths	0	0	-	-
				Puerperal sepsis	1	2	*RR: 0.50 (0.05-5.51)*	-
				Deaths from neonatal sepsis, meningitis, pneumonia	3 (all underlying conditions)	4 (no underlying conditions)	*RR: 0.76 (0.17- 3.37)*	-
				Apgar scores at birth	0: 6 1-6: 8	0: 6 1-6: 5	-	-

Reference Country	Design	Participants	IAP treatment details	Outcomes reported	Findings Treatment	Control	Summary measures (95%CI)	Factors adjusted for in the analysis
		415 women and 424 infants not treated)			7-10: 402	7-10: 408		
Svare 1997[329] Denmark	Randomised controlled trial	110 intrapartum women (59 treated, 51 not treated)	Preterm labour Ampicillin 2grams intravenously every six hours for 24 hours followed by pivampicillin 500 mg orally every eight hours for seven days, plus metronidazole 500 mg intravenously every eight hours for 24 hours followed by metronidazole 400 mg orally every eight hours for seven days	Maternal Chorioamnionitis - endometritis	3	0	-	None
				5 minute APGAR score < 7	5	1	*RR: 4.32 (0.52-35.79)*	None
				Admission to neonatal department	23/58	32	*RR: 0.63 (0.43-0.93)*	None
				Days in neonatal department	Median: 15.5 (R: 1-60)	Median: 27 (R: 2-121)	-	-
				Oxygen /NCPAP /ventilation	M: 9.7 (SD: 15.7)	M: 10.8 (SD: 17.2)	-	-
				Neonatal antibiotic days	M: 5.9 (SD: 2.8)	M: 6.6 (SD: 4.2)	-	-
				Meningitis, septicaemia, pneumonia	6/58	11	*RR: 0.48 (0.19-1.20)*	-

E coli Escherichia coli, CI confidence interval, CFU colony forming units, g grams, GBS group B *Streptococcus,* IAP intrapartum antibiotic prophylaxis, IQR interquartile range, IV intravenous, M mean, NICU national intensive care unit, OR Odds ratio, p probability level, R range, RR relative risk, *S. aureus Staphylococcus aureus*, S. *pneumoniae Streptococcus pneumoniae*, SEM Standard error of mean, NS not significant, UK United Kingdom, US United States of America, UTI urinary tract infection

* p=0.037

Figures in italics have been calculated by myself

Appendix 12. Study information sheet for ecological studies (objective 4-6)

THE UNIVERSITY OF WARWICK

STUDY INFORMATION LEAFLET

Study Title:	Importing international data on Group B Streptococcus to inform Group B Streptococcus screening policy in the UK
Investigator(s):	Ms Farah Seedat, Dr Saverio Stranges, Dr Ngianga-Bakwin Kandala, Dr Sian Taylor Phillips

Introduction
You are invited to take part in a research study. Before you decide, you need to understand why the research is being done and what it would involve for you. Please take the time to read the following information carefully. Talk to others about the study if you wish.

(Part 1 tells you the purpose of the study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study)
Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

PART 1

What is the study about?
When Group B Streptococcus is passed on from a mother to baby during childbirth, there is a small risk that it can cause early onset GBS (EOGBS) disease in infants, which has serious life threatening complications. Due to the severe complications of GBS in infants, some groups have been campaigning for antenatal screening to be introduced. The aim of antenatal screening is to identify GBS colonised mothers for treatment with intrapartum antibiotic prophylaxis treatment (IAP). At present the UK National Screening Committee does not consider that there is enough evidence that the benefits of antenatal screening would outweigh the harms and therefore their policy is not to routinely screen all women in pregnancy. Most importantly, there is a lack of randomised controlled trial (RCT) evidence assessing the effectiveness of screening. Other countries take a range of approaches to antenatal screening for GBS. As there is no robust RCT evidence on the effectiveness of GBS screening, analysis of the approaches taken in different countries and their effectiveness may be an important source of information to aid UK decision-making. In this project, we aim to investigate how data on group b streptococcus (GBS) in countries with different screening programmes can be imported and factored into policymaking about whether and how to commence screening programmes within the UK. To do this, we are kindly asking you to provide aggregate national and regional GBS related outcomes in your country.

Do I have to take part?
It is entirely up to you to decide. We will describe the study and go through this information sheet, which we will give you to keep. By sending us any of the data requested, you are giving your consent for the information that you have supplied to be used in this study and formal signed consent will not be collected. You will be free to withdraw your data at any time, without giving a reason and this will not affect you or your circumstances in any way.

What will happen to me if I take part?
If you agree to take part in the study you will be asked to provide available annual figures for the following GBS related outcomes in your country/region:
Early onset neonatal sepsis incidence (culture positive or negative, < 7 days of life) per 1000 livebirths
Overall neonatal sepsis incidence (culture positive or negative, < 90 days) per 1000 livebirths
Early onset GBS incidence (culture positive or negative, < 7 days) per 1000 livebirths
Overall neonatal GBS incidence (culture positive or negative, <90 days) per 1000 livebirths
Case fatality from EO (< 7 days)/overall neonatal GBS (as a % of diagnosed cases)
Case fatality from early-onset (< 7 days)/overall neonatal sepsis (as a % of diagnosed cases)
Neonatal mortality per 1000 livebirths
Number and percentage of early-onset (< 7 days)/overall neonatal sepsis cases resistant to different antibiotics
Number and percentage of EO (< 7 days)/overall neonatal GBS cases resistant to different antibiotics
Early-onset (< 7 days)/overall neonatal Escherichia coli (*E. coli*) incidence per 1000 livebirths
Number and percentage of early-onset (< 7 days)/overall neonatal *E. coli* resistant to different antibiotics
Maternal anaphylaxis incidence per 10,000 women treated with IAP
GBS screening and IAP uptake (as a percentage of all pregnant women offered)

GBS maternal colonisation prevalence (as a percentage of all pregnant women)
Percentage of pregnant women with prolonged rupture of membranes
Percentage of women with intrapartum fever
Percentage of women who underwent a caesarean section
Percentage of women who received clindamycin and % who received penicillin for GBS prevention
Late onset sepsis incidence (culture positive or negative, 7-89days) per 1000 livebirths
Late onset group B streptococcus incidence (culture positive or negative, 7-89 days) per 1000 livebirths
Incidence of peripartum/postpartum clostridium difficile per 1000 deliveries
GBS prevention protocol

What are the possible disadvantages, side effects, risks, and/or discomforts of taking part in this study?
There are no risks in providing data for this study. Your rights will not be affected.

What are the possible benefits of taking part in this study?
By providing data for this study, you may help to influence and improve future GBS healthcare in the UK and abroad. For your assistance, your organisation will be acknowledged in all publications of the study and you will have access to any intellectual property generated.

Expenses and payments
Reimbursement is not available for data provision. For your assistance, the organisation will be acknowledged in all publications of the study and you will have access to any intellectual property generated.

What will happen when the study ends?
The data provided will be saved in a database of GBS related outcomes across all countries with available data. This database will be securely stored on a password protected laptop that will be stored in a locked cabinet overnight in the Department of Health Sciences at the Warwick Medical School campus of University of Warwick, and not removed from the site. After the study has finished anonymous data will be stored for in the locked cabinets and then archived as per University policy.

Will my taking part be kept confidential?
Yes. We will follow strict ethical and legal practice and all information about you will be handled in confidence. Further details are included in Part 2.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm that you might suffer will be addressed. Detailed information is given in Part 2.

This concludes Part 1.
If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

PART 2

Who is organising and funding the study?
University of Warwick is organising the study. The Principal Investigator is Ms Farah Seedat. The study has been reviewed and approved by an independent research ethics committee. The Economic and Social Research Council and the UK National Screening Committee funded this study.

What will happen if I don't want to carry on being part of the study?
Participation in this study is entirely voluntary. Refusal to participate will not affect you in any way.

If you agree to participate, you may nevertheless withdraw from the study at any time without affecting you in any way.
You have the right to withdraw from the study completely and decline any further contact by study staff after you withdraw. Withdrawing from the study will not affect you in any way.

What if there is a problem?
This study is covered by the University of Warwick's insurance and indemnity cover. If you have an issue, please contact Jo Horsburgh (details below).

Who should I contact if I wish to make a complaint?

Any complaint about the way you have been dealt with during the study or any possible harm you might have suffered will be addressed. Please address your complaint to the person below, who is a Senior University of Warwick official entirely independent of this study:
Jo Horsburgh, Deputy Registrar, Deputy Registrar's Office, University of Warwick, Coventry, UK, CV4 8UW. T: +00 44 (0) 2476 522 713 E: J.Horsburgh@warwick.ac.uk

Will my taking part be kept confidential?
The data that we require from you are all annual aggregated data at the national or regional level of your country therefore it will not contain any patient level data and must be anonymous. Once emailed to us data will be saved in an anonymous password protected document. Personal data will not be stored by the research team. During the study, this anonymous data file will be stored in a password locked laptops that will be stored in a locked cabinet overnight in the Department of Health Sciences at the Warwick Medical School campus of University of Warwick, and not removed from the site. The anonymous data will be stored for after the study has finished in the locked cabinets and then archived, as per University policy.

