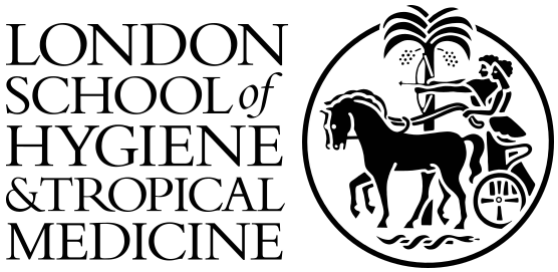


Epidemiology of *Staphylococcus aureus* carriage and disease among under five children in rural Gambia

ADERONKE ODUTOLA

Thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy of the University of London

March 2020

Department of Infectious Disease Epidemiology

Faculty of Epidemiology and Population Health

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

Funded by the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine

Abstract

Background: Data on the burden of severe *Staphylococcus aureus* disease, a significant cause of invasive bacterial infections among children aged <5 years in The Gambia and Africa at large, are lacking. The work in this thesis estimates the burden (cases and deaths) of severe *S. aureus* disease in Africa, the incidence of *S. aureus* bacteraemia (SAB) in children aged <5 years and assesses the risk factors for neonatal *S. aureus* acquisition in rural Gambia.

Methods: This thesis includes three separate studies. First, following a systematic literature review and meta-analysis, raw and processed data were synthesised to estimate the numbers of cases and deaths due to severe *S. aureus* diseases in children aged <5 years in Africa. Second, the incidence and case fatality ratio (CFR) of SAB in <5 years Gambian children were estimated from a population-based study. Lastly, a longitudinal study of newborn-contact pairs was used to evaluate the risk factors associated with neonatal *S. aureus* carriage at birth as well as with its acquisition at one-week of age.

Results: In Africa, an estimated 392,066 cases and 46,467 deaths due to severe *S. aureus* disease occurred among children aged <5 years with 20.4% and 58.4% of these occurring among neonates, respectively in 2015. The incidence of SAB among Gambian children aged <5 years and neonates was 78/100,000 person-years (95%CI 67–91) and 3.5/1,000 live-births (95%CI 2.9–4.7), respectively. The CFR was 14.1% (95%CI 9.6-19.8). *S. aureus* carriage at birth was associated with the midwife's report of handwashing before delivery while carriage acquisition one week after delivery was associated with maternal and household child nasal carriage.

Conclusion: The burden of severe *S. aureus* disease among children aged <5 years in Africa is substantial. Developing new, and implementing existing, strategies urgently to tackle this will contribute to achieving the sustainable development goals.

Statement of Own Work

I have read and understood the School's definition of plagiarism and cheating given in the Research Degrees Handbook. I declare that this thesis is my own work and that I have acknowledged all results and quotations from the published or unpublished work of other people.

I have read and understood the School's definition and policy on the use of third parties (either paid or unpaid) who have contributed to the preparation of this thesis by providing copyediting or proofreading services. I declare I did not seek such advice and therefore, no changes to the intellectual content or substance of this thesis were made.

I have exercised reasonable care to ensure that the work is original and does not to the best of my knowledge break any UK law or infringe on any third party's copyright or other intellectual property right.

Name: Aderonke Odutola

Signed January 2020

Acknowledgements

A very special gratitude goes out to all at the Medical Research Council Unit, The Gambia at London School of Hygiene and Tropical Medicine (MRCG) for your unfailing support and assistance, and for funding the PhD research. I would like to thank my supervisors, Dr Christian Bottomley, Dr Grant Mackenzie, Dr Syed A. Zaman and Professor Jodi Lindsay, for the patient guidance, encouragement, and advice they provided. I have been fortunate to have supervisors who were committed to my work, and who responded to my questions and queries so promptly. My sincerest appreciation goes out to the members of my advisory committee, Professor Brian Greenwood and Dr Anna Roca, for their patience and support in overcoming the numerous obstacles I faced through my research, and for being dedicated mentors.

Many thanks to the Pneumococcal Surveillance Project (PSP) doctors, Dr Malick Ndiaye, Dr Ilias Hossain, Dr Muhammed Shah, Dr Biliques Shah, Dr Yekini Olatunji, and nurses for their cooperation, last-minute favours and of course friendship. I would also like to thank the PSP field and laboratory staff (under the leadership of Alagie Konateh, Mustapha Darboe and Rasheed Salaudeen). I am thankful for the support I received from the MRCG Research Support Office, especially from Dembo Kanteh, Elizabeth Stanley-Batchilly, Isatou Cham, Felix Badji, Pa Cheiboh, who provided the necessary assistance required to complete my research work. My heartfelt thanks to the MRCG Basse field site drivers, (under the leadership of Boye Jallow and Buba Fadera) for all the rides to the various participants' homes and at odd hours! These trips were sometimes a bit overwhelming, but having such dedicated, funny and generous professionals made them so much pleasure. I heartily thank my field staff, Alieu Komma, Belal Keita, Demba Njie, Fatou Baldeh, Foday Conteh, Momodou Njie, Muhammed Lahai, Mustapha Jaiteh, Mustapha Sankano, and Philip Musa Sarjo, the

laboratory staff, Alasana Bah and Joseph Kanu and the data managers, Abdoullahi Nyassi and Sarwar Golam for their integrity, commitment, timely support, and hard work.

I want to thank my friends, Michael, Regina, Femi, Uduak, Crystal, Bola, Ify, Bola, Bolaji, Patrick, Effua, Henry, Bashirat, Joseph, Alberta, Bernard, Sarah, Tosin, and Taiwo for their friendship and words of encouragement. Many thanks to Pastor Joseph Kanu and Pastor and Mrs Musa-Kormayea for your spiritual and moral support. I thank Professor Brian Greenwood, Professor Richard Adegbola, Professor Tumani Corrah, Professor Martin Ota, and Professor Martin Antonio for their continuous interest in my development. Special thanks to Professor Judith Glynn and Jenny Fleming for coming to my aid at a crucial moment.

I am grateful to my siblings, who supported me morally and emotionally along the way, and my late parents, Adeola and Adunola Odutola, without whose inspiration I would never have pursued an advanced course in epidemiology. Many thanks for all your encouragement. Finally, all thanks to God, the Father of our Lord Jesus Christ, for His unspeakable gift.

Dedication

This work is dedicated to every child in Africa who has had, currently has, or will have any of the spectrum of Staphylococcal diseases.

Table of Contents

List of Tables

List of Figures

Abbreviations

ABHR	Alcohol-based hand rub
AMR	Antimicrobial resistance
ARI	Acute Respiratory Tract Infection
BDHSS	Basse Health and Demographic Surveillance System
BHC	Basse Health Centre
CA	Community-associated
CA-MRSA	Community-associated methicillin-resistant *S. aureus*
CFR	Case fatality ratio
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
CXR	Chest X-ray
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EMBASE	Exerpta Medica Database
FWHDSS	Fuladu West Health and Demographic Surveillance System
GATHER	Guidelines for Accurate and Transparent Health Estimates Reporting
GBS	Group B *streptococcus*
GDP	Gross domestic product
GI	Gastrointestinal
H. influenzae	*Haemophilus influenzae*
HA	Hospital-acquired
HAI	Hospital-acquired infections
HCW	Health care workers
HDSS	Health and Demographic Surveillance System
HH	Household
HHH	Household head

Hib	*Haemophilus influenzae* type b
HIC	High income countries
HIV	Human Immunodeficiency Virus
IBI	Invasive bacterial infections
IAP	Intrapartum antibiotics prophylaxis
IL-8	Interleukin-8
IPC	Infection Prevention and Control
IPSC	International Paediatric Sepsis Consensus
LMIC	Low- and middle-income countries
LOC	Loss of consciousness
LSHTM	London School of Hygiene and Tropical Medicine
MALDI-TOF MS	Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry
MeSH	Medical Subject Headings
MIC	Minimum inhibitory concentration
MICS	Multiple Indicator Cluster Surveys
MRC	Medical Research Council
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
NA	Not applicable
NHS	National Health Service
NR	Not reported
NPNMNS	Non-pneumonia non-meningitis non-septicaemia disease
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PICOS/PECOS	Population, intervention or exposure, comparison group, outcome and study design
PMF	Peptide mass fingerprints

RCTs	Randomised controlled trials
RDT	Rapid diagnostic test
ROB	Risk of bias
RVH	Royal Victoria Hospital
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*
SAB	*Staphylococcus aureus* bacteraemia
SDG	Sustainable Development Goals
sSA	Sub-Saharan Africa
SSTI	Skin and soft tissue infections
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TBAs	Traditional Birth Attendants
TSS	Toxic shock syndrome
TSST-1	Toxic shock syndrome toxin-1
U5MR	Under-five mortality rates
UK	United Kingdom
UNAIDS	The Joint United Nations Programme on HIV and AIDS
UNICEF	United Nations Children's Fund
URR	Upper River Region
USA	United States of America
UTI	Urinary tract infection
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
VSSA	Vancomycin-sensitive *Staphylococcus aureus*
WASH	Water, sanitation, and hygiene
WAZ	Weight-for-age
WCR	West Coast Region
WHO	World Health Organisation

WHZ	Weight-for-height

Chapter 1: Introduction

1.1 Overview of *Staphylococcus aureus* disease in children

In 2018, 5.3 million and 2.5 million childhood and neonatal deaths occurred worldwide, respectively.[1] In the last three decades, there has been a substantial reduction (59%) in under-five mortality globally.[1] The decline is likely attributable to several interventions including exclusive breastfeeding, adequate nutrition, prevention and case management of diarrhoea and pneumonia, immunisation and malaria control. Despite the gains, there are geographic variations with sub-Saharan Africa and South Asia accounting for about 80% of these deaths in 2018.[1]

Neonatal mortality rates have declined at a slower pace compared to the overall under-5 mortality (the neonatal period is defined as the first 28 days of life). As a result, neonatal mortality now accounts for two-thirds of deaths in children under one year of age and nearly 47% of all deaths in children under five years of age.[1] Furthermore, the United Nations has projected that neonatal mortality rates would continue to increase as a proportion of under-five deaths over the next ten years[1] unless mitigation actions are taken urgently. To meet the third Sustainable Development Goal (SDG 3.2), additional interventions are required to reduce neonatal and under-5 mortality in Africa, and reliable estimates of cause-specific childhood disease burden will be necessary to guide policy.

Globally, preventable infections are responsible for a third of childhood deaths,[2] and all-cause invasive infections such as pneumonia, meningitis, and sepsis account for 23% of neonatal deaths.[1,3,4] Neonatal infections are the second leading cause of deaths (after pneumonia) among children aged less than five years.[3] More neonates die from infections than from all childhood deaths from HIV and malaria infections combined.[1,3] In sub-Saharan Africa (sSA), preventable infections are responsible for about 40% of these childhood

deaths.[2] Available evidence suggest that in sSA deaths from invasive bacterial infections (IBI) (n=500,000) outnumber malaria deaths (n=250,000) among children less than five years of age.[5]

Until recently, the main bacteria implicated in childhood IBI were *Streptococcus pneumoniae* and *Haemophilus influenzae.*[6-8] However, following the widespread use of conjugate vaccines against *H. influenzae* type b (Hib) and *S. pneumoniae* in children, the disease burden due to Hib has decreased considerably,[9,10] and that due to vaccine-serotype *S. pneumoniae* is declining.[11-13] The decrease in the incidence of diseases due to these pathogens has led to *Staphylococcus aureus* becoming a relatively more important cause of IBI among children aged less than five years.[14]

Staphylococcal disease can present in a wide variety of ways ranging from local infections of the superficial skin and soft tissues (SSTI) and pyomyositis to toxin-mediated diseases such as scalded skin syndrome and food poisoning to systemic and more life-threatening diseases like septicaemia, endocarditis, meningitis, brain abscess and toxic-shock syndrome (TSS). Worldwide, hospital-based reports rank *S. aureus* among the top two leading Gram-positive bacteria causing neonatal sepsis[14-19] and a leading cause of IBI in older children.[20-23]

S. aureus carriage, a significant risk factor for *S. aureus* disease, is highly prevalent in the early neonatal period.[24,25] Thus, knowledge of the risk factors for *S. aureus* carriage acquisition in newborns is essential in finding ways to reduce the incidence of neonatal *S. aureus* bacteraemia (SAB). However, there is a scarcity of up-to-date population-level data on SAB and no data on country-specific estimates of disease burden (number of cases and deaths) due to *S. aureus* diseases in sub-Saharan Africa. Population-level data are important

because they are representative, demonstrate true disease burden and are less subject to bias than hospital-based data. Such data help to inform decision-making, allocation of funds and planning processes in the healthcare sector of a nation. It is therefore essential to describe the population-based burden of the disease, and associated risk factors in children aged less than five years, to inform further research and the design of interventions required to reduce childhood and neonatal morbidity and mortality due to *S. aureus* disease.

1.1.1 The global incidence of *S. aureus* disease in children

The global burden of *S. aureus* disease in children is unknown. Regional variations in incidence exist due to differences in the populations studied and unavailability of routinely collected high-quality data in most low- and middle-income countries (LMIC).