What will happen to the results of the study?
We hope to disseminate the results of the study by publishing it in a peer-reviewed journal and presenting the study at relevant conferences. We also intend to provide reports for the UK National Screening Committee to publish on their website and hold a seminar at their premises to communicate specific findings and policy recommendations to the staff. We will also send a report to the institutions across the world that provided data.

Who has reviewed the study?
This study has been reviewed and given favourable opinion by the University of Warwick's Biomedical and Scientific Research Ethics Committee (BSREC): REGO-2014-777, 20/06/2014

What if I want more information about the study?
If you have any questions about any aspect of the study or your participation in it not answered by this participant information leaflet, please contact:

Dr Sian Taylor Phillips
E: S.Taylor-Phillips@warwick.ac.uk
T: + 44 (0) 2476 575882

Thank you for taking the time to read this participant information leaflet.

Appendix 13. Questionnaire survey for ecological studies (objective 4-6)

THE UNIVERSITY OF WARWICK
WARWICK MEDICAL SCHOOL

Using International Data To Inform Group B Streptococcus Screening Decisions
Data Form

Thank you very much for your interest in collaborating on, and contributing data to, our project investigating the contextual predictors of Group B Streptococcus (GBS) disease. We hope the aims and methods of the project, as well as the data we are requesting, are clear to you from the introductory emails and study information sheet. If you have any questions, please feel free to contact Ms Farah Seedat on f.seedat@warwick.ac.uk or +447943826123.

Annual figures on GBS related outcomes

In the study information sheet you will find the overall list of outcomes we are interested in. We would like to know the annual figures for these outcomes. We understand that you may not have data for all of the years, or for all of the outcomes. Please feel free to send us data on any of the outcomes across any years, as we are keen to compile as much of the available international data as possible. This will not only enable us to perform the analysis, but will also be a useful resource in future.

The table on the next page shows the data that we require. Please complete the table with your data and email it back to us. Please fill in the data for each year in separate columns, specifying the year in the first row. For outcomes that you do not have data, please put a 'N' in the box.

Alternatively, using the table as a guide, you can send us your corresponding data in whichever format it is in, and we can fill out the table with your data.

Thank you very much for your time and data.

Data table

Outcome	Culture positive, negative or both	Definition	Setting (Country, region)	1996	1997	1998	1999		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of livebirths																						
Group B Streptococcus																						
Number/percentage of pregnant women colonised with GBS (by year or overall estimated prevalence)																						
Number of early-onset group B streptococcus cases		< x days																				
Number of late-onset group B streptococcus cases		Specify x-xx days																				
Number of all neonatal group B streptococcus cases		Specify: <xx days																				
Number of deaths from early-onset group B streptococcus																						
Number of deaths from late-onset group B streptococcus																						
Number of deaths from all neonatal group B streptococcus																						
Number of early-onset group B streptococcus cases resistant to clindamycin																						
Number of early-onset group B streptococcus cases resistant to erythromycin																						
Number of early-onset group B streptococcus cases resistant to other antibiotics (please specify)		Specify antibiotic																				

Outcome	Culture positive, negative or both	Definition	Setting (Country, region)	1996	1997	1998	1999		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of late-onset group B streptococcus cases resistant to clindamycin																						
Number of late-onset group B streptococcus cases resistant to erythromycin																						
Number of late-onset group B streptococcus cases resistant to other antibiotics (please specify)		Specify antbiotic																				
Number of all neonatal group B streptococcus cases resistant to clindamycin		Specify antibiotic																				
Number of all neonatal group B streptococcus cases resistant to erythromycin																						
Number of all group B streptococcus cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Neonatal Sepsis																						
Number of all-cause early onset sepsis cases		Specify early: < x days																				
Number of all-cause late onset sepsis cases		Specify late: x-x days																				
Number of all-cause overall neonatal sepsis cases		Specify: <x days																				

Outcome	Culture positive, negative or both	Definition	Setting (Country, region)	1996	1997	1998	1999		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of deaths from early-onset neonatal sepsis																						
Number of deaths from late-onset neonatal sepsis																						
Number of deaths from all neonatal sepsis																						
Number of early-onset neonatal sepsis cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Number of all neonatal sepsis cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Number of all sepsis cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Neonatal Escherichia coli (*E. coli*)																						
Number of early-onset *E. coli* cases		Specify early: < x days																				
Number of late-onset *E. coli* cases		Specify late: x-x days																				
Number of all neonatal *E. coli* cases		Specify: <x days																				
Number of deaths from early-onset *E. coli*																						

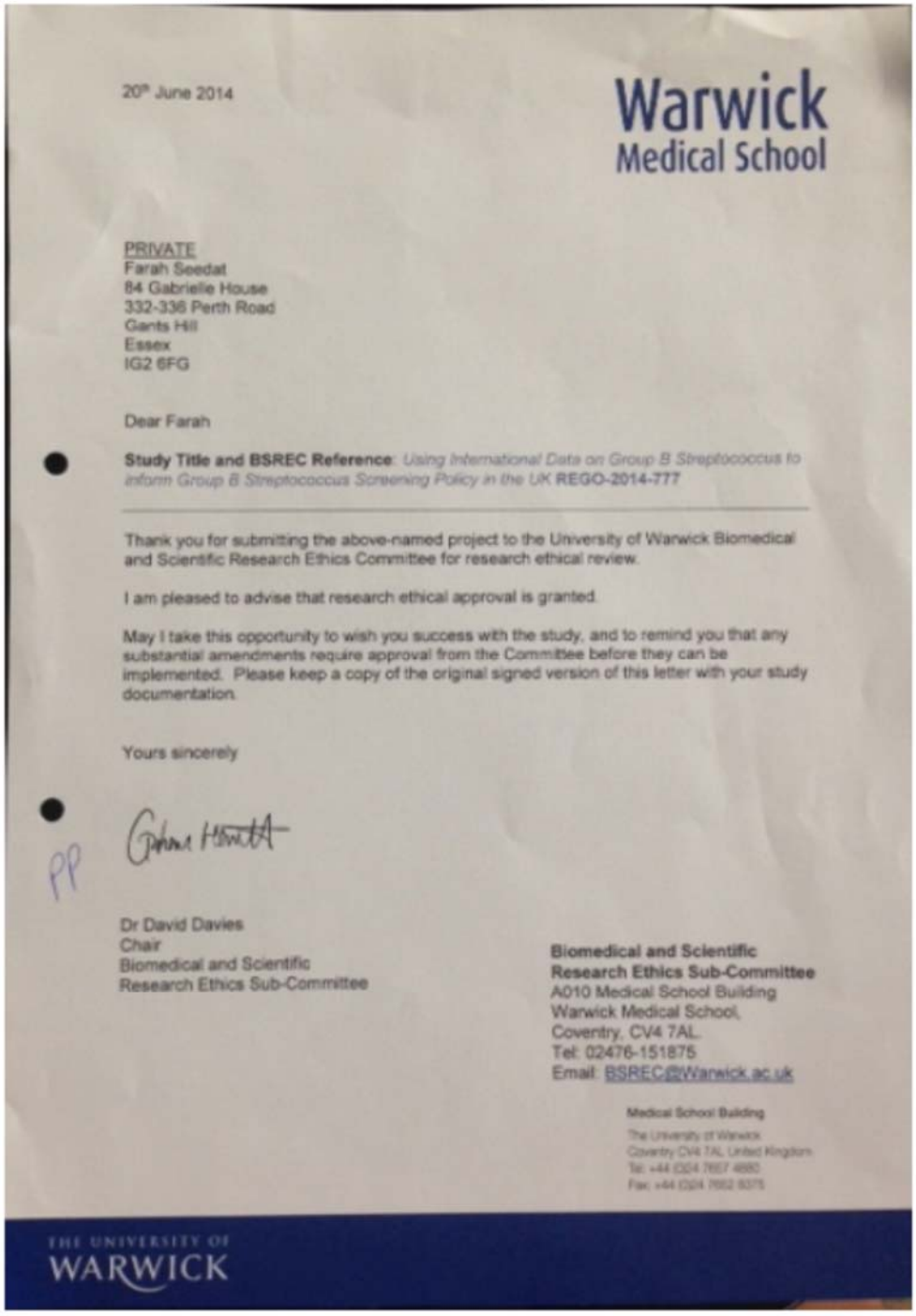
Outcome	Culture positive, negative or both	Definition	Setting (Country, region)	1996	1997	1998	1999		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of deaths from late-onset *E. coli*																						
Number of deaths from all neonatal *E. coli*																						
Number of early-onset *E. coli* cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Number of all neonatal *E. coli* cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Number of all *E. coli* cases resistant to antibiotics (specify each antibiotic individually in separate rows)		Specify antibiotic																				
Uptake rates																						
GBS screening uptake rate		Definition of uptake																				
GBS intrapartum antibiotic prophylaxis uptake		Definition of uptake																				
Number of women given intrapartum antibiotic prophylaxis for GBS (if different to uptake rate)																						
Number of women who received penicillin for GBS prevention																						