High-income countries

Most population-based and hospital-based studies on *S. aureus* disease burden have been conducted in high-income countries (HIC).[26-29] These studies reported an increase in the incidence of *S. aureus* disease from 1971 to 2007.[26-28] A population-based study in Denmark among subjects aged 0 - 20 years reported an increase in the incidence of SAB from 4.6 cases annually per 100,000 population in the period 1971 - 1975 to 8.4 cases per 100,000 population in the period 1996 – 2000 with the highest rates among infants.[26] Similarly, a hospital-based study in Spain among children aged 0 - 16 years reported an increase in the incidence of SAB from 1.3 cases per 1,000 admissions in the period 1995 – 1999 to 3.7 cases per 1,000 admissions in the period 2006 – 2008 with the highest rates seen among neonates.[27] Both studies linked the rise with an increase in the use of central venous catheters, increased survival rates of premature and low birth weight infants, children with

metabolic disorders, immunodeficiencies and haematological diseases.[26,27] The majority of cases are nosocomial infections. Since 2008, the incidence of SAB has remained stable in Denmark[30] or decreased in Spain.[27] The decline in Spain is attributable to improved infection prevention and control measures.

In HIC, *S. aureus* was the third most common cause of neonatal sepsis after group B *streptococcus* and *Escherichia coli*[16,31] and a leading cause of IBI in older children.[20-22] In the UK, Ladhani and colleagues,[31] in a prospective national surveillance study of IBI in infants, reported an increase in the incidence from 412 per 100,000 population in 2010 - 2011 to 552 per 100,000 population in 2016 - 2017 with the highest rates seen in the first week of life. *S. aureus* was the third most common cause of IBI in infants (responsible for 10.8% of isolates recovered) after *E. coli* and group B *streptococcus.* Although, this study was unable to differentiate between community-acquired and hospital-acquired infections or between early-onset or late-onset neonatal infections due to lack of access to clinical data, *S. aureus* remained a significant cause of childhood IBI.

In the USA, hospital-based reports of *S. aureus* disease among children aged less than 18 years showed a significant increase in incidence between 2002 and 2007 from 20.8 to 35.8 annual cases per 1,000 admissions.[28] The investigators attributed the increase to a surge in the frequency of methicillin-resistant *S. aureus* (MRSA) disease,[28] although an increase in the burden of SSTI and other chronic complex conditions could have contributed to the rise.

Data on infective endocarditis are rare in children, and population-based incidence data are even more limited. Retrospective hospital-based studies reported an incidence of 0.05 – 0.78 per 1,000 paediatric admissions over eight decades with no definite trends.[32-34] Traditionally, *Streptococcus viridians* was the dominant pathogen recovered from children

with endocarditis. However, current data suggest that *S. aureus* is now the leading pathogen (32.0% - 51.6% of isolates recovered),[35] and this is attributable to the increasing use of vascular catheters and prosthetic materials.[35]

The incidence of paediatric osteomyelitis ranges between 1.9 and 24.0 cases per 100,000 person-years[36-39] and *S. aureus* was responsible for 72% of cases.[39] In the USA, there was an increase in the incidence of SSTI up until 2008 as a result of an epidemic of community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains.[40] More recently, the incidence of SSTI has declined, but the proportion caused by *S. aureus* remains largely unchanged.[40,41]

Low- and middle-income countries

S. aureus disease is also a common cause of IBI in children in low- and middle-income countries (LMIC). Two recent reviews of the bacterial causes of neonatal sepsis - one in 157 LMIC and another in 26 sub-Saharan African (sSA) countries found that *S. aureus* was the most commonly isolated bacterium.[14,19] In Asia, available data from both population-based and hospital-based studies indicate that *S. aureus* is the leading cause of IBI in neonates[42,43] and children aged less than 15 years.[44] Unlike in the HIC, these are community-acquired infections.

There are relatively few population-based data on the incidence of SAB in sSA. Available studies report an incidence of SAB ranging from 27 to 118 per 100,000 person-years in children aged less than five years between 1998 and 2010.[6,45,46] This variation may be related to the prevalence of HIV in the communities. Similar to the observations in the HIC, the highest rates are seen among neonates. Population-based data from Kenya and Mozambique showed that *S. aureus* was the third and first most common Gram-positive bacteria among neonates with sepsis, respectively.[6,45] Among children aged less than five

years, *S. aureus* ranked the fifth and third most common, respectively. The most common causes being *S. pneumoniae*, non-typhoidal salmonella species, *H. influenzae*, and *E. coli*. Hospital-based studies show that *S. aureus* is the principal cause of neonatal sepsis[19,47-49] and the second leading cause in children aged less than five years in both Nigeria and Ghana (after *Salmonella* spp).[50,51]

Data on the aetiology of pneumonia in children in Africa are also limited. Among the cases of bacteraemic pneumonia, determined by isolating a bacterial pathogen from blood, in children aged less than five years, the prevalence of *S. aureus* ranged between 0.8% to 34.0%.[52-54] In contrast, that of non-bacteraemic pneumonia (established by isolation of the pathogen from the lung parenchyma) was 2.0% – 45.2%.[54-56] In a multi-centre study in Africa and Asia, *S. aureus* was the third most prevalent bacterial cause of pneumonia after *S. pneumoniae* and *H. influenzae*[57] and was more commonly isolated in severe cases.[58]

Globally, there is geographical variation in the incidence of meningitis. Data from a systematic review reported that between 1980 and 2010, the highest incidence of acute bacterial meningitis among children aged 0 - 4 years (143.6 cases per 100,000 child-years) was seen in Africa and the lowest (16.6 cases per 100,000 child-years) in the American region.[59] This review may have over-estimated the incidence of meningitis in Africa because it did not differentiate between data collected routinely and those from outbreak investigations. However, *S. aureus* meningitis (patients with clinically suspected meningitis from whom *S. aureus* was isolated from the cerebrospinal fluid) is relatively uncommon, representing about 0.6% – 0.9% of the isolates identified from patients aged less than 15 years[60,61] and 12% from newborns.[19] No country in Africa has data on the incidence of *S. aureus* meningitis.

There are few hospital-based studies on endocarditis in African children. However, among the studies that have reported data on the epidemiology of endocarditis in children, *S. aureus* was either the leading or second leading pathogen identified.[62-64] Hospital-based studies on osteoarticular infections among African children[65-67] reported *S. aureus* as the predominant pathogen isolated, representing between 36% - 50% of patients investigated. However, population-based data on *S. aureus* endocarditis or osteoarticular infections among African children are lacking. Similarly, there are no population-based studies on *S. aureus* SSTI.

S. aureus remains a principal causative agent of several clinical syndromes, yet there are few population-based studies describing the burden of disease due to the pathogen. Furthermore, the burden – the number of cases and deaths - among African children is unknown. This information is not only needed for policymakers as they seek to reduce neonatal and child mortality, but it also serves as baseline data.

1.1.2 Predisposing factors

In HIC, the risk of *S. aureus* disease is significant at the extremes of life; that is newborns, infants, and older people are at the most risk.[27,68-70] With advances in sophisticated supportive care, more premature babies are more likely to survive, and the immature immune systems in these babies put them at high risk of infection. Similarly, in LMIC, the risk is highest among infants.[45,71-73] Apart from their immature immune responses, poor hygiene, and high carriage rate of *S. aureus* may contribute to the increased risk. The increased risk in older age groups is attributable to a rise in the ageing population with the attending co-morbidities, which increase their exposure to the healthcare facilities. Underlying co-morbidities such as cancer,[74] diabetes,[74] haemodialysis,[27,74,75] heart disease and stroke[76] are known risk factors.

In HIC, ethnicity has been reported as a risk factor for *S. aureus* disease in a community setting[77,78], but this is likely to be an indirect effect from overcrowding, poor hygiene, and delayed access to health care. Some investigators have related the increased risk in some ethnic groups to a higher prevalence of skin lesions.[79,80] In both HIC and LMIC, HIV infection is a risk factor for *S. aureus* disease.[71,81,82] Other factor associated with *S. aureus* disease in LMIC is sickle cell disease.[83]

Other risk factors for *S. aureus* disease in HIC include male sex,[68,70] the use of central venous catheters,[27,84] previous admission to intensive care,[27] administration of parenteral nutrition.[27] Although the risk factors for *S. aureus* disease are well described in HIC, there are limited data on the risk factors associated with *S. aureus* disease in African children. Knowledge of the risk factors for *S. aureus* disease will help shape the development of effective interventions against this disease.

1.1.3 Carriage as a risk factor for infection

The relationship between *S. aureus* carriage and infection was first reported in 1954 when Roodyn found that 77% of his patients with stye and 37% of those with boils carried the same type of *S. aureus* in their nose.[85] After that, several reports on the *S. aureus* carrier state as a risk factor for disease in hospital and community settings were published.[86-88] These studies and others have provided further evidence that between 82% and 86% of carriage strains are indistinguishable from those causing disease.[24] Some studies also showed that decolonisation of patients resulted in a reduction in the prevalence of *S. aureus* disease.[89]

More recently, data from a meta-analysis of 38 articles involving 5,596 chronic renal disease patients undergoing dialysis reported an 11-fold increase in the risk of developing an

infection in those who carried *S. aureus* compared to those who did not.[90] Furthermore, another meta-analysis reported that participants who carried community-acquired *S. aureus* were twice as likely to progress to disease compared to those who did not.[91] However, prediction of progression of carriers to disease is challenging because of the complexity of the interplay between carriage, pathogenesis and host susceptibility.

1.2 Carriage, acquisition and transmission

1.2.1 Carriage

S. aureus is carried on multiple sites on the human body, namely the nares, hands, throat, axilla, vagina, skin, groin, and umbilicus.[92] *S. aureus* detection rate varies by site of the body[93] and increases with the number of body sites (maximum three sites) screened.[93-95] The most frequent carriage site is the anterior nares of the nose.[92] In newborns, *S. aureus* is commonly carried on the umbilicus, which leads to an increased risk of superficial and systemic infection.

Carriage rates vary with age and geographical location. Estimates from HIC indicate carriage rates between 7.6% in 0 - 40-month-old children in Israel[96] and 13.5% in 24-month-olds in the Netherlands.[97] Carriage rates are higher in LMIC, ranging from 20% in 0 - 23-month-old children in The Gambia[98] to 35% in 12 - 72-month-old children in India.[99] The prevalence of *S. aureus* carriage is typically higher in young compared to older infants[96,100] with the highest values seen in neonates.[25,101] The nares are believed to be pathogen-free at birth, but *S. aureus* is rapidly acquired after birth sometimes as early as two hours after birth.[102] In HIC, nasal *S. aureus* carriage in infants peaks at about 40% - 50% between six and eight weeks of life[100,102] but then drops to about 21% by 24 weeks.[100] On the other hand, in low-income countries, carriage in infancy peaks much earlier at between 50% – 63% by 6 - 7 days of life[101,103] then falls to about 21% at 20 weeks.[25,101] These differences suggest a higher force

of infection (the rate at which susceptible individuals acquire an infection) in LMIC but may also be a reflection of the sources and route of acquisition of *S. aureus* in the infant in the two settings. Further research is required to confirm this.

1.2.2 Acquisition and transmission

Acquisition describes the presence of a pathogen that was previously absent at a site on a host while transmission describes the spread of a pathogen from a reservoir or host to another host. The transmission pathways, and therefore, acquisition of *S. aureus* among newborns are not well characterised. Still, they have been linked to multiple factors in the mother (for example, carriage status and intrapartum antibiotic use).[104,105] Transmission may be from the mother during delivery, person-to-person contact post-delivery, or through exposure to contaminated materials. Transmission by the airborne route is considered less likely. In Africa, the sources of *S. aureus* acquisition by the newborn are not known; knowledge of what these are will inform the development of strategies to reduce acquisition during the neonatal period.

Mother-to-newborn transmission

As with other pathogens associated with early-onset sepsis, it is assumed that the most significant source of neonatal acquisition of *S. aureus* is the mother. In particular, it is believed that transmission takes place during delivery as the newborn passes through the vaginal tract. Maternal vaginal carriage of *S. aureus* has been associated with neonatal carriage using conventional microbiological methods.[94,106,107] Furthermore, molecular genotyping has shown that among mother-newborn pairs who carried *S. aureus* at the same time points (concordance), about 45% - 84% of them carried the same genotype.[94,108]

Maternal *S. aureus* transmission to her newborn can also occur in the post-natal period either during handling[94,100] or breastfeeding.[100,107] Studies have shown that babies born to carrier mothers via caesarean section carried strains with the same genotype as those carried by the mother,[94,105] and breastfeeding is positively associated with neonatal *S. aureus* carriage.[100,107] However, the concordance between a mother and her newborn's carriage status and strain typing may also be a reflection of their shared environment or from a person who is in close contact with both of them. Additionally, in 23% - 80% of mother-infant pairs, *S. aureus* carriage discordance has been reported, in which mothers were non-carriers, but newborns were carriers.[94,105-108] In such discordant *S. aureus* carrier mother-infant pairs, potential sources of transmission to the newborn include healthcare workers (HCW), other patients or environmental sources such as delivery surfaces.

In Africa, only two studies (one conducted in Gabon and the other in The Gambia) have investigated neonatal *S. aureus* acquisition. In The Gambian study, 21% of neonatal carriage was attributable to maternal carriage, whereas, in the study conducted in Gabon, only 5.6% was attributable to maternal carriage.[104,107] The attributable fraction may have been lower in the Gabonese study because vaginal samples were not collected or by antibiotic use by both the mothers and their newborns in the immediate postpartum period or both.[104] Despite the differences, these studies underscore the fact that there are other sources of transmission of *S. aureus* to the newborn.

Transmission in the healthcare facility

Transmission of *S. aureus* in the healthcare facility in outbreak settings is well documented.[109,110] These studies have shown that many aspects of the hospital environment are associated with the risk of transmission including other patients (especially if their

carriage status is unknown),[111] inanimate objects, body fluids, HCW, and unhygienic practices in the health facility.[112]

The hands of HCW are the primary source of cross-transmission of carriage and infection in the hospital.[113] In the absence of optimal hand hygiene, the degree of contamination increases as patient care continues,[112] and the probability of transmitting also increases. *S. aureus* hand carriage correlates well with nasal carriage.[92] Furthermore, *S. aureus* is resistant to desiccation, surviving on surfaces for many months, if not adequately cleaned with antiseptics**.** Overcrowded and understaffed wards and unavailable or unhygienic sanitary protocols for managing infections on the ward aid transmission.[109] Therefore, infection-control measures, including optimal hand hygiene and adequate cleaning of hospital surfaces, reduce the risk of *S. aureus* transmission in the hospital.[111]

Household transmission

In epidemiology, a reservoir is defined as "*one or more epidemiologically connected populations or environments in which the pathogen can be maintained, and from which carriage or infection is transmitted to the defined target population*".[114] The household plays an insidious role as a reservoir in the transmission, acquisition, and persistence of successful clones of *S. aureus* in a community.[86,95,115,] Clustering of *S. aureus* carriage and infection are observed in households and can often be traced to carriage in an index member.[95,116] These carriers are usually asymptomatic and serve as reservoirs for *S. aureus* transmission to the rest of the household.

Transmission between household members can occur through the sharing of contaminated fomites,[117-119] direct skin-to-skin contact or indirect contact with an individual who is a

carrier or infected.[95, 120] The most common route of transmission in the household is direct contact with the carrier or infected person.[121] The ability of *S. aureus* to survive for months on surfaces, lack of personal and environmental hygiene, and the ubiquitous nature of *S. aureus* propagate environmental contamination.[122] In particular, domestic pets and livestock have been associated with *S. aureus* transmission in the household. Strain similarities between pets and their owners have been demonstrated;[119,123] however, most of these studies have been unable to ascertain the direction of transmission.

Personal hygiene such as regular baths, hand hygiene (including handwashing and use of alcohol-based hand gels), regular laundering of bed linen and clothes, and environmental cleaning has been shown to reduce household transmission of *S. aureus.*[124] In HIC, the risk of transmission of *S. aureus* increases with family size (≥ 5 members)[125] and the presence of a child aged less than five years in the household.[117] In LMIC, household transmission maybe even more common than in HIC due to poor hygiene, overcrowding and high ambient temperatures and humidity.

1.3 Microbiology and pathogenesis of *S. aureus* diseases

1.3.1 History of *S. aureus*

In 1880, a Scottish surgeon, Alexander Ogston (1844 – 1929) was concerned by the alarming rate of postoperative deaths due to the suppuration of surgical wounds. The contemporary theory that suppuration was necessary for wound healing did not convince him.[126] He therefore stained and examined pus from the abscess of one of his patients under a microscope. He observed:

"*My delight may be conceived when there were revealed to me beautiful tangles, tufts and chains of round organisms in great numbers, which stood out clear and distinct among the pus cells and debris...*"[127]

He concluded that micrococci caused abscesses. He further went on to inject pus from abscesses into guinea pigs and demonstrated the formation of new abscesses; the guinea pigs later developed features of septicaemia. He named the micrococci "*Staphylococci*" from a Greek word "*staphyle"* which means a bunch of grapes.

In 1884, a German surgeon, Anton J. Rosenbach (1842-1923), isolated two types of staphylococci which he named according to the pigmentation of their colonies – *Staphylococcus aureus* (from the Latin word "*aurum*" which means gold) and *Staphylococcus albus* (from the Latin word "*albus*" which means white).[128]

1.3.2 The bacterium – *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, non-motile, non-spore-forming, catalase-positive, oxidase-negative, facultative anaerobe belonging to the *Staphylococcaceae* family.[129] The bacterium has a cell wall which consists of proteins, teichoic acid and a thick layer of peptidoglycan, outside of which lies a polysaccharide layer called the capsule. The bacteria are approximately 0.5 - 1.5 µm in diameter and occur singly, in pairs or irregular clusters. On nutritive media, the colonies are well defined, yellow, smooth, opaque, convex with a diameter of about 1 to 3 mm and they are haemolytic on blood agar. *S. aureus* grows between 15^0C and 45^0C, in a pH range of 4.2 – 9.5 and a maximum sodium chloride concentration of 15%.[129] Incubation at room temperature in daylight for about 24 to 48 hours will enhance pigment production. *S. aureus* appears as mucoid, sticky colonies when encapsulated. It is

differentiated from other *Staphylococcus* species by its gold pigmentation and positive reactions to the coagulase test, mannitol fermentation test and deoxyribonuclease test.[129] Positive reaction to mannitol fermentation test differentiates it from *Staphylococcus epidermidis* while sensitivity to novobiocin differentiates from *Staphylococcus saprophyticus.*

1.3.3 Host defense mechanisms

Typically, carriage and colonisation are used interchangeably and refers to the establishment of *S. aureus* at the portal of entry of disease. However, some investigators believe that they are separate entities. Carriage is usually defined as the presence of *S. aureus* on the body surface without causing tissue invasion or symptoms. By contrast, during colonisation, there is tissue invasion eliciting host immune responses without causing symptoms.[130] Disease is said to occur when there is an invasion of the body eliciting host immune responses and causing symptoms.

Intact skin and mucosa of body tracts

The body's first lines of defense against infection are the epithelial surfaces of the skin and the mucosal linings of the body tracts. The outer layer of the intact skin, the stratum corneum, forms a physical and chemical barrier between the body's internal milieu and the external environment.[131] Tight junctions connect the epithelial cells of the skin, respiratory, gastrointestinal, and urogenital tracts and form a seal against pathogens.

Furthermore, the linings of these tracts secrete mucus containing immunoglobulins and lysozyme that prevent adherence of pathogens to the epithelium.[132] The epithelia of the respiratory tract have cilia which move to expel mucus and pathogens from the tract and the

mucosal epithelia of the stomach produce acid and digestive enzymes which degrade pathogens.[132] As long as these defense mechanisms are not interrupted, the pathogen is carried on the skin or epithelium asymptomatically.

1.3.4 Pathogenesis of disease

Anti-staphylococcal antibodies are produced during colonisation episodes. However, most are not capable of killing the bacteria or neutralising their toxins.[133,134] Therefore, colonised persons and carriers are not protected from disease;[135-137] at best, they are protected against the severe types of the disease.[134] The reason for less severe disease in colonised persons is not known, although they tend to have higher anti-staphylococcal antibodies than non-colonised.[138]

S. aureus usually enters the human body where there is a breach in the integrity of the skin or mucosal lining of the respiratory or urinary tract. Subsequently, there may be haematogenous spread of *S. aureus* from a localised infection to other tissues. This exposure of the human tissues to *S. aureus* may initiate the up-regulation of bacterial virulence genes.[139] Host pattern recognition molecules "sense" the bacteria and peptidoglycan on their cell wall, causing both the human host and the pathogen to release chemokines and exotoxins, respectively. The exotoxins released by *S. aureus* include enterotoxin A, enterotoxin B, and toxic shock syndrome toxin (TSST-1) which induce human monocytes and endothelial cells to produce interleukin-8 (IL-8), a potent chemoattractant.[140]

The host cytokines (granulocyte chemotactic protein 2, IL-8 and complement component 5a) attract neutrophils and phagocytes from the blood and bone marrow to the site of infection. Some of these host chemokines and cytokines prime neutrophils for reduced

mobility, firm adhesion to the endothelium, facilitate migration through the endothelial junction out of the blood vessel (diapedesis) to the infection site and stimulate phagocytosis, degranulation and bactericidal activity (Figure 1.1).[141,142] Anti-staphylococcal antibodies produced by plasmablasts initiate the complement systems leading to the production of C3 convertases.[142] These convertases lead to a cascade of reactions in the complement systems causing opsonisation of the bacteria. Additionally, anti-staphylococcal antibodies also cause opsonisation of the bacteria.[142] At the infection site, neutrophils quickly engulf opsonised *S. aureus* by releasing a host of antimicrobial substances such as lysozyme, proteases, and reactive oxygen species.[143] These antimicrobial substances also cause the non-specific host tissue destruction leading to the formation of necrotic tissue which may be walled off with the development of an abscess. This process of infection and consolidation of the disease takes about 1 - 2 weeks.[143]

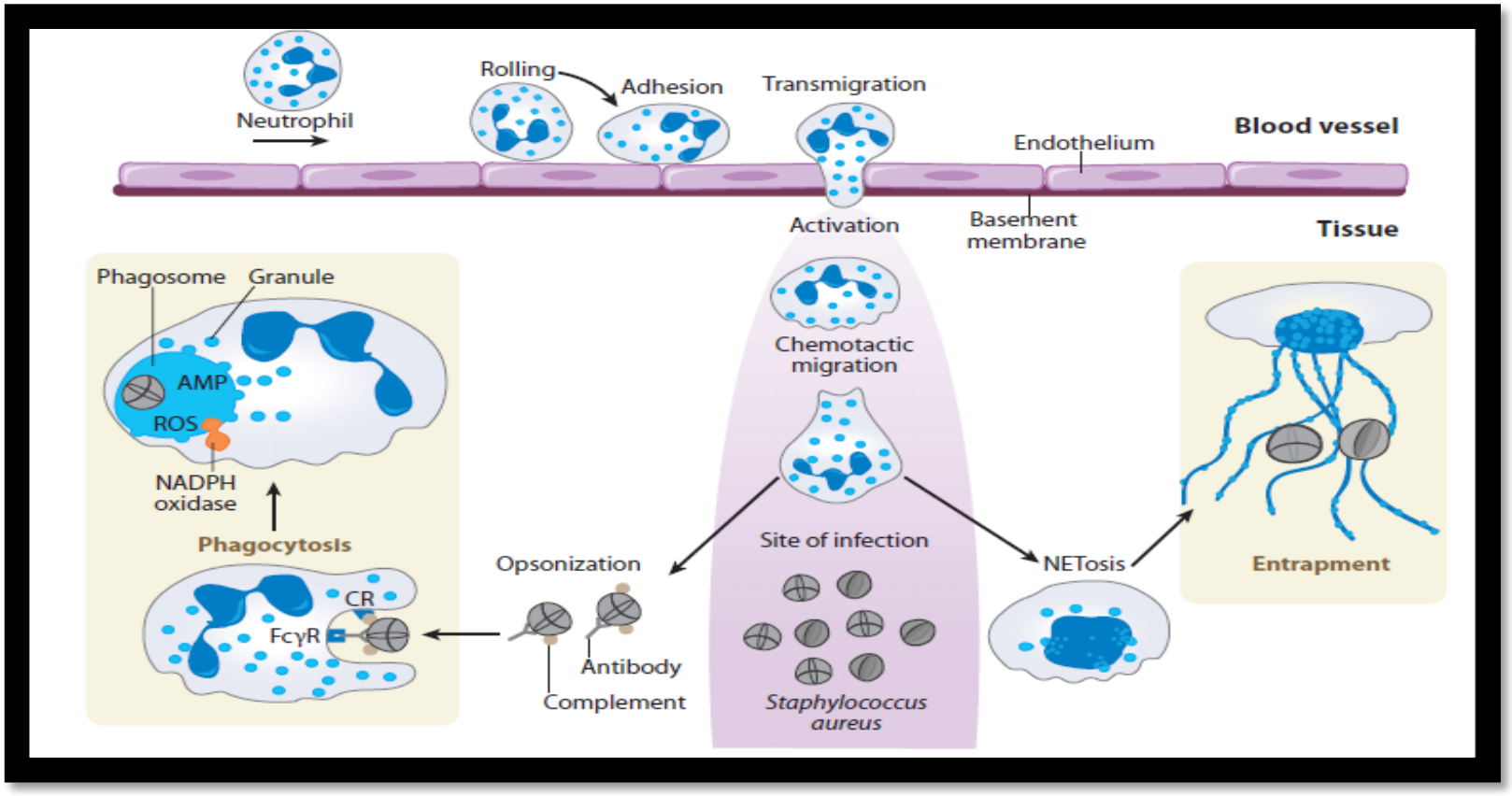


Figure 1.1: Movement of neutrophils to infection site and phagocytosis of *S. aureus*
AMP=Antimicrobial proteins; CR=complement receptors; FcγR=Fcγ receptors; NADPH=Nicotinamide adenine dinucleotide phosphate hydrogen; ROS=reactive oxygen species; NETosis=expulsion of DNA by neutrophils

Source: Spaan et al. Neutrophil versus *Staphylococcus aureus*: A biological tug of war. Annual Review of Microbiology. 2013. Used with permission from Science Direct[142]

Apart from causing disease by tissue invasion, *S. aureus* release exotoxins such as enterotoxins, exfoliating, and toxic shock syndrome toxins leading to gastroenteritis, scalded skin syndrome and toxic shock syndrome, respectively.