Outcome	Culture positive, negative or both	Definition	Setting (Country, region)	1996	1997	1998	1999		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Number of women who received clindamycin for GBS prevention																						
Number of women who received other intrapartum antibiotic prophylaxis (specify antibiotic)		Specify antibiotic																				
Other																						
Number of preterm deliveries		Specify premature: < xx weeks																				
Number of low birth weight babies		Definition of low birth weight																				
Number of Peripartum/postpartum clostridium difficile cases																						
Number of maternal anaphylaxis cases																						
Number of pregnant women with intrapartum prolonged rupture of membranes		Specify rupture duration																				
Number of women with preterm pre-labour rupture of membranes		Specify preterm (< xx weeks) and rupture duration																				

Outcome	**Culture positive, negative or both**	**Definition**	**Setting (Country, region)**	**1996**	**1997**	**1998**	**1999**		**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Number of women with intrapartum fever		Specify fever degree																				
Number of women who underwent a caesarean section																						
Most prevalent GBS strain																						
Number of multiple births																						
National/regional GBS prevention protocol (Please attach protocol to your email)		Universal, risk-based, combined, none																				

Thank you very much for taking the time to fill out this form and providing data.

Appendix 14. Ethical approval for ecological studies (objective 4-6)



20th June 2014

Warwick
Medical School

PRIVATE
Farah Seedat
84 Gabrielle House
332-336 Perth Road
Gants Hill
Essex
IG2 6FG

Dear Farah

Study Title and BSREC Reference: *Using International Data on Group B Streptococcus to Inform Group B Streptococcus Screening Policy in the UK* **REGO-2014-777**

Thank you for submitting the above-named project to the University of Warwick Biomedical and Scientific Research Ethics Committee for research ethical review.

I am pleased to advise that research ethical approval is granted.

May I take this opportunity to wish you success with the study, and to remind you that any substantial amendments require approval from the Committee before they can be implemented. Please keep a copy of the original signed version of this letter with your study documentation.

Yours sincerely

PP

Dr David Davies
Chair
Biomedical and Scientific
Research Ethics Sub-Committee

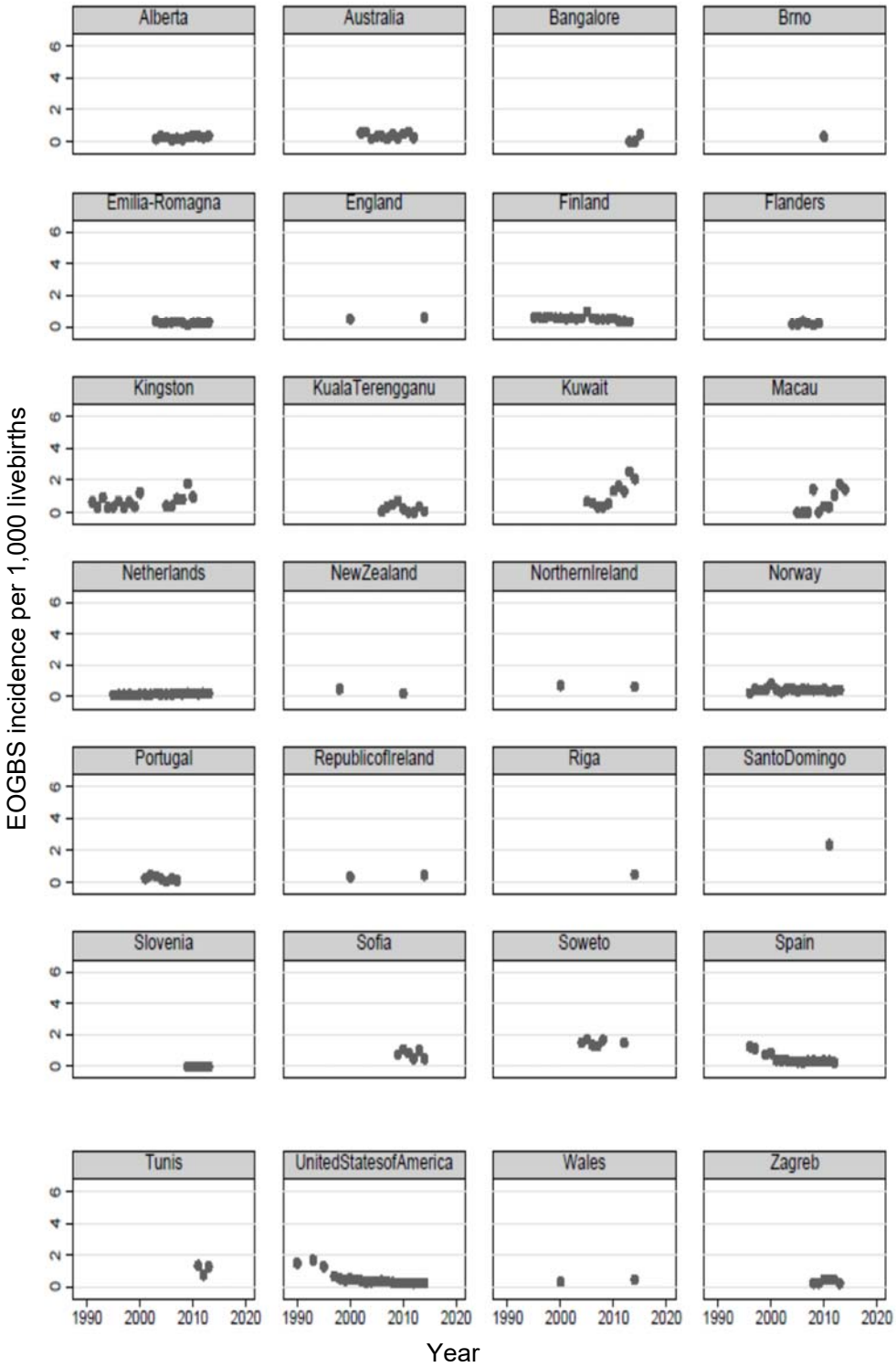
Biomedical and Scientific
Research Ethics Sub-Committee
A010 Medical School Building
Warwick Medical School,
Coventry, CV4 7AL
Tel: 02476-151875
Email: BSREC@Warwick.ac.uk

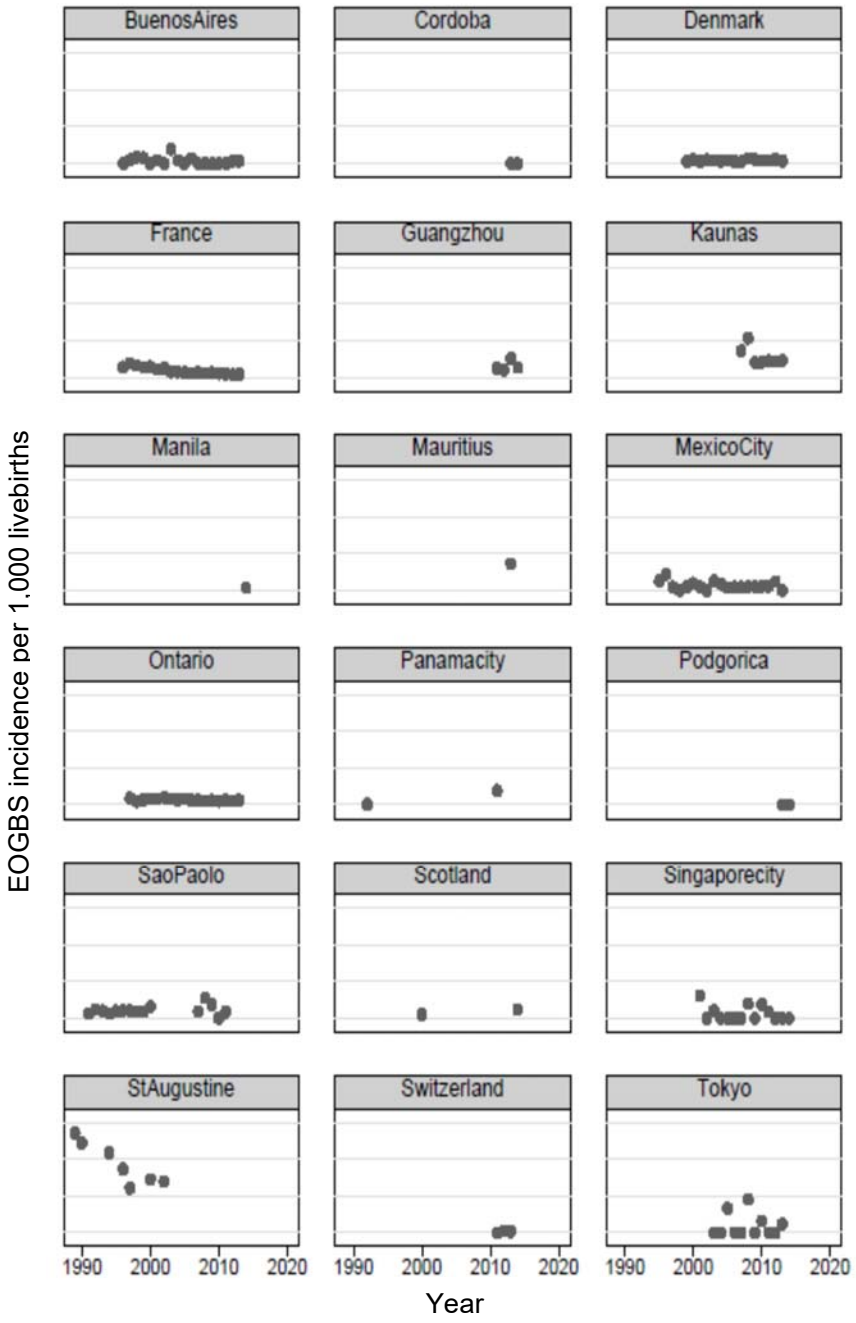
Medical School Building
The University of Warwick
Coventry CV4 7AL United Kingdom
Tel: +44 (0)24 7657 4680
Fax: +44 (0)24 7652 8375