1.3.5 Host immune evasion pathways

Neutrophil evasion

All stages of neutrophil recruitment and functions are targeted by *S. aureus* in an attempt to evade human immune responses. *S. aureus* prevents neutrophil chemotaxis by secreting chemotactic inhibitory proteins and staphylococcal superantigen-like substances. These substances inhibit the reduced mobility of neutrophils before adhesion to the endothelium, which is an essential step of neutrophil migration. Additionally, *S. aureus* reduces or prevents opsonisation by targeting the complement pathways and blocking C3 convertases.[144] *S. aureus* also prevents opsonisation by antibodies by up-regulating proteins such as staphylococcal protein A which block the effector domain of the opsonising antibodies.[144] It evades reactive oxygen substances by converting hydrogen peroxide to water and oxygen through the activities of catalase and staphyloxanthin.[145] Apart from avoiding innate immunity, *S. aureus* also secretes pore-forming toxins which cause cellular destruction leading to the disruption of the endothelial and epithelial linings and lyse phagocytic cells.[146]

Formation of biofilm

Development of biofilms is another way by which *S. aureus* evades the host immune systems. *S. aureus* biofilms are adherent sessile communities of bacteria embedded in an extracellular polymeric matrix attached to a host cell surface. Biofilms are formed on damaged host

epithelium like heart valves and fitted medical devices and are capable of manipulating and evading host immune responses. The matrix inhibits cytokine production and the ability of macrophages to penetrate the matrix and phagocytose bacteria.[147]

Additionally, pro-inflammatory Th1/Th17 responses are up-regulated early in *S. aureus* infections and promote the development of biofilms and chronic infections.[148,149] In the absence of nutrients and oxygen, *S. aureus* enter a dormant state evading the host immune system and also becoming intolerant of antibiotics.[150] *S. aureus* may also act as an intracellular pathogen in osteoblasts and epithelial cells, effectively concealing itself from immune detection.[151,152]

1.4 Classification of *S. aureus* diseases

S. aureus diseases can be classified according to its focus of infection, place of acquisition, antibiotic susceptibility, the clinical syndrome, and severity (see Box 1).

1.5 Diagnosis

1.5.1 Conventional microbiological method

Early detection or identification of the pathogen from human specimens, especially in severe cases, is crucial to the management of *S. aureus* disease. Pathogen identification is usually not required for mild cases as they may resolve spontaneously without treatment. Available diagnostic tools vary in terms of their sensitivity and specificity, ease of use, cost, and speed.

Box 1: Definition of terms

Community-acquired disease refers to a patient who is culture positive for *S. aureus* at admission (that is, culture positive on a sample obtained within 48 hours of hospital admission)[a].

Healthcare-associated disease refers to a patient who is culture positive for *S. aureus* at admission and has had prior contact with the healthcare environment in the previous 2 - 30 days, for example, attended a hospital-based clinic or an Accident and Emergency department.

Hospital-acquired disease refers to a patient who is culture positive for *S. aureus* in a sample collected more than 48 hours after hospital admission[a].

Methicillin-resistant *S. aureus* (MRSA) are strains of *S. aureus* resistant to a wide range of β-lactam antimicrobial agents due to the presence of the *mecA* gene and detected by testing for susceptibility to cefoxitin or oxacillin[b] while those susceptible to all β-lactam antimicrobial agents are **methicillin-sensitive *S. aureus* (MSSA)**[b], however most MSSA are penicillin-resistant due to β-lactamases.

Vancomycin-resistant *S. aureus* (VRSA) are strains of *S. aureus* with a minimum inhibitory concentration (MIC) for vancomycin of ≥ 16 μg/mL, those with a MIC between 4 and 8 μg/mL are referred to as vancomycin-intermediate *S. aureus* (VISA) while vancomycin-sensitive *S. aureus* (VSSA) are those with a MIC for vancomycin of ≤ 2 μg/mL[b]

***S. aureus* septicaemia/bacteraemia** cases are patients with clinically suspected septicaemia from whom *S. aureus* was isolated from the blood.[c]

***S. aureus* bacteraemic pneumonia** cases are patients with clinical, with or without radiological signs of pneumonia, from whom *S. aureus* was isolated from the blood.

***S. aureus* non-bacteraemic pneumonia** cases are patients with clinical signs of pneumonia and radiological evidence of lung consolidation from whom *S. aureus* was isolated from lung parenchyma.

***S. aureus* meningitis** cases are patients with clinically suspected meningitis from whom *S. aureus* was isolated from the cerebrospinal fluid.

***S. aureus* osteomyelitis/osteoarticular infections** cases are patients with clinically suspected osteomyelitis or septic arthritis from whom *S. aureus* was isolated from the blood, bone, or joint fluid.

***S. aureus* endocarditis** cases are patients with clinically suspected endocarditis from whom *S. aureus* was isolated from the blood or valve vegetations.

Uncomplicated *S. aureus* disease cases are patients with clinical signs of disease and who are culture-positive for *S. aureus* but without endocarditis and implanted prostheses; negative follow-up cultures after 2 - 4 days, clinical resolution within 72 hour of effective therapy and no evidence of metastatic infections. (*Infectious Disease Society of America*)

Complicated *S. aureus* disease refers to any patient who does not meet the criteria for uncomplicated disease and who is culture-positive for *S. aureus*.

[a] *Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM: CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988, 16(3):128-40.*

[b] *The Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. In., Twelfth Edition (M02-A1 edn). Wayne, Pennsylvania, USA.; 2017.*

[c] Note that in all the above definitions isolation were by conventional microbiological methods. Although the sensitivity of conventional microbiology is lower than molecular methods, the latter has a higher probability of detecting staphylococcal DNA from other sources, hence the choice of method.

The ideal diagnostic test for *S. aureus* should have high sensitivity, high specificity, rapid time to detection, reliability, and be inexpensive while being less affected by prior antibiotic use or blood volume.

1.5.2 Microbiological culture

Microbiological culture is the gold standard for the diagnosis of *S. aureus* disease,[153,154] and also allows antibiotic susceptibility testing. Information on antibiotic susceptibility enables modification of empiric antibiotic treatment for the proper management of patients and reduces the selection of resistant strains, especially in the era of high rates of antimicrobial resistance. However, the turn-around time for culture is two to five days.

Septicaemia

Blood cultures are positive in 5% – 10% of children with features suggestive of invasive infections.[155] This figure may be lower in neonates (due to smaller blood volume obtained for the test) and in resource-poor settings, especially in sSA.[155] Prior antibiotic use, laboratory capability, the number of blood cultures done, the volume of blood obtained, the ratio of the volume of blood to that of the medium in the culture bottle, blood culture sampling technique, blood culture bottle and the system used influence the yield of positive blood culture.[156-158] The use of automated blood culture systems has improved the yield because of their antibiotic-removal resins and charcoal content and also reduced the time to detection of pathogens.[159] Despite this, the yield of *S. aureus* in blood cultures is still considered low. Sensitivity and specificity of blood cultures concerning *S. aureus,* or any

other pathogen detection cannot be evaluated because there is no “gold standard” test for comparison.

Meningitis

Cerebrospinal fluid (CSF) culture is considered the reference test for the diagnosis of bacterial meningitis. The detection rates of CSF culture are over 80% in symptomatic patients with no history of antibiotic use before sampling.[160] In LMIC, some children with meningitis are likely to have received antibiotics before presentation[161,162] reducing the culture positivity rate.[160] In such cases, molecular methods such as polymerase chain reaction (PCR)-based tests may be useful for the identification of *S. aureus*.

Pneumonia

The diagnosis of pneumonia is discussed in chapter 2.

1.5.3 Molecular detection

New tools for pathogen identification are being developed to improve on the sensitivity, time to detection and clinical benefit to the patients - the prompt administration of appropriate antibiotics which improves survival. Molecular methods such as PCR-based tests have high sensitivity ranging from 72% to about 100% and specificity ranging from 80% to 100%; they are rapid and reliable, even with a history of pre-treatment antibiotic use.[163] In addition, molecular methods may be used to detect antimicrobial resistance which makes them attractive. Rapid PCR-based tests have been developed for the detection of MRSA and MSSA based on the detection of the *mecA* gene. Molecular methods might be useful in clinical practice, though they are currently not in use. A drawback of these methods

is that they are often too sensitive, detecting deoxyribonucleic acid (DNA) from dead microbes, other non-microbial nucleic acid, or contaminant DNA. The presence of PCR inhibitors may also influence them. Other limitations of these tests include the cost and skills required to perform these tests. Additionally, isolates are not available for further research and tests. However, these tests are widely used in research.

1.5.4 Mass spectrometry method

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) is a new method for pathogen identification. It works by ionisation of the specimen, measuring the mass to charge ratio and generating peptide mass fingerprints (PMF) which are compared with known PMF stored in a database.[164] This method is accurate, fast and cost-effective in pathogen (including *S. aureus*) identification[165] up to species level from human specimens. Although this method depends on microbiological culture, it is fast gaining grounds in clinical use.

1.5.5 Methods for characterising *S. aureus* strains

Phenotypic tests

The most reliable method for the detection of methicillin-resistant strains of *S. aureus* is the detection of *mecA* gene.[166] Most laboratories are unable to perform molecular biology techniques, therefore, phenotypic tests (oxacillin or cefoxitin disc diffusion or E test) are the most widely used to screen for methicillin resistance. Cefoxitin is preferred above oxacillin because it is more stable and accurate.[166]

Genotyping tests

Molecular typing methods are essential in the evaluation of the global evolution of *S. aureus* and genetic relatedness of its strains. Based on the detection of the *mecA* gene, techniques such as PCR tests can distinguish between MRSA and MSSA. Other methods used for molecular characterisation of *S. aureus* strains include pulsed-field gel electrophoresis, staphylococcal protein A typing, multi-locus sequence type, and SCC *mec* typing. Pulsed-field gel electrophoresis (PGFE) is used to separate large DNA molecules into distinct fragments. It is discriminatory, easy to perform, reproducible, relatively cheap; however, it is laborious and time-consuming. Staphylococcal protein A (s*pa*) typing relies on a single-locus (polymorphic X region of protein A gene) sequencing, therefore prone to misclassification. It is, however, portable, rapid, cheap, discriminatory and reproducible.[167] Multi-locus sequence type (MLST) is a DNA sequence-based typing that depends on seven conserved house-keeping genes. It lacks the power to resolve small evolutionary differences as might be seen during outbreaks and is more expensive than the spa typing or PGFE. Whole-genome sequencing analyses the entire genome of the bacteria, therefore highly discriminatory and has transformed outbreak investigations. The preferred method depends on the objective of the study.

1.5.6 Biomarkers

Biological markers (or biomarkers) are "*biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention*".[168] They are useful in clinical practice as an early diagnostic tool, for monitoring response to therapy and treatment outcomes. There is no single biomarker with 100% sensitivity, though combining them has the potential to achieve higher sensitivity and specificity. The well-established and

commonly used biomarkers in infections are white cell count, C-reactive proteins, and procalcitonin. They are useful markers of infections but not specific for *S. aureus* infections. Some biomarkers such as interleukin-10 and interleukin-1β[169,170] have been correlated with the outcome of *S. aureus* bacteraemia, but these were small studies, and the results need to be validated in larger populations.

1.6 Management

1.6.1 Specific treatment

The drug of choice for the treatment of *S. aureus* depends on the country, the methicillin-susceptibility type of *S. aureus* infection, the severity of the infection and the local antimicrobial susceptibility patterns. In patients with MSSA infections, the preferred drugs include flucloxacillin, amoxicillin, oxacillin, and nafcillin, while vancomycin and daptomycin are recommended in patients with MRSA. Vancomycin is not recommended for the treatment of MSSA because of the increased risk of relapse, treatment failure[171] and death.[172] Other drugs used include linezolid and clindamycin. Uncomplicated cases of systemic infections should be treated with at least 14 days of parenteral therapy and complicated cases (presence of prostheses, metastatic disease, or deep focus of disease) for 28 days while for confirmed endocarditis the standard course of treatment is 42 days.[171] Sometimes, these patients receive a combination of two or more antibiotics, and they are more likely to receive combination therapy if the focus of infection is not removable. New drugs are available for the treatment of SSTI; these are semi-synthetic glycopeptides such as dalbavancin, oritavancin, and telavancin. Clinical responses to these antibiotics are similar to the standard care and have reduced cost compared to standard care.[173]

In Africa, the first-line drugs for the treatment of *S. aureus* diseases include cloxacillin and flucloxacillin. The WHO recommends the use of cloxacillin or flucloxacillin for the treatment of *S. aureus* diseases.[174] In The Gambia, the drug of choice is flucloxacillin;[175] uncomplicated cases are treated for 5 – 7 days and complicated cases for longer.[175]

Antimicrobial resistance (AMR)

The World Health Organization (WHO) has deemed antimicrobial resistance to be a significant threat to global health.[176] Antimicrobial resistance is increasing worldwide and threatens the gains of childhood survival programmes that have been established over the last three decades. Projections of deaths attributable to AMR will reach 10 million deaths a year by the year 2050 if current trends are unchecked.[177] The primary drivers of AMR are unregulated antibiotic use in human (hospitals and community) as well as veterinary medicine.[178] In LMIC, scarcity of supportive diagnostic resources, availability of over-the-counter antibiotics, poverty and ignorance, contribute to the overuse and misuse of these drugs. Reports on AMR have shown an association with increased adverse patient outcomes such as morbidity, cost of treatment and even mortality.[179] Additionally, AMR is associated with lost productivity, missed days from school and work by parents.

The first report of *S. aureus* antimicrobial resistance was in 1930.[180] Today, almost a century later, about 90% - 95% of clinical isolates of *S. aureus* are resistant to penicillin,[181] and about 60% of isolates from intensive care units in the USA are methicillin-resistant.[182] Pockets of resistance to vancomycin have been reported mainly in high-income countries.[183] VRSA strains carry the *vanA* gene and are exceedingly rare; no VRSA transmission between patients has been documented,[184] suggesting that these bacteria may have impaired fitness.

MRSA

Since the isolation of MRSA in 1961, it has been a significant cause of nosocomial infections (HA-MRSA),[185] and a community-acquired strain (CA-MRSA) emerged 20 - 25 years ago causing SSTI.[186] Methicillin-resistance has been described in both hospital and community strains, but it is reported that HA-MRSA are generally resistant to a wide range of antibiotics compared to CA-MRSA. Nowadays, the distinguishing features between the two strains are fading with CA-MRSA strains causing nosocomial infections and being resistant to a wide variety of antibiotics, just like HA-MRSA.[186] A few staphylococcal lineages are responsible for both HA-MRSA and CA-MRSA, and they have evolved, by acquiring genetic materials through horizontal gene transfer, thus altering the gene expression and function.[185]

There are geographical variations in the strains of MRSA. Based on MLST, MRSA population structure associated with humans has ten dominant lineages called clonal complexes (CC) within which many more minor ones arise.[185] Some strains are widespread, while others are limited to some countries. For example, CC30 is commonly seen in Australia, Canada, Spain, the UK, and USA while Germany, Italy, and the UK have reported CC22.[185] The common clonal complexes in Africa include CC5, CC30, CC80 and CC88,[187] and in The Gambia, CC5 and CC15.[98] This suggests global dissemination of *S. aureus* or high adaptation to new environments. Despite the broad geographic variations, isolates from the same lineage have conserved genes, while those from different lineages differ by hundreds of genes.

In the USA, the incidence of MRSA bacteraemia declined between 2005 and 2014,[188,189] while other studies report a stable incidence between 2010 and 2014.[190] The converse is the case in Australia, Canada and the Scandinavia where Laupland and colleagues[191] reported an increase in the incidence of MRSA bacteraemia between 2000 and 2008. In LMICs, there is a wide variation in the prevalence of MRSA. A prospective multinational surveillance study in 17 teaching hospitals in eight Asian countries reported that the prevalence of CA-MRSA and

HA-MRSA was 25.5% and 67.4%, respectively.[192] In Africa, the prevalence of MRSA ranges from 16% to 44% of all clinical isolates;[19,193] the higher rates are seen in areas of high HIV prevalence due to repeated exposure of HIV patients to healthcare facilities. In Africa, the resistance of *S. aureus* isolates to penicillin and ampicillin is high, up to 89% or even 100% (in the case of penicillin)[178] and relatively low to gentamicin.[178,194] This high antimicrobial resistance rate is not surprising given the unregulated access and use of antibiotics. The most frequently prescribed antimicrobial in The Gambia includes amoxicillin[195] exerting selective pressure on the *S. aureus* carriage isolates in this country, thus increasing the risk of resistance.

Local AMR patterns usually acquired through continuous surveillance inform the decision on the empirical therapy for infections like *S. aureus* diseases. However, in Africa, where the burden of *S. aureus* community-acquired diseases is high, there is limited information on AMR patterns. The WHO recommends the use of ampicillin and gentamicin as empirical treatment of suspected neonatal and childhood bacterial infections in the absence of local AMR data.[174] Given that *S. aureus* is a significant cause of neonatal and childhood IBI it is, therefore, imperative to document the susceptibility of the *S. aureus* isolates seen in Africa to these antibiotics.

1.6.2 Supportive treatment

In addition to administering antibiotics, the management of *S. aureus* infection should involve repeated cultures and the removal of an identifiable focus of infection in cases where this can be identified. The former is important because of the risk of developing endocarditis which increases with persistent bacteraemia, and pyelonephritis and renal scarring in *S. aureus* UTI. Other supportive treatments depend on the clinical syndrome and the severity

of the disease and include, but are not limited to, resuscitation, the administration of intravenous fluid and circulatory support in septic shock,[196] supplementary oxygen in pneumonia, and control of seizures in meningitis and brain abscess.[196]

1.7 Morbidity/Mortality

1.7.1 Length of hospital stay

S. aureus disease is associated with extended hospital stays. Patients with *S. aureus* disease have, on average, longer hospital stays compared to those with the same clinical syndrome due to other pathogens.[197] The duration of hospital stay is further increased in patients with associated comorbidities and those with hospital-acquired infections (HAI) due to *S. aureus*.[74]

It is unclear whether resistance impacts the duration of hospital stay. Some authors have reported similar durations in patients with methicillin-resistant *S. aureus* (MRSA) and those with methicillin-susceptible *S. aureus* (MSSA)[198] while others have reported longer durations in patients with MRSA.[71,199,200]

1.7.2 Cost

In HIC, the cost of treating *S. aureus* disease varies according to the severity of infection, admission status (in-patient or out-patient), the presence of antimicrobial resistance and the country. In HIC, the cost ranges from $12,216 to $84,436 per patient[201,202] with the cost of treating MRSA being particularly high.[199] There are no data on the cost of treating *S. aureus* diseases in adults or children in Africa.

1.7.3 Case fatality ratios

In the pre-antibiotic era, mortality from invasive *S. aureus* disease was as high as 80%,[203] but this has drastically reduced since the introduction of antibiotics and better protocols for *S. aureus* disease management. In HIC, CFR ranges between 1.4% and 6.0%,[27,70,79] and the predictors of fatality include older age,[27] black/indigenous race,[78,79] immunosuppression,[204] the presence of co-morbidities,[70,79] and acquisition of the disease in the hospital.[27] Fatality ratios are higher in patients with MRSA compared with those with MSSA, even after adjusting for age and the presence of co-morbidities.[200,205] In LMIC, CFR ranges between 6% and 32% in children[45,47,71,72,206] and the factors associated with fatality include younger age,[73] absence of a focus of infection,[73] malnutrition,[72,73] not receiving appropriate antibiotics,[73] and HIV infection.[72] Patients who present with non-specific symptoms such as a history of fever or elicited fever without evidence of localization of the infection in any organ or system of the body are usually regarded as not having focus of infection. These patients without focus of infection are unlikely to receive appropriate antibiotics because they have not presented with other clinical features traditionally associated with *S. aureus* infections which reduces the physician's suspicion to treat as such. More information on risk factors for fatality in LMIC are required.

1.8 Prevention

1.8.1 Infection prevention and control

Cross-infection in the hospital settings

Simple and inexpensive interventions can reduce most staphylococcal hospital-acquired infections (HAI) and carriage. These infections occur when patients with no evidence of an infection and not incubating infections at the time of admission acquire infections during

their hospital stay. Most HAI occur as a result of unhygienic practices in the hospital. These aid the transmission of *S. aureus* or other pathogens from patient to patient via contaminated clothing and hospital equipment[207] or through the contaminated hands of HCW.[208] These HAI are associated with high mortality and increased health care costs.

The burden of HAI in LMIC is significantly higher than reported in HIC.[209] Worldwide, the leading pathogen responsible for HAI is *S. aureus.*[111] Other pathogens include extended-spectrum-beta-lactamase-producing Enterobacteriaceae, *Klebsiella* spp, *Pseudomonas aeruginosa*, and *Acinetobacter* species amongst others.[210]

Carriage in the patient after contact with a contaminated person or environment usually precedes the development of diseases. Although strategies to reduce the transmission of pathogens and HAI are complex, simple intervention such as hand hygiene (including handwashing and the use of alcohol-based hand sanitisers), use of personal protective equipment such as gloves, masks and gowns by staff, disinfecting and decontaminating hospital equipment, and the isolation of infected patients have been suggested.[211] Even though there are no data on the effectiveness of personal protective equipment,[212] their role in the prevention of the spread of carriage and HAI cannot be overlooked.

Hand washing is a cost-effective intervention for the transmission of carriage and prevention of HAI in the hospital setting[213] however, compliance with hand hygiene practices is variable in both HIC and LMIC.[214,215] In HIC, the primary reason for non-compliance is behavioural.[214] In contrast, in most LMIC, non-compliance may also be attributable to the scarcity or irregular availability of essential hand hygiene resources such as water, soap, alcohol-based hand sanitisers, and single-use towels. Besides, there is often a lack of knowledge of infection prevention and control (IPC) by hospital staff and

inadequate hospital policy on hygiene.[216,217] These findings cut across all levels of healthcare in Africa.[215] Improvement in the compliance of hand hygiene is essential for the reduction of the incidence of HAI and carriage in hospital settings.[218]

In HIC, apart from hand hygiene, other interventions in the IPC programmes include activities such as screening for *S. aureus* on admission, isolating carriers or patients with the disease, contact precautions and decolonisation of patients. These measures are well accepted in outbreak settings, and combinations of interventions have been shown to prevent transmission.[219] However, they have their limitations. Screening has not yet been standardised because there is no consensus on the patient population, body sites, frequency of screening, and cost-benefits of screening all patients. Additionally, there is no evidence for an increase in the incidence of *S. aureus* infection rates following the cessation of isolation and contact precautions in the nursing of infected patients.[220] In contrast, isolation and contact precautions led to reduced contact times with isolated patients by HCW, causing anxiety and depression in such patients or their parents. Good compliance with these effective infection control measures leads to infrequent transmission of *S. aureus* in the hospital setting.[111]

Autoinfection

Standard IPC measures alone may not be 100% effective against *S. aureus* transmission. Autoinfection is the development of a disease in one body site following the transfer of a pathogen previously carried in another body site. It can occur in the hospital as well as in the community settings. The demonstration of strain similarities between invasive and carriage *S. aureus* isolates from the same patient supports the importance of autoinfection.[24]

Therefore, a review recommended decolonisation of patients to prevent autoinfection,[221] but the evidence for this intervention is weak.[222] A potential problem with decolonisation is that it leads to the temporary clearance of *S. aureus* carriage. Repeated decolonisation has been proposed to overcome this problem, but the effect of the decolonising agent such as mupirocin diminishes over time. There is also the possibility that antimicrobial resistance to the decolonising agent may develop. Currently, decolonisation is used for patients with recurrent SSTI and when the risk of *S. aureus* transmission remains high despite optimal infection control measures.[171]

1.8.2 Vaccination

Vaccination is one of the most effective public health intervention against diseases. However, there is no effective vaccine against *S. aureus*. The high burden of *S. aureus* disease, increasing prevalence of *S. aureus* antimicrobial resistance, high case fatality ratios and the ease of transmissibility of the pathogen in the hospital and community highlight the urgent need for an effective vaccine. Various *S. aureus* proteins have been used as targets for vaccine development for passive or active immunisation,[223-225] and some have been evaluated in early phase clinical trials. However, none of the five vaccine candidates that have progressed into clinical trials so far has demonstrated efficacy against disease.[226] Possible reasons for the failure of these vaccines include the use of a single protein as a target, the absence of adjuvants, antibodies not being assessed for functionality or opsonophagocytic activity, and critically ill trial participants.

Barriers to the development of an effective vaccine against *S. aureus* disease or carriage include a lack of information on correlates or surrogates of protection, antigenic variation,

complex invasion, carriage and disease mechanisms amongst others.[226] A vaccine that induces both humoral and cellular immune responses will be required. Vaccine development is currently focusing on the use of multiple antigens as targets in the same vaccine.[226] Unfortunately, however, the most recent candidate, SA4Ag vaccine, which included four antigens, also failed to demonstrate efficacy against disease.

Monoclonal antibodies appear to be a promising adjunctive treatment to reduce the mortality rates in cases of severe *S. aureus* infection, especially in immunocompromised patients.[227] However, to date, none of the five candidate monoclonal antibodies evaluated in clinical trials has demonstrated efficacy against the disease.[228] In the absence of an effective vaccine for active or passive immunisation, efforts should be made to optimise and scale-up the other preventive measures.

1.9 The rationale for the studies

Part of the Sustainable Development Goal 3.2 requires that countries reduce childhood and newborn mortality to 25 per 1,000 live births and 12 per 1,000 live births by 2030, respectively. The current childhood and neonatal mortality rates in Africa are 78 per 1,000 live births and 28 per 1,000 live births, respectively. Achieving these targets would entail as much as a threefold and two-fold reduction of the current rates in the region. Hence, significant and innovative efforts are required to increase the momentum of recent years further and achieve better health outcomes in this age group.