THE UNIVERSITY OF
WARWICK

Appendix 15. Scatterplots for EOGBS incidence

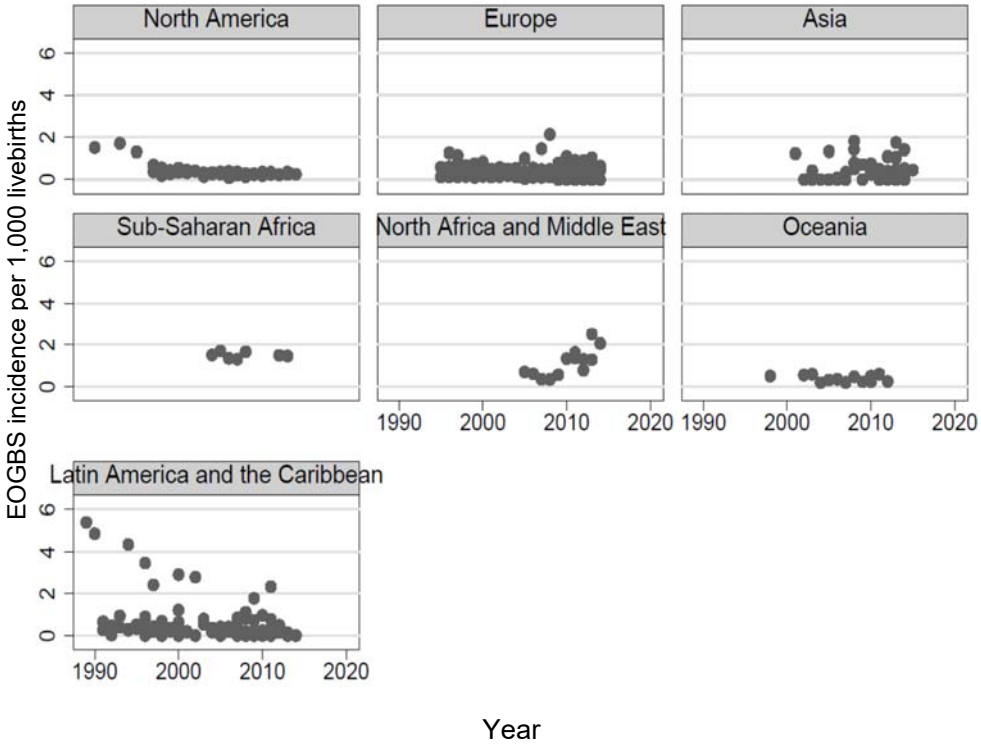
A. By geographical area





EOGBS early-onset group B *Streptococcus* disease

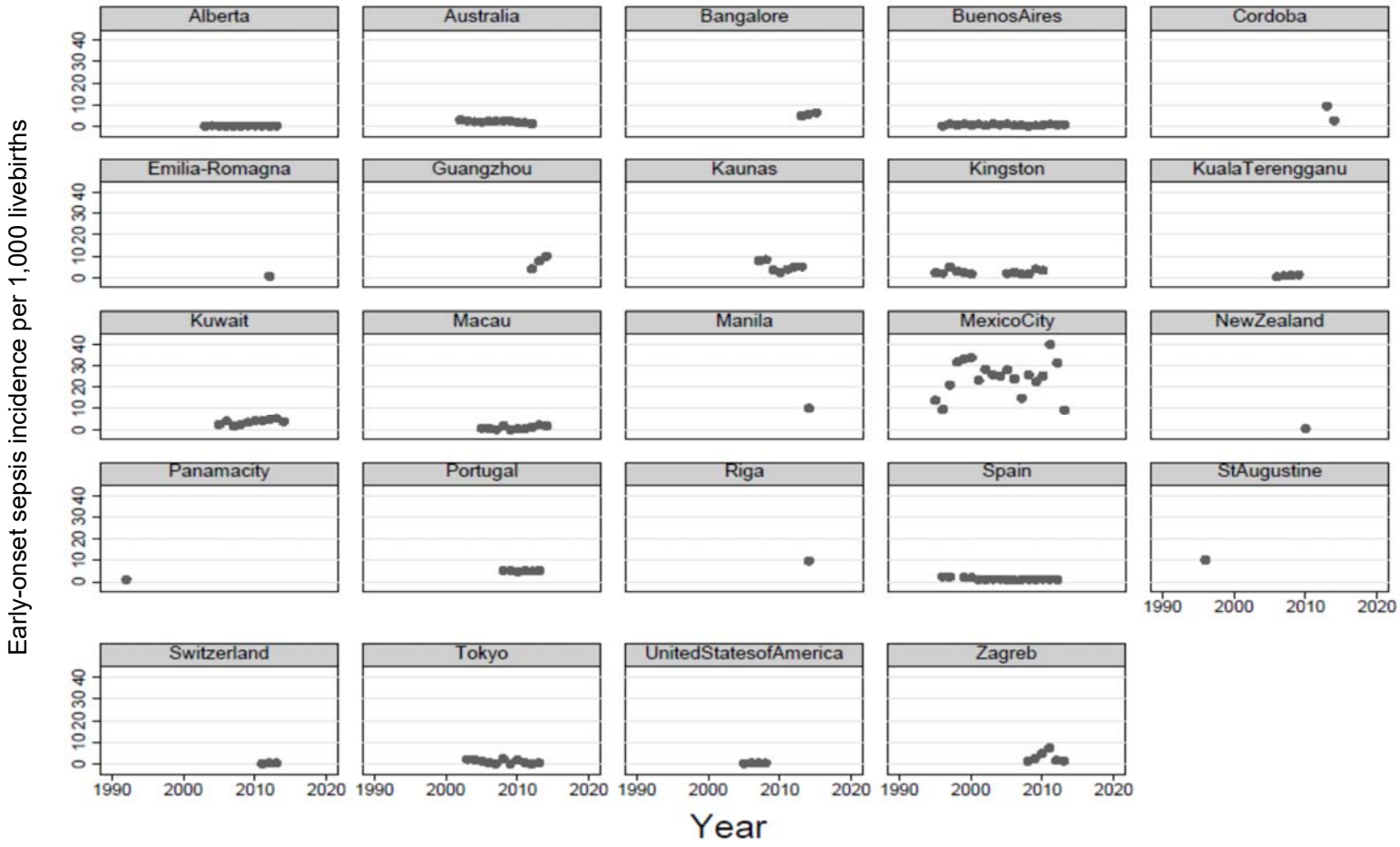
B. By world region



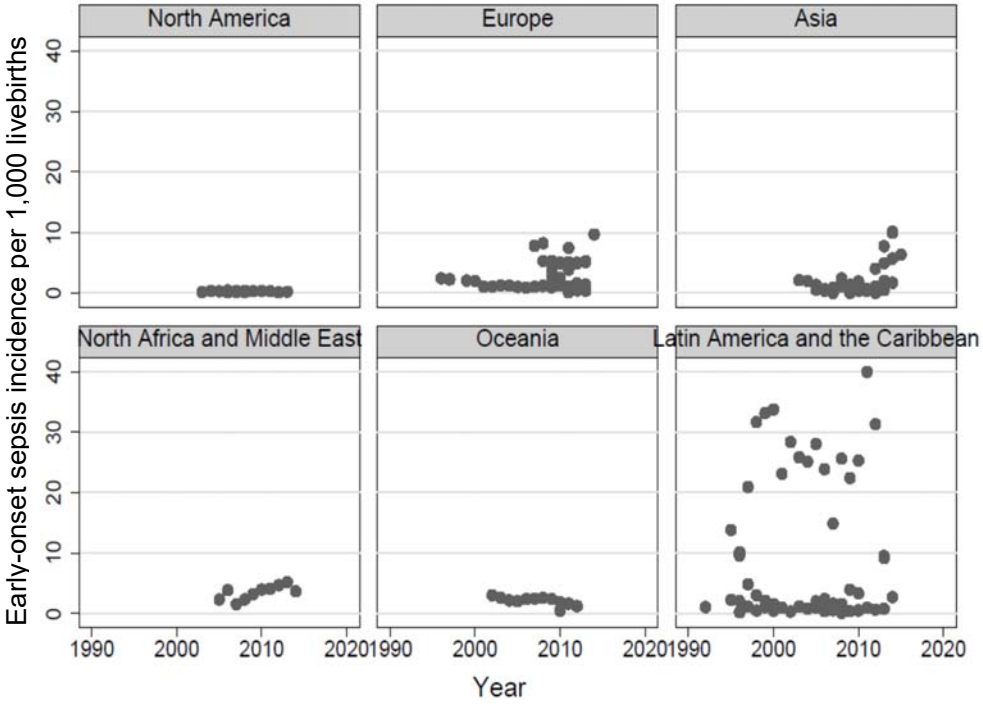