Preventable infections contribute significantly to the unacceptably high childhood and neonatal mortality rates in Africa. *S. aureus* is a significant cause of IBI (ranking among the top two leading gram-positive bacteria) in childhood and neonates with morbidity and

mortality worsened by antimicrobial resistance. However, there are limited, recent population-based country-representative data on the burden and risk factors for *S. aureus* disease in children aged less than five years in Africa. Furthermore, newborns rapidly acquire high carriage rates of *S. aureus*, a significant risk factor for disease. Besides, there are differences in AMR within and between countries driven by antibiotic use, co-morbidities such as HIV and socioeconomic factors. Information on the burden of *S. aureus* disease among under-5 children in Africa, as well as the risk factors for neonatal carriage acquisition and the prevalence of antimicrobial strains of both carriage and invasive isolates in The Gambia and Africa at large, will serve as baseline and are necessary for the evidence-informed allocation of health resources and developing strategies for control, treatment, and prevention.

1.10 Aim and objectives

The collection of studies presented in this thesis aims to estimate the burden of severe *S. aureus* disease in African children and identify the risk factors for carriage acquisition among neonates in rural Gambia.

The specific objectives are to:

1. Systematically review the literature on the burden of *S. aureus* disease and estimate the number of cases and deaths attributable to *S. aureus* disease in children aged less than five years in Africa.
2. Estimate the incidence and case fatality ratio of SAB among children aged less than five years in rural Gambia using prospective data collected over eight years in a health and demographic surveillance system setting.

3. Assess the risk factors for neonatal carriage acquisition of *S. aureus* among a cohort of newborns and their contacts in the immediate peripartum period in rural Gambia.

Chapter 2: The burden of community-acquired *Staphylococcus aureus* disease in children aged less than five years in Africa.

Preamble

This chapter describes the burden (number of cases and deaths) of severe *S. aureus* disease among children aged less than five years in Africa. The first step was to conduct a systematic literature review and meta-analysis of the incidence, prevalence, and case fatality ratio of childhood *S. aureus* disease in Africa. Then, the information from this meta-analysis was synthesised with country-specific population estimates to estimate the burden (number of cases and deaths) of severe *S. aureus* disease in Africa.

2.1 Introduction

Credible estimates of cause-specific childhood morbidity and mortality can help to formulate regional and national health policy. In LMIC, *S. aureus* is a significant contributor to childhood and neonatal morbidity and mortality; however, it ranks low among diseases of public health importance.[206] The burden of *S. aureus* morbidity and mortality is made more substantial by the increasing prevalence of antimicrobial resistance and the lack of an effective vaccine. Resources geared towards the improvement of child survival are increasing, and in the past three decades, much progress has been made in reducing childhood morbidity and mortality. However, the reduction of neonatal morbidity and mortality has been slower than that in the under-5 years' age group. Although mortality rates in African countries are still unacceptably high, the lack of valid estimates of the burden (number of cases and deaths) of *S. aureus* disease in children aged less than five years at

national and sub-regional levels could account for the low level of attention and priority given to *S. aureus*. Due to considerable geographical variations in cause-specific disease burden, national and sub-regional data are required to develop appropriately focused interventions. Therefore, knowledge of the burden of *S. aureus* disease in Africa will serve both as the baseline for monitoring trends and to guide policy decisions on the allocation of health resources and the development of strategies for treatment, control, and prevention at both national and sub-regional levels. This study estimates the number of cases and deaths of severe *S. aureus* disease in children aged less than five years in each sub-region in Africa in 2015 to fill the knowledge gap.

The approach described below is consistent with methods used by others to estimate the burden of disease caused by *S. pneumoniae* in children aged less than five years.[229]

The road map for the chapter

This chapter describes two distinct methods sections - one for the systematic literature review and meta-analysis (pages 59 – 65) and the other for estimating the burden of severe disease (pages 65 – 72). Then, the results of the systematic literature review (pages 72– 89) are presented, followed by the burden of severe disease (pages 89 - 101) results. Finally, the last section of the chapter discusses the results of both the systematic review and the disease burden estimates (pages 101 - 111).

Box 2: Definition of terms

Aetiologic fraction for pneumonia is the proportion of *S. aureus*-positive cases among patients diagnosed with clinical pneumonia who had blood culture or lung aspirate investigation. For septicaemia and meningitis, it is the proportion of *S. aureus*-positive cases among all culture-confirmed bacteraemia and meningitis patients, respectively. Different definitions were used because of the case ascertainment methods employed to calculate incidence for the clinical syndromes. For septicaemia and meningitis, the cases were patients with culture-confirmed disease while for pneumonia they were those with clinical pneumonia.

Burden is the impact on population health as measured by the number of cases (morbidity) and deaths (mortality) attributable to *S. aureus* disease.

Case fatality ratio (CFR) is the proportion of cases of *S. aureus* disease who died of the disease before discharge from the hospital.

Clinical syndrome is a complex of symptoms and signs associated with a specific condition for which the cause may not necessarily be known. Those associated with *S. aureus* include pneumonia, septicaemia, meningitis, endocarditis, osteomyelitis, toxic shock syndrome, food poisoning, skin and soft tissue infections and urinary tract infection, among others.

Disease refers to a clinical syndrome with a known causative agent or process.

Incidence is the number of new cases in a defined population at risk per year.

Non-pneumonia Non-meningitis Non-septicaemia (NPNMNS) clinical syndromes associated with *S. aureus* other than pneumonia, meningitis, or septicaemia, for example endocarditis, osteomyelitis, urinary tract infections, brain abscess, skin and soft tissue infections among others.

Population-based studies are studies conducted in a well-defined geographical location and everyone in the specified age group in the area is followed-up in time for the event of interest. Population-based studies are designed to detect all cases that occur in the given geographical location.

See Box 1 (Chapter 1, section 1.4) for other definitions

Hypothesis

The burden of community-acquired *Staphylococcus aureus* disease in children aged less than five years in Africa is high.

2.2 Objectives

Aim

To estimate the burden of community-acquired *S. aureus* disease in children aged less than five years in Africa.

Objectives

- To estimate the number of cases of community-acquired *S. aureus* disease in children aged less than five years in Africa in 2015
- To estimate the number of deaths due to community-acquired *S. aureus* disease in children aged less than five years in Africa in 2015 and
- To describe antimicrobial susceptibility patterns of clinical *S. aureus* isolates recovered from children aged less than five years in Africa

2.3 Methods

This report follows the Guidelines for Accurate and Transparent Health Estimates Reporting (GATHER) statements.[230] The GATHER statement provides guidelines for the reporting of studies synthesising multiple studies or data sources to report on global, regional, or national health estimates. The key elements of GATHER include describing (i) the objectives and funding, (ii) data inputs, (iii) data analysis, and (iv) results and discussion (Appendix 1).

2.3.1 Methods for the systematic review

A systematic literature review was conducted to obtain country representative data on the estimates of incidence, aetiologic fraction and case fatality ratio (CFR) of each of the clinical syndromes caused by *S. aureus*. These parameters were required for the models for the estimation of the number of cases and deaths due to *S. aureus* disease.

Eligibility

The criteria for inclusion of the studies used the population, intervention or exposure, comparison group, outcome, and study design (PICOS/PECOS) framework.[231]

Population: Children aged less than five years in Africa

Outcomes: 1) incidence of meningitis, toxic shock syndrome, neonatal septicaemia, septicaemia, peritonitis, scalded skin syndrome, endocarditis, pericarditis, septic arthritis, osteomyelitis, empyema, lung abscess, pyomyositis, urinary tract infections, conjunctivitis, otitis media, skin infections; 2) aetiologic fraction of community-acquired *S. aureus* disease in cases of meningitis, toxic shock syndrome, neonatal septicaemia, septicaemia, peritonitis, scalded skin syndrome, endocarditis, pericarditis, septic arthritis, osteomyelitis, empyema, lung abscess, pyomyositis, urinary tract infections, conjunctivitis, otitis media, skin infections; 3) case fatality ratio of *S. aureus* meningitis, toxic shock syndrome, neonatal septicaemia, septicaemia, peritonitis, scalded skin syndrome, endocarditis, pericarditis, septic arthritis, osteomyelitis, empyema, lung abscess, pyomyositis, urinary tract infections, conjunctivitis, otitis media, skin infections; 4) resistance to antibiotics (ampicillin, amoxicillin, amoxicillin-clavulanate, cloxacillin, chloramphenicol, cotrimoxazole, cefotaxime, cefoxitin or oxacillin, erythromycin, gentamicin, ofloxacin, and penicillin)

Study designs included randomised controlled trials (RCTs), case-control, cohort, and cross-sectional studies.

Inclusion criteria

Studies on the aetiologic fraction or incidence of community-acquired *S. aureus* bacteraemia and serious non-bacteraemic illnesses, for example, conjunctivitis, empyema, endocarditis, lung abscess, meningitis, osteomyelitis, otitis media, pericarditis, peritonitis, pyomyositis, scalded skin syndrome, septic arthritis, skin infections, toxic shock syndrome, urinary tract

infections were included. Studies conducted among children aged less than five years in an African country between January 1980 and May 2019 and published in English or French were included.

Exclusion criteria

Studies were excluded if they assessed infections in specific risk groups (for example, HIV-infection, sickle cell disease, haemodialysis patients, or malnutrition among others), or if they were specific to methicillin-resistant *S. aureus* (MRSA) or methicillin-sensitive *S. aureus* (MSSA) or the identification of molecular clones of clinical isolates. Other studies excluded were case reports of less than 50 cases, conference abstracts, outbreak investigations, retrospective studies, review articles, editorials, policy statements, and those on secondary data analysis of a dataset already described in a comprehensive paper. Studies that did not stratify isolates by age or sample type or where the methods of data collection or laboratory methods were unclear (for example, the method of selection of participants) were also excluded.

Data sources

A systematic search of articles reported in English and French was undertaken using Medline (Medical Literature Analysis and Retrieval System Online), EMBASE (Excerpta Medica Database, Amsterdam, The Netherlands), Web of Science, Global Health, and Africa Wide Information. In addition, the reference lists of included articles were searched to identify additional studies that met the eligibility criteria.

Search strategy

A combination of both Medical Subject Headings (MeSH) terms and free text terms were combined to capture concepts related to clinical syndromes, pathogen, and geographic area

for the literature search. The terms included “abscess”, “acute respiratory infection”, “bacter(a)emia”, “bloodstream infection (s)”, “cellulitis”, “conjunctivitis”, “empyema“, “endocarditis”, “fever”, “meningitis”, “osteomyelitis”, “otitis media”, “pericarditis”, “peritonitis”, “pneumonia”, “pyomyositis”, “septic(a)emia”, “septic arthritis”, “skin infection”, “toxic shock syndrome”, “urinary tract infection”, “*Staphylococcus aureus”,* “*S aureus”*, “*Staph aureus*” and a list of all African countries. See Appendix 2 for the search strategy for Medline, EMBASE, and Global Health which was undertaken through Ovid®. The United Nations list and African Union classification of the 55 African countries were utilised[232] for the search. The French and English names of Cote d’Ivoire and Botswana and Batswana (name of the citizens) were used. “Congo”, “Guinea” and “Sudan” were used to represent the countries with the similar names - Democratic Republic of Congo and Congo, Guinea, Guinea-Bissau and Equatorial Guinea, and Sudan and South Sudan. Swaziland was renamed Eswatini in 2018; therefore, both names were included in the updated search.

Data management and extraction

The search was first performed in April 2016 and updated in May 2019. AO compiled retrieved articles in Endnote referencing software and removed duplicates. AO developed a checklist of inclusion and exclusion criteria to assess the eligibility of articles and trained Natalie Edgar (NE) and Ese Ebruke (EE) on the use of the checklist to screen article titles and abstracts. AO and EE assessed abstracts, titles and titles of citations in articles in English while NE assessed articles in French. The references of relevant articles were placed in a folder in Endnote. Full-text articles fulfilling eligibility criteria were retrieved and assessed by the investigators individually with documentation of reasons for excluding an article. Malick Ndiaye (MN) and NE assessed full articles in French. An independent review by a third investigator, Effua Usuf (EU) resolved disagreements between assessors about

eligibility. Data extraction forms created using Microsoft Excel were piloted. EE, NE and MN were trained and extracted data onto validated forms. Assessment for duplicate data was done by cross-referencing reports from the same country for the period of data collection, location, and sample size. When information was missing, the investigators contacted the authors for clarification. AO collated completed data extraction forms.

The extracted data depended on the study type and included first author's name, study site (country), setting (level of hospital), year of study or year published, eligibility criteria, clinical syndrome studied, age of participants, number of subjects studied, type of sample obtained, number of participants positive for *S. aureus* and for all bacteraemia, person-time of follow-up, method of detection (molecular or conventional microbiology) and incidence or prevalence of *S. aureus* meningitis or septicaemia. Other variables included antibiotic use before sampling, antimicrobial resistance data, additional tests (for example, HIV or malaria testing), number of deaths in cases and the elements of study quality assessment. Data were extracted independently by three investigators (AO, NE, and EE). Incidence was extracted directly or computed based on the information on the number of cases and person-years of follow-up. The aetiological fraction was calculated as the proportion of *S. aureus*-positive cases among all patients with clinically suspected disease who were investigated and had true bacteraemia. CFR was the proportion of cases of *S. aureus* disease that died of the disease before discharge from the hospital and antimicrobial resistance was calculated as the proportion of resistant isolates among the tested ones.