EOGBS early-onset group B *Streptococcus* disease

Appendix 16. Scatterplots for early-onset sepsis incidence

A. By geographical area

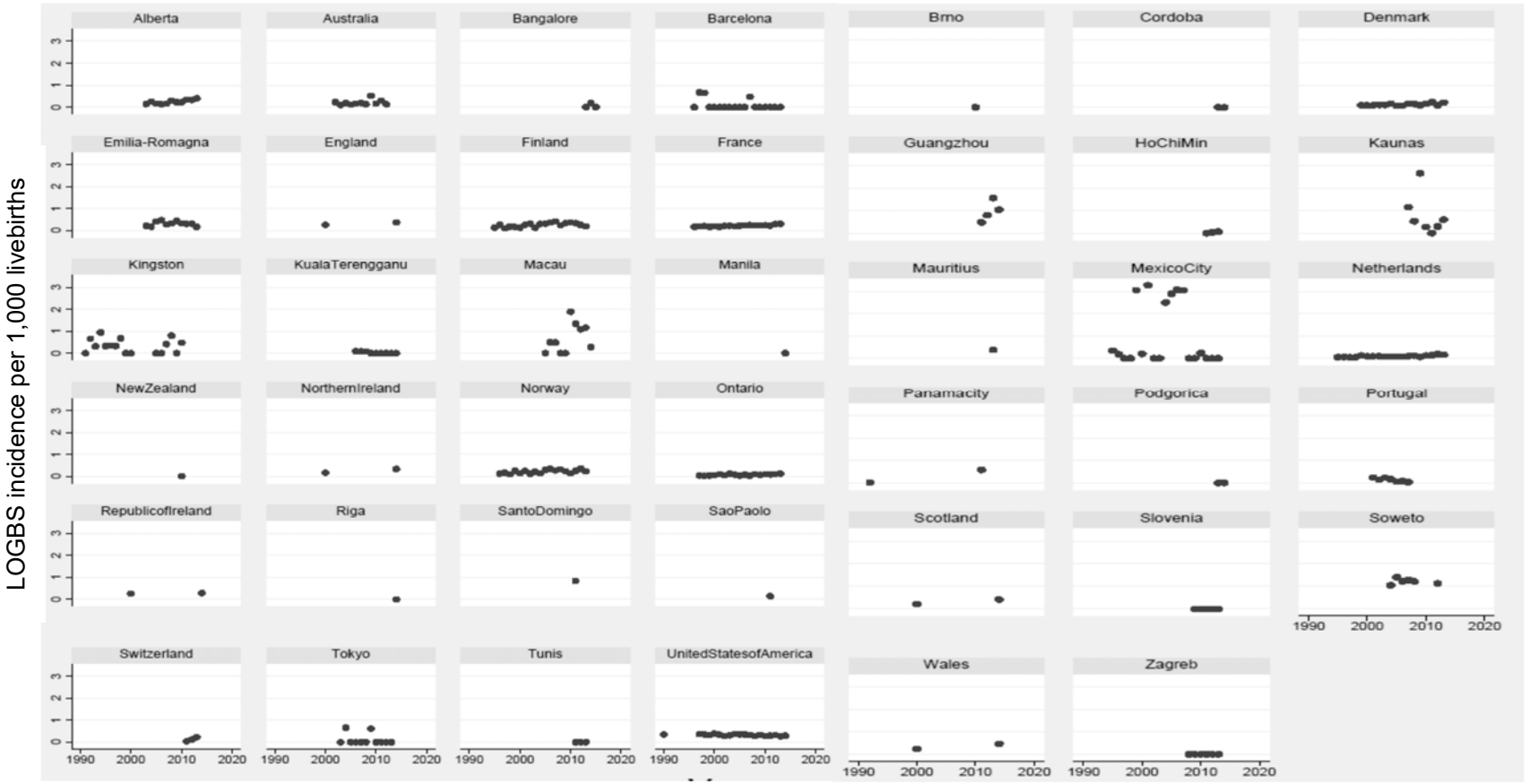


B. By world region



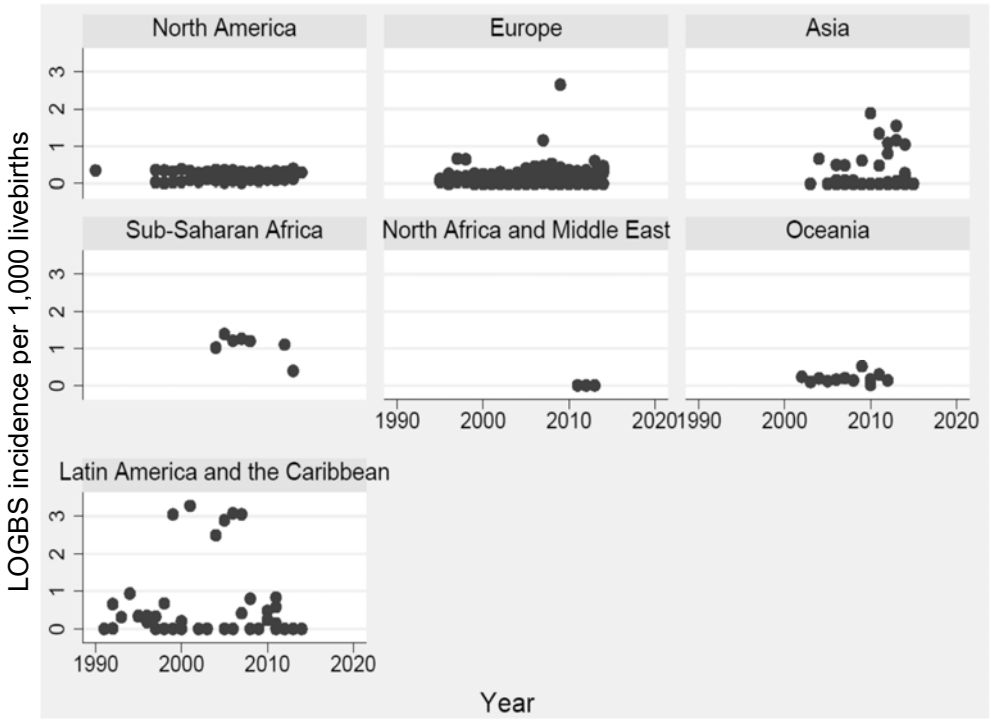
Appendix 17. Scatterplots for late-onset group B Streptococcus incidence