Assessment of the risk of bias of literature

Assessment of the study quality was done independently by AO and EE. Due to the constraints of resource-limited settings as seen in most African countries, microbiological

methods (transport method or media used) for the isolation of *S. aureus* and non-reporting of contaminants for the studies which alluded to performing blood cultures were not used as criteria for quality assessment. Factors considered during quality assessment were:

i. The use of pre-defined inclusion criteria
ii. Systematically or consecutively recruited and sampled paediatric hospital admissions
iii. Evaluation of all admissions, all febrile admissions or all patients meeting the pre-defined eligibility criteria with microbiology culture of body fluid.

In accordance with the Cochrane Collaboration's assessment for risk of bias (ROB),[233] the quality of each article was graded as low, unclear or high risk of bias established on two domains; i) the possibility of missing cases and ii) the use of pre-defined eligibility criteria. Low ROB was considered if pre-defined inclusion criteria were stated, and at least 80% of all eligible participants were investigated and reported. Articles were categorised into three groups:

A: Both investigators agreed on the two domains of ROB

B: Both investigators agreed on one of the domains of ROB

C: Both investigators disagreed on the two domains of ROB, or there was no information on which to base a judgement.

Only articles where both investigators agreed on at least one of the domains of ROB were used to estimate the incidence, aetiologic fraction or case fatality ratio of *S. aureus* disease. All included articles of sufficient quality were used to estimate CFR and antimicrobial resistance patterns.

Antimicrobial resistance

The prevalence of resistance was defined as the proportion of resistant clinical isolates among all *S. aureus* isolates tested for susceptibility. Estimates were reported for each antibiotic stated in section 2.3.1 by region, when feasible.

A DerSimonian-Laid random effects meta-analysis[234] was used to obtain summary estimates of incidence, aetiologic fraction, case fatality ratio and proportion of resistant isolates. Statistical analyses were done in Stata 14.0 (College Station, Texas 77845 USA) and RStudio Team (Boston, MA).

2.3.2 Methods for estimating the burden of disease

Information required for this analysis was available from three United Nations agencies. The United Nations Population Division[235] and the Joint United Nations Programme on HIV and AIDS (UNAIDS)[236] provided information on the population sizes of under-5 children and the prevalence of HIV among children aged 0 - 4 years in African countries in 2015, respectively. The United Nations Children's Fund (UNICEF)[237] provided information on country-specific mortality rates, number of live births, and the proportion of children with pneumonia in the preceding two weeks and had access to health care.

Categorisation of countries

In countries where model parameters (incidence, aetiologic fraction or CFR estimates) were missing, these were imputed by hierarchical expansion using data from similar countries. Similar countries were identified by creating categories by 1) mortality strata[238] according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75

per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births) and 2) sub-regions as defined by the African Union[239] – central, east, north, southern and west. A neighbouring country is one that shares a border with the nation of interest.

The methods used to estimate the number of cases by clinical syndrome

Pneumonia cases

The number of *S. aureus* bacteraemic pneumonia cases among children aged 0 – 59 months was estimated by multiplying the country-specific estimates of hospitalised cases of all-cause clinical pneumonia as modelled by McAllister and colleagues[240] by the aetiologic fraction for bacteraemic pneumonia. Aetiologic fraction estimates from population-based studies from each country were used, when available. Where more than one estimates were available from one country, the pooled estimate was used. Where no country-specific estimates of aetiologic fraction were available, these were imputed using the algorithm in Figure 2.1.

The number of cases of non-bacteraemic pneumonia (as defined in section 1.4) was calculated similarly using the estimates of the aetiologic fraction of non-bacteraemic pneumonia due to *S. aureus* instead of that due to bacteraemic pneumonia.

Meningitis cases

Data on country-specific incidence of *S. aureus* meningitis were not available. The number of *S. aureus* meningitis cases was calculated by multiplying the country-specific incidence of all-cause bacterial meningitis (from the systematic review) by the population size and the

country-specific aetiologic fraction of meningitis attributable to *S. aureus* from population-based studies.

$$No.S.aureus\ cases = Meningitis\ incidence \times Pop_{2015} \times P_{Cases,Disease} \quad \text{equation 1)}$$

where $P_{Cases,Disease}$ is the proportion of meningitis cases attributable to *S. aureus,* meningitis incidence is the incidence of all-cause bacterial meningitis per 100,000 population while Pop_{2015} is the population size of children less than five years of age in each country.
In the absence of country-specific information, estimates of incidence and aetiologic fraction were imputed based on the algorithm in Figure 2.1.

Septicaemia cases

The incidence, aetiologic fraction and CFR of septicaemia were derived from the systematic review. Incidence of *S. aureus* septicaemia (per 100,000 population) was applied to the estimates of country-specific population size[235] to determine the number of *S. aureus* cases.

$$S.aureus\ cases\ = S.aureus\ septicaemia\ Incidence \times Pop_{2015} \quad \text{equation 2)}$$

The number of cases of neonatal septicaemia was derived by applying the neonatal *S. aureus* incidence (per 1,000 live births) to the estimate of live births for each country.

$$S.aureus\ cases = Neonatal\ S.aureus\ septicaemia\ Incidence \times\ LB_{2015} \quad \text{equation 3)}$$

where *S. aureus* septicaemia Incidence is the incidence of *S. aureus* septicaemia in neonates (per 1,000 live births) and LB_{2015} is the number of live births per country in 2015.[238]

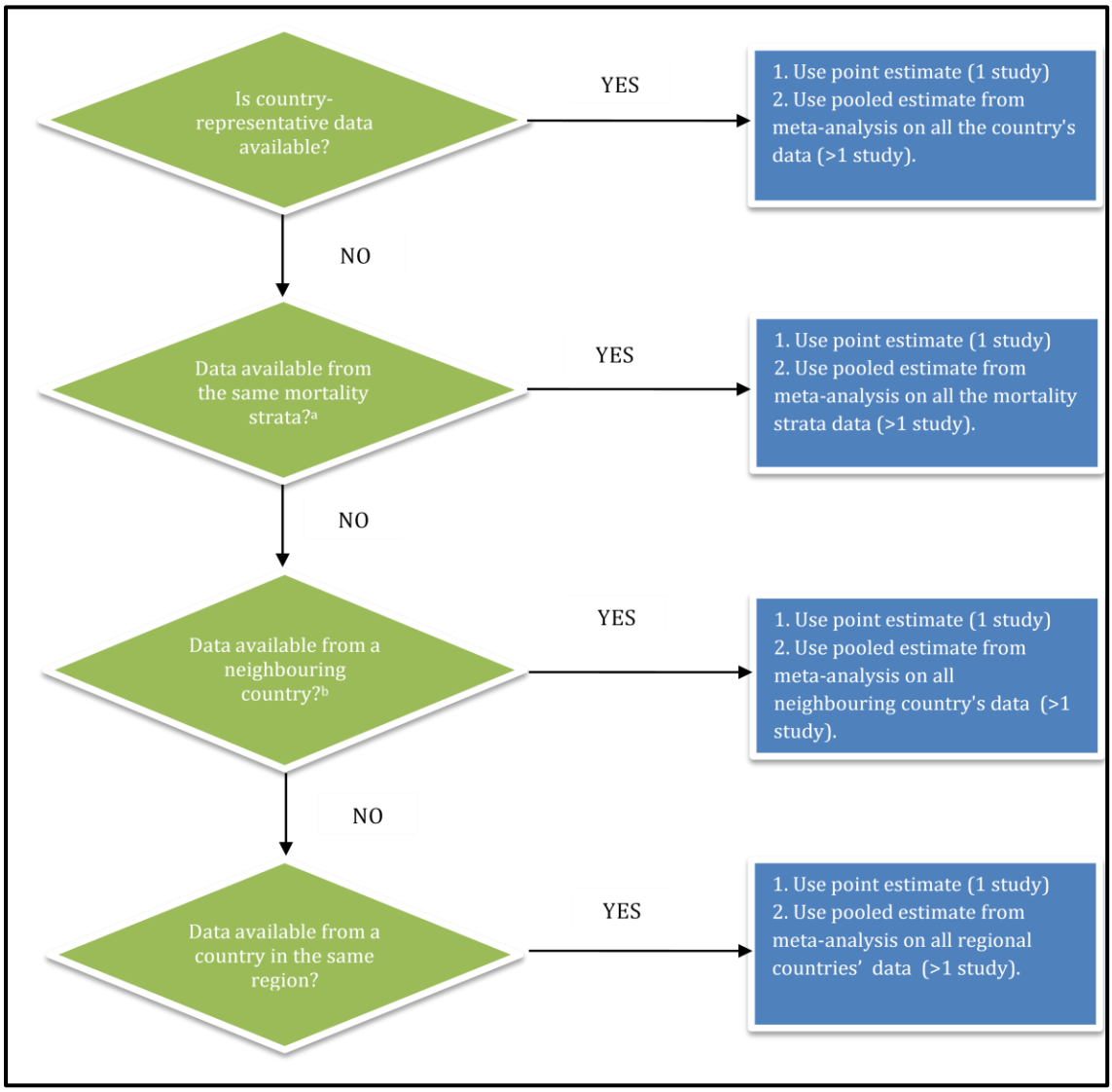


Figure 2.1: The algorithm for imputing country-specific estimates of incidences, aetiologic fraction, case fatality ratio for meningitis and septicaemia and aetiologic fraction for pneumonia

[a] Countries were categorised by 1) mortality strata according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30- <75 per 1,000 live births), c) high (75 - <150 per 1,000 live births), d) very high (> 150 per 1,000 live births) and 2) African Union sub-regions used– central, east, north, southern and west.

[b] A neighbouring country is one that shares a border with the country of interest.

Country-specific data were used when available, and values were imputed for countries without information using estimates (or pooled estimates) from the countries in the same

under-5 mortality stratum or from that of a neighbouring country with data (see the algorithm in Figure 2.1). Incidence rates adjusted for the proportion seeking healthcare[51,241] were used whenever available. This was done to account for the under-estimated incidences reported by other studies because some children would not have been taken for a health facility visit.

Non-pneumonia non-meningitis non-septicaemia disease (NPNMNS) cases

Disease burden due to non-pneumonia non-meningitis non-septicaemia clinical syndromes such as endocarditis, osteomyelitis, pericarditis, peritonitis, pyomyositis, scalded skin syndrome, septic arthritis, toxic shock syndrome, and urinary tract infections could not be obtained because there were no incidence data on these diseases and no prospective study reported on the relative proportion of septicaemia to NPNMNS disease among children in Africa.

Number of deaths by clinical syndrome

Pneumonia deaths

Due to the scarcity of data on *S. aureus* pneumonia CFR, the number of *S. aureus* pneumonia deaths was calculated by multiplying the WHO estimates for all-cause pneumonia deaths in children aged 0 – 59 months[3] by the aetiologic fraction of bacteraemic pneumonia. This approach assumes that the proportion of pneumonia cases due to *S. aureus* was similar to the proportion of deaths due to *S. aureus* and that the CFR for *S. aureus* pneumonia is the same as pneumonia caused by other pathogens. The CFR for *S. aureus* pneumonia was taken from independent estimates of deaths and cases.

Meningitis deaths

None of the studies reported on the CFR for *S. aureus* meningitis cases; therefore, the number of *S. aureus* meningitis deaths was calculated by multiplying the estimates of the number of *S. aureus* meningitis cases by the all-cause bacterial meningitis CFR. For cases without access to healthcare, a CFR of 90% was used. The proportion of children under the age of five years with pneumonia in the preceding two weeks and had access to health care obtained from UNICEF[237] was used as a proxy for the proportion of meningitis cases who had access to health care and received treatment. For countries without data, the coverage for the third dose of Diphtheria-Pertussis-Tetanus vaccine for the country also obtained from UNICEF,[237] was used.

Septicaemia deaths

The number of deaths due to *S. aureus* septicaemia was calculated by multiplying the number of cases by the country-specific CFR from the systematic review (observed or imputed).

$$No.S.aureus\ septiceamic\ deaths = S.aureus\ septicaemic\ CFR \times No.S.aureus\ septicaemic\ cases$$

Equation 4)

Inferring the rate (incidence or deaths) in children < 5 years from the rate in children < 2 year

Although, the majority of studies reported incidence in children less than five years of age, some reported incidence for those less than two years of age. For these studies, incidence estimates for children less than five years were derived by using the formula:

$$Rate_{<5} = \frac{Rate_{<2}}{P_{<2}} \times (\%U5Pop_{<2})$$

Equation 5)

where $Rate_{<5}$ and $Rate_{<2}$ represent estimates of incidence in children less than 5 years and less than two years respectively; $P_{<2}$ is the proportion of all cases in children less than two years (89.9%)[242] and $\%U5Pop_{<2}$ is the proportion of under-five population that are less than two years of age (50.7%).[243]

Sensitivity analysis

Sensitivity analyses were performed to assess the difference between using aetiologic fractions from hospital-based compared to population-based studies.

For bacteraemic pneumonia, aetiologic fractions derived from hospital-based studies were applied to the country-specific number of hospitalised cases of all-cause severe pneumonia modelled by McAllister and colleagues.[240] For meningitis, aetiologic fractions derived from hospital-based studies were applied to the country-specific number of cases of all-cause bacterial meningitis.

For septicaemia, the number of *S. aureus* septicaemia cases was calculated by applying country-specific population size estimate (number of live births) to the incidence of all-cause septicaemia (neonatal septicaemia) and the aetiologic fractions derived from hospital-based studies. (Equation 1).

The number of *S. aureus* deaths for each of the clinical syndrome was determined as described above.