A. By geographical area



LOGBS late-onset group B *Streptococcus* disease

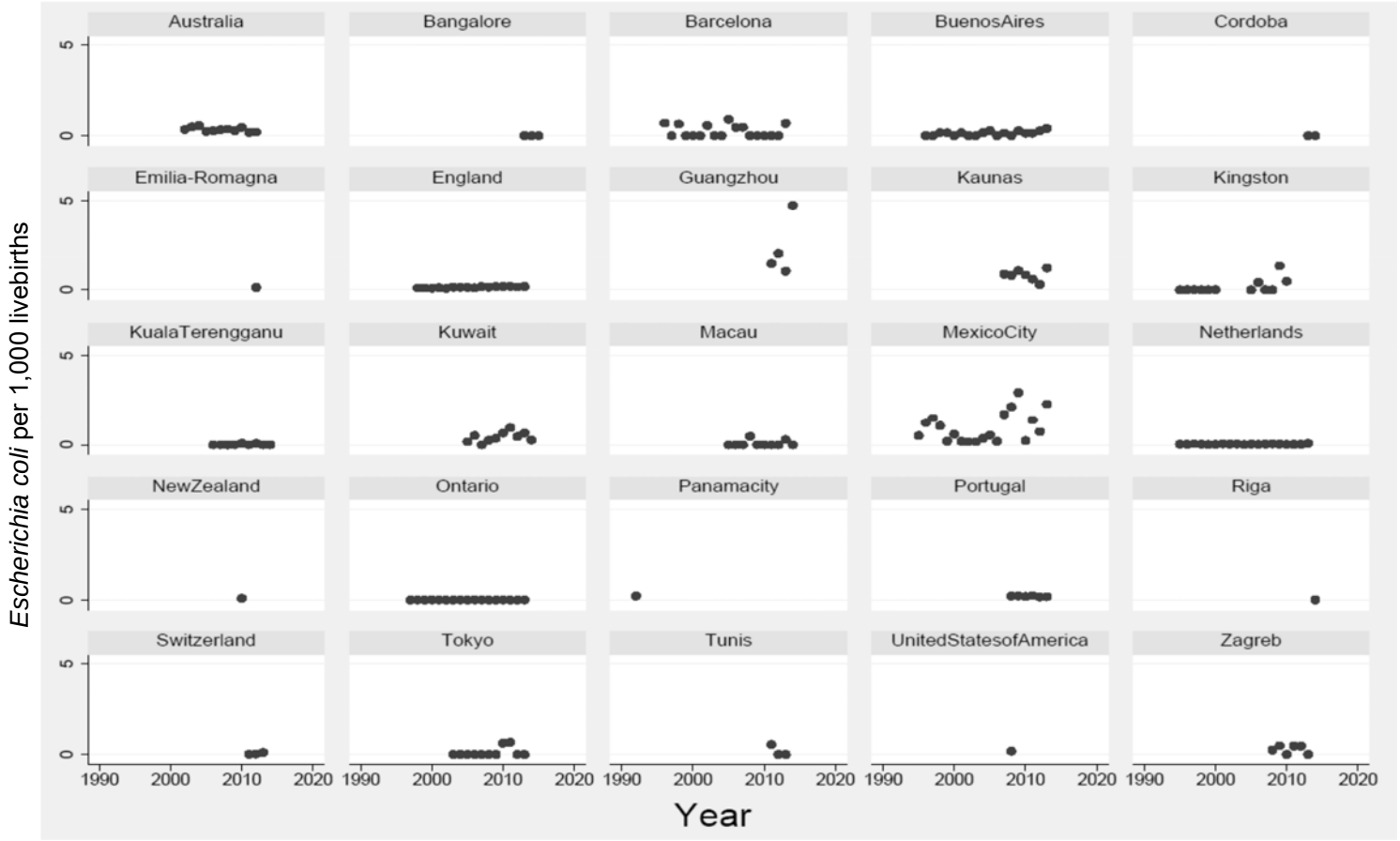
B. By world region



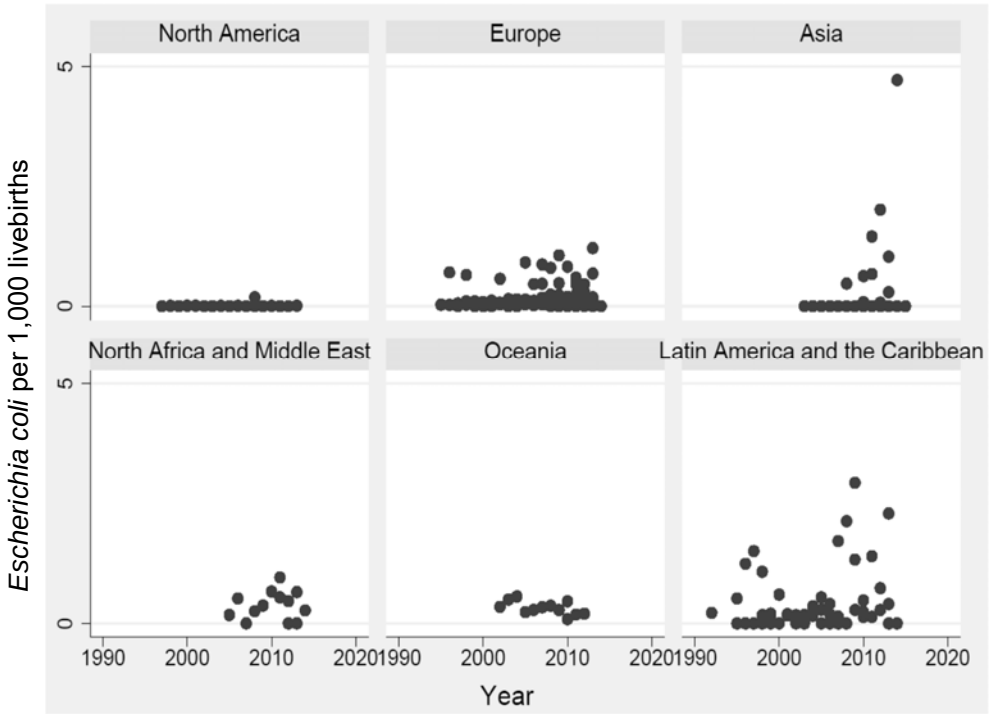
LOGBS late-onset group B *Streptococcus* diseass

Appendix 18. Scatterplots for *Escherichia coli* incidence

A. By geographical area

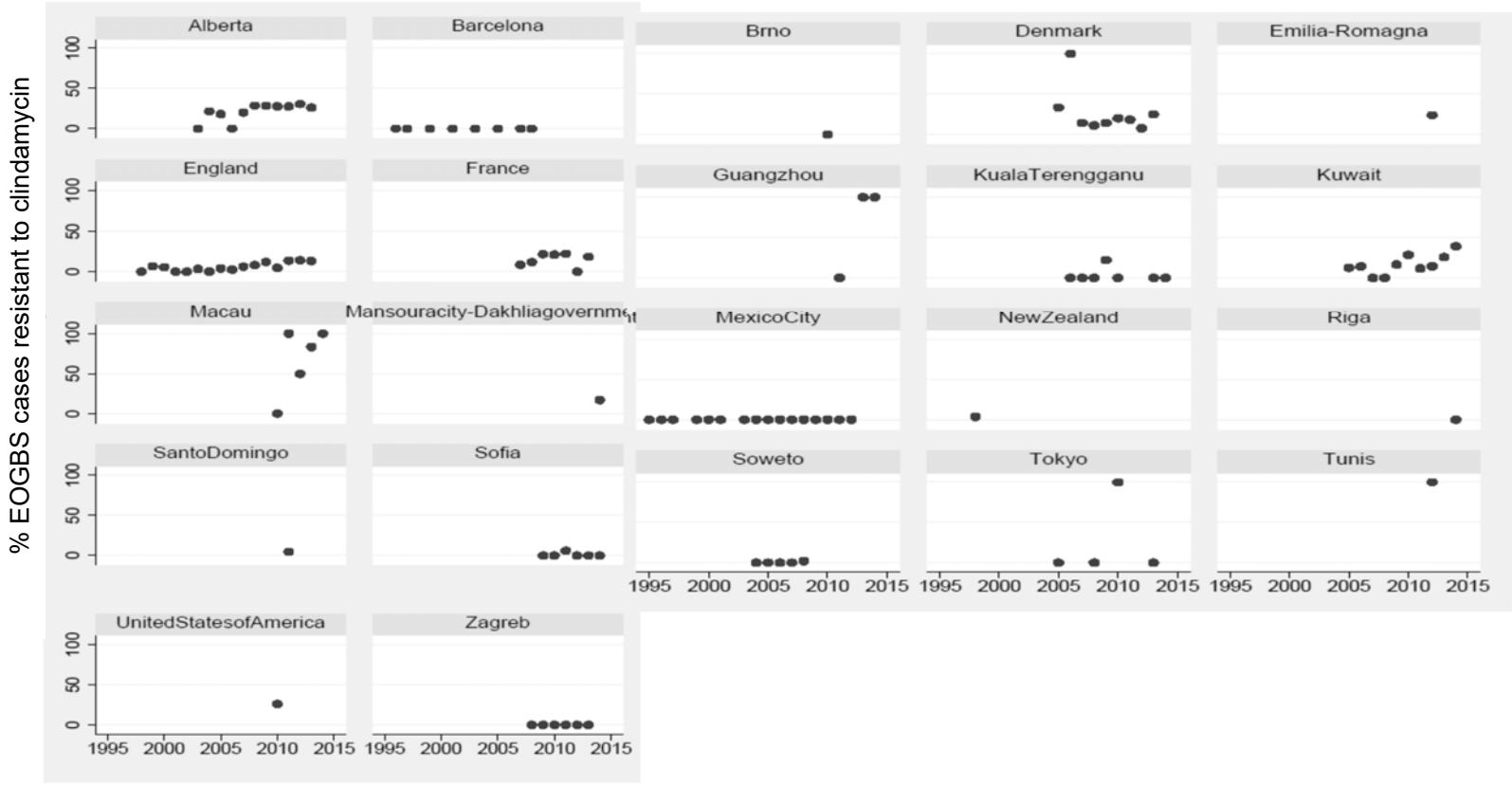


B. By world region

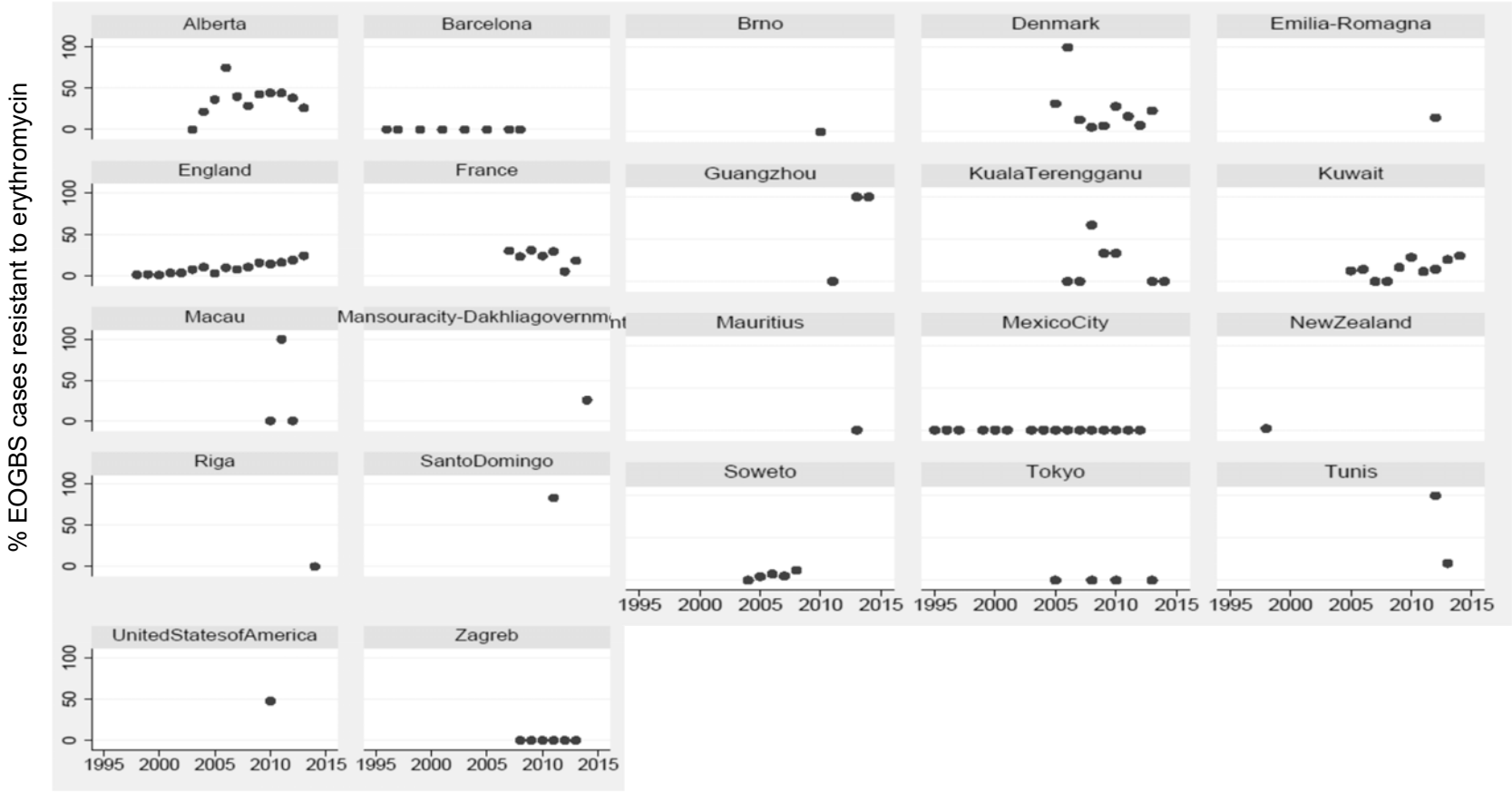


Appendix 19. Scatterplots for EOGBS antibiotic resistance

A. By geographical area

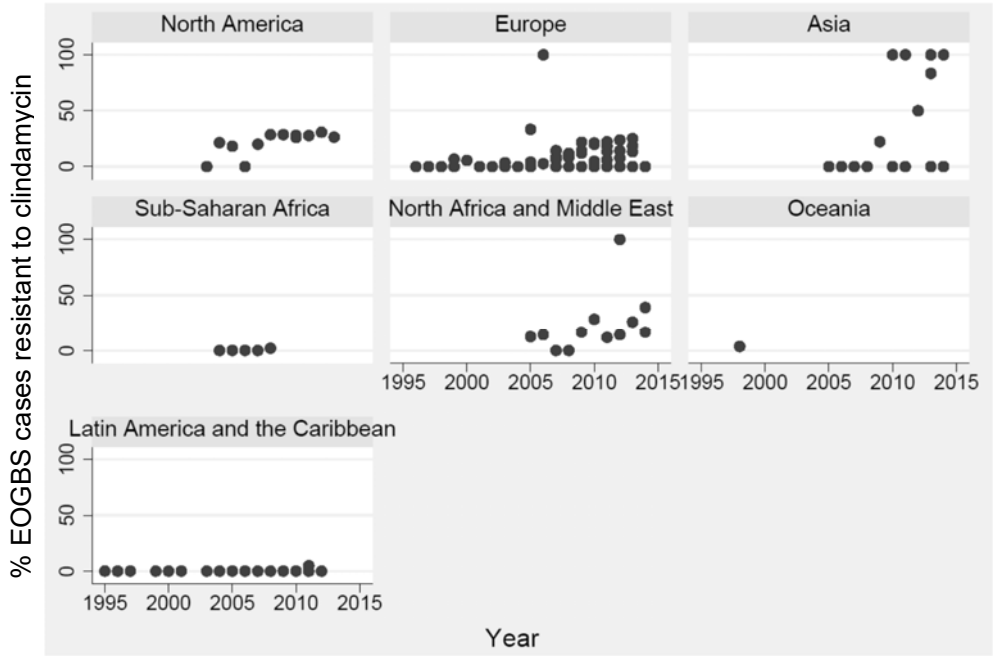


EOGBS early-onset group B *Streptococcus*

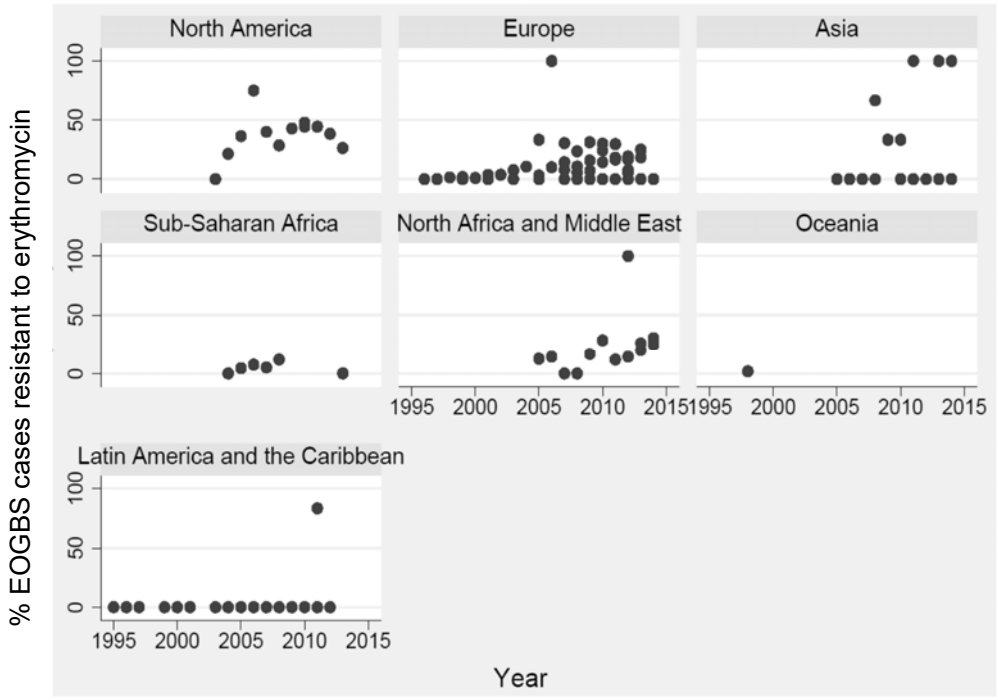


EOGBS early-onset group B *Streptococcus*

B. By world region



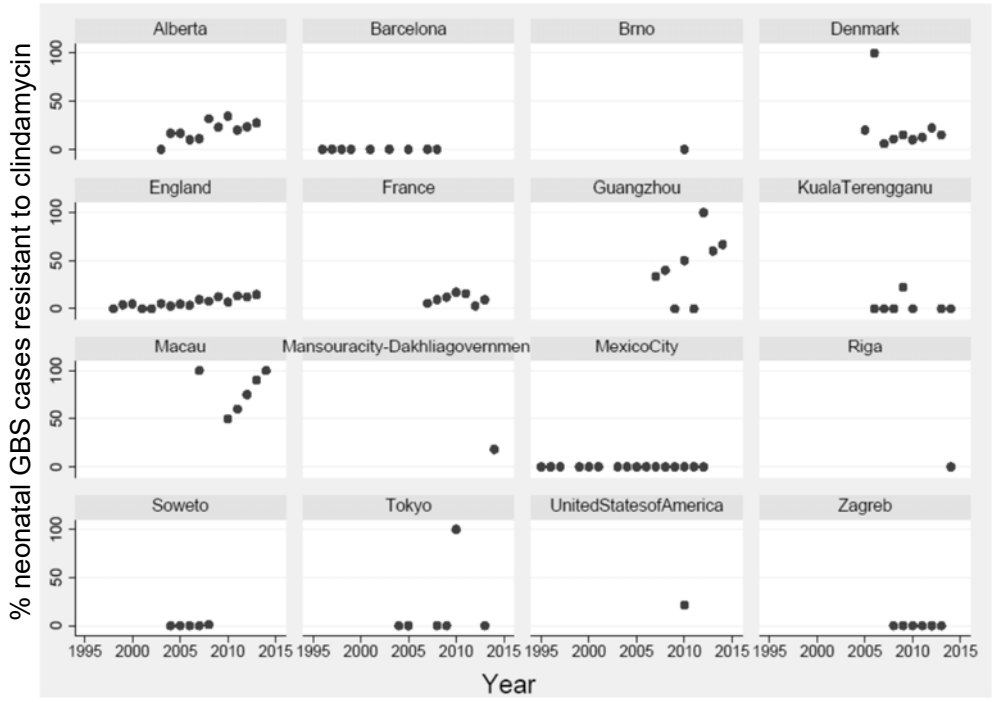
EOGBS early-onset group B *Streptococcus*

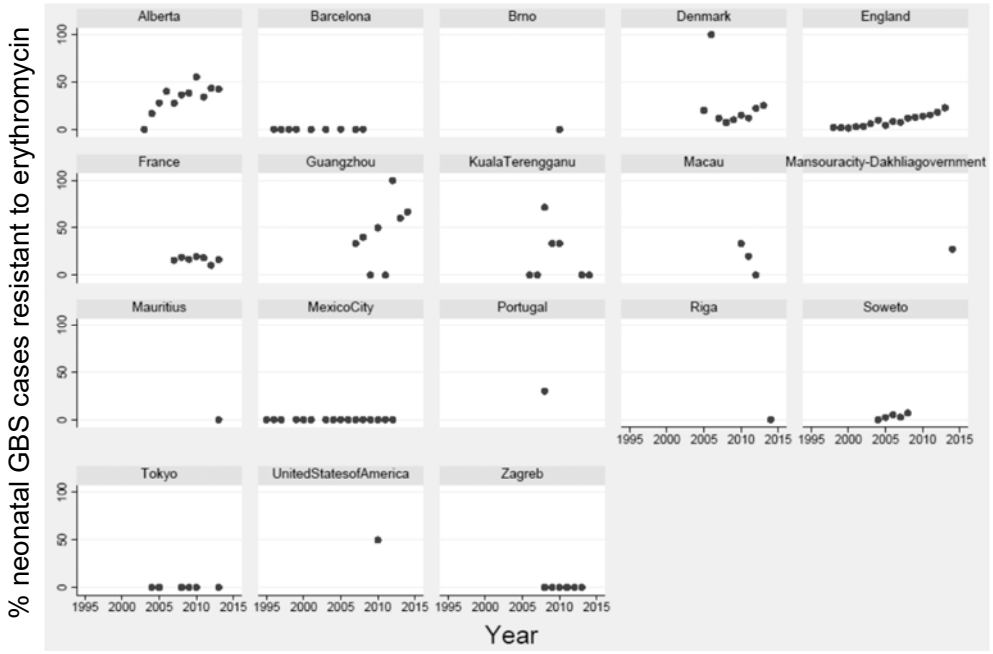


EOGBS early-onset group B *Streptococcus*

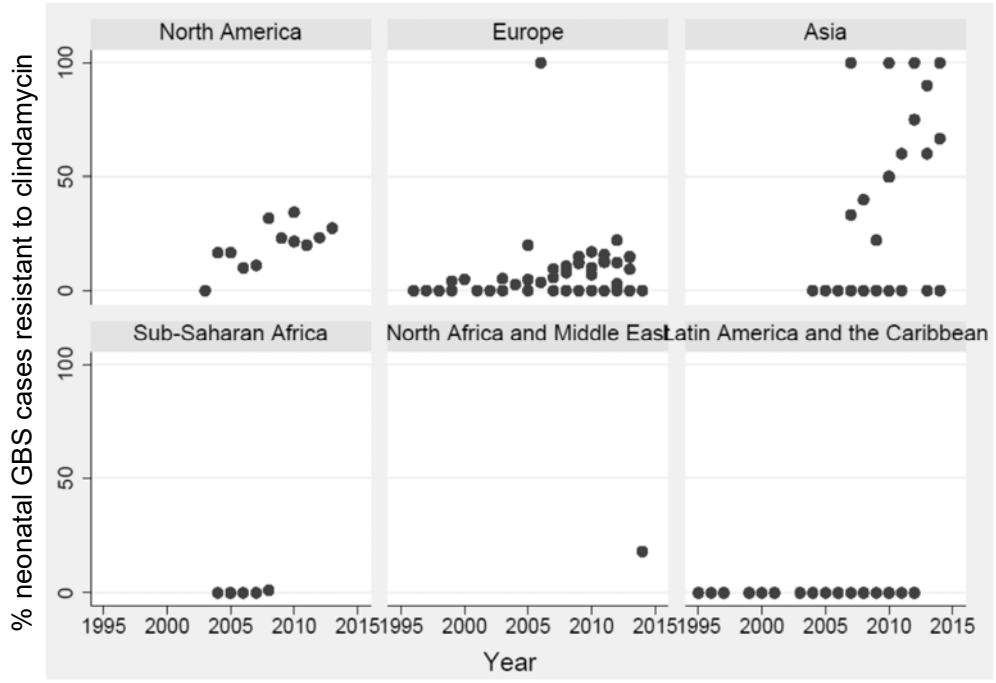
Appendix 20. Scatterplots for neonatal GBS antibiotic resistance

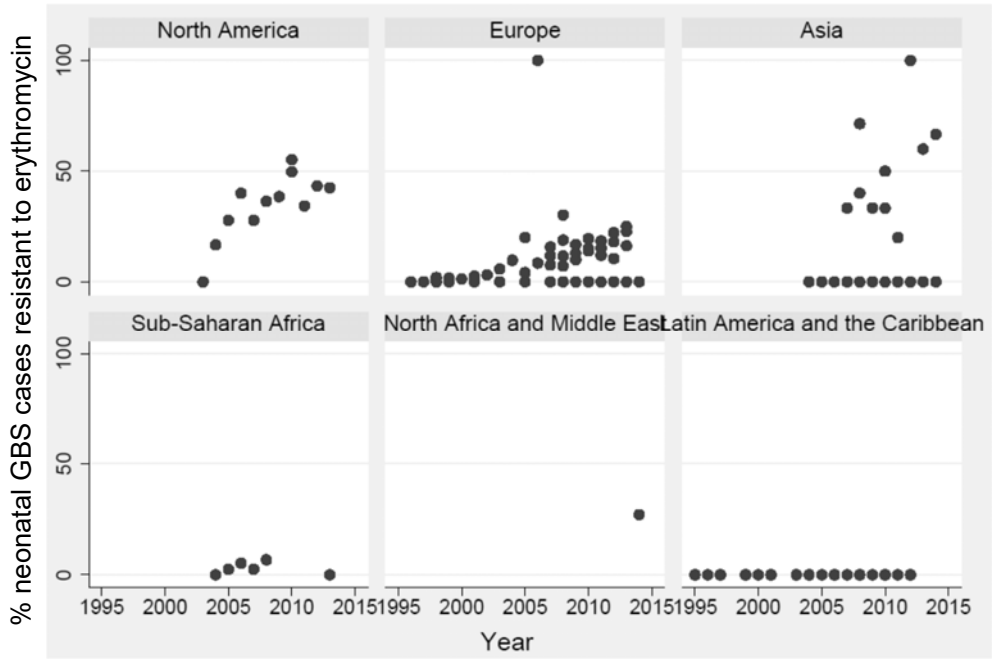
A. By geographical area





B. By world region





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