Sub-regional population estimates were sums of the country-specific figures.

Uncertainty ranges

The data used for the derivation of the estimates were characterised by substantial uncertainty. However, there were few studies which threaten the precision of the estimates of uncertainty ranges; therefore, this step was omitted.

Data cleaning and the algorithm in Figure 2.1 were implemented in a Microsoft Excel Spreadsheet (Version 16.5).

2.4 Results of the systematic review

2.4.1 Summary of included studies

The search yielded 90,524 titles which were narrowed down to 55,759 after excluding duplicates. Most of the further exclusions (54,213) were because the titles were unrelated or, to a lesser extent, because the full text was not found (Figure 2.3). Of the full articles retrieved, 345 were excluded. Reasons for exclusion included retrospective studies (70), isolates not disaggregated by samples or age (39), methodological issues (35), study participants less than 50 cases (29), hospital-acquired diseases (16) and 13 studies which did not differentiate between community-acquired and hospital-acquired diseases. Articles on the incidence of pneumonia, early neonatal septicaemia and the aetiologic fraction of urinary tract infection were also excluded. Figure 2.3 shows the full screening outcome. Thirty-nine studies from four of the five African regions and eleven countries were included in the quantitative synthesis. There was one study from Central Africa which did not disaggregate data by age and so was excluded.[244] A third of the studies (12/39; 30.8%) were from Nigeria and four studies from Kenya (Table 2.2). The majority of the included studies were hospital-based (26/39; 66.7%), one was in a randomised controlled trial setting (1/39;

2.6%), and one a case-control study (1/39; 2.6%). The population-based studies were from Burkina Faso and Togo (1),[245] The Gambia (1),[242] Ghana (1),[51] Kenya (3),[6,46,246] Mozambique (3),[45,60,247] Nigeria (1),[54] and Tanzania (1).[241] The articles had information on at least one of the following estimates: incidence, aetiologic fraction, case fatality ratio, and neonatal septicaemia incidence and aetiologic fraction. Nine articles reported on two estimates,[6,46,49,53,241,248-252] four on three[45,51,54,60] and one article on six estimates.[242] All the studies were prospective. Tables 2.1 and 2.2 summarise the studies included.

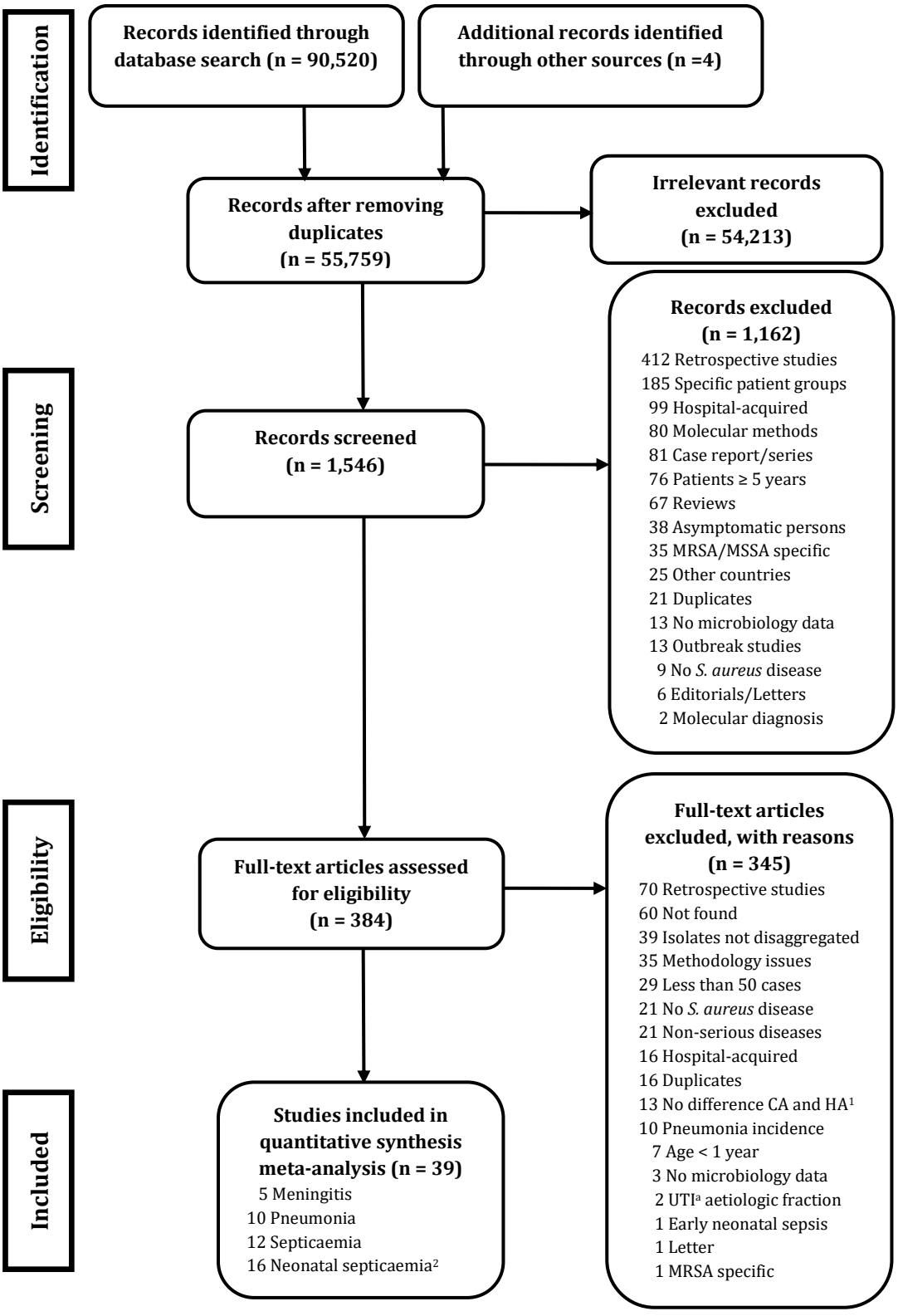


Figure 2.2: Flow chart of the study selection process

[1] CA=Community-acquired; HA=Hospital-acquired; UTI=urinary tract infection

[2] Four articles reported on both septicaemia and neonatal septicaemia incidence/aetiologic fraction estimates

2.4.2 Risk of bias

Thirty-five studies were considered poor quality based on selection bias (21/35; 60.0%), no or vague clinical definitions (9/35; 25.7%) or issues with case ascertainment (5/35; 14.3%) and were excluded from the analysis. Of the included studies, 100% stated pre-defined eligibility criteria, while 95% reported investigating over 80% of eligible cases (Figure 2.4).

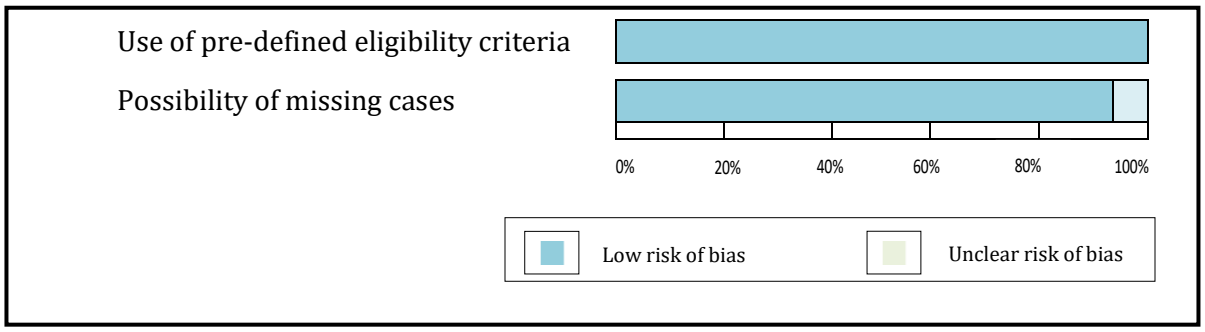


Figure 2.3: Risk of bias graph of included studies

S. aureus disease articles

Although there was a large body of literature on *S. aureus* disease in Africa, only 1.3% (11/869) of the studies were prospective and population-based among children under the age of five years. Of the full articles assessed for eligibility, 20.3% (70/345) were retrospective (Figure 2.3).

Pneumonia

Two population-based (one from Kenya[246] and the other from Mozambique[247]) and four hospital-based studies (two each from Nigeria[54,56] and Gambia[53,251]) provided information on the aetiologic fraction of bacteraemic and non-bacteraemic pneumonia, respectively (Table 2.2). Four hospital-based studies reported on the hospital-based aetiologic fraction of bacteraemic pneumonia (Supplementary Table 2.1).[253-256] All these studies except one were from mortality stratum 3.

Meningitis

Four prospective studies – two population-based[60,252] and two hospital-based[161,257] - reported on the aetiologic fraction of *S. aureus* meningitis and the incidence and CFR of all-cause meningitis (Table 2.2 and Supplementary Table 2.1). Three studies were from mortality stratum 3 and one from mortality stratum 2.

Septicaemia

Five population-based studies reported on the incidence of all-cause septicaemia and *S. aureus* septicaemia – two from Kenya,[6,46] and one each from The Gambia,[242] Ghana,[51] and Mozambique (Table 2.1).[45] Two of these articles also provided information on the incidence and aetiology fraction of neonatal septicaemia.[6,242] Two other studies reported on the incidence of all-cause septicaemia alone.[8,241] In hospital-based settings, five studies from three countries[23,258-261] and ten studies from three countries[48,49,250,262-268] provided data on the aetiologic fraction of *S. aureus* septicaemia among children less than five years of age and neonates, respectively (Supplemental Table 2.1). Information on CFR of septicaemia cases among under-5 children[45,242] and neonates[242 49,248-250] were obtained from two and five studies, respectively.

The forest plots are shown in Appendix 3.

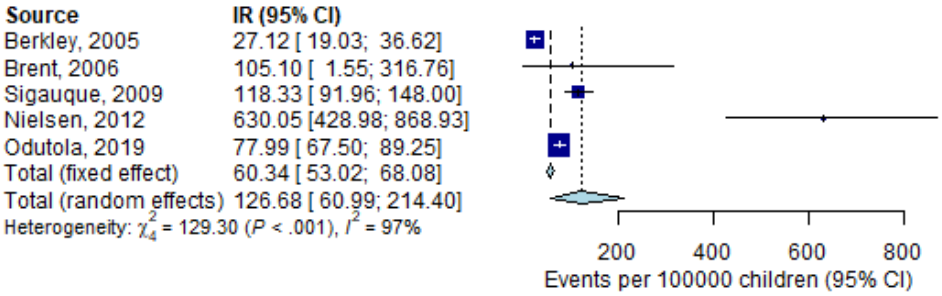


Figure 2.4: Random-effects pooled incidence of community-acquired *S. aureus* bacteraemia in population-based studies in mortality strata 3 in Africa

Table 2.1: Number of studies by country, mortality stratum and region (population-based studies)

Parameter	Country	Region	Mortality Stratum	No of studies	Effect size (95%CI)
Pneumonia					%
Bacteraemic aetiologic fraction	Pooled[1]		3	2	1.6 (0.9 – 2.4)
	Kenya	East	3	1	1.7 (0.9 – 2.8)
	Mozambique	South	3	1	1.5 (0.7 – 2.8)
Non-bacteraemia aetiologic fraction	Pooled		3	4	25.0 (4.0 – 46.0)
	Gambia	West	3	2	5.0 (1.0 – 10.0)
	Nigeria	West	3	2	33.0 (26.0 – 40.0)
Meningitis					per 100,000 person-years
All-cause incidence[2]	Pooled		3	3	85.1 (69.2 – 102.6)
	Mozambique	South	3	1	171.9 (110.4 – 246.3)
	Mali	West	3	1	76.9 (69.0 – 85.4)
	Togo/Burkina Faso	West	3	1	79.8 (73.0 – 87.0)
Aetiologic fraction	Mozambique	South	3	1	16.0 (5.0 – 36.0)
All-cause CFR[3]	Mozambique	South	3	1	24.0 (11.0 – 42.0)
Septicaemia					per 100,000 person-years
Incidence[2]	Pooled		3	5	126.7 (61.0 – 214.4)
	Gambia	West	3	1	78.0 (67.5 – 89.3)
	Ghana	West	3	1	630.1 (430.0 – 868.9)
	Kenya	East	3	2	44.8 (12.4 – 161.7)
	Mozambique	South	3	1	118.3 (92.0 – 148.0)
CFR	Pooled		3	3	9.0 (6.0 – 12.0)
	Gambia	West	3	2	14.0 (10.0 – 20.0)
	Mozambique	South	3	1	6.0 (3.0 – 11.0)
Neonatal septicaemia					per 1,000 live births
Incidence[4]	Pooled		3	2	1.3 (0.2 – 9.6)
	Gambia	West	3	1	3.5 (2.9 – 4.7)
	Kenya	East	3	1	0.4 (0.2 – 0.8)
CFR	Pooled		3	5	21.0 (14.0 – 26.0)
	Gambia	West	3	1	18.0 (10.0 – 28.0)
	Nigeria	West	3	4	23.0 (14.0 – 32.0)
	Tanzania	East	2	1	33.3 (4.0 – 78.0)

[1] Summary estimate of the meta-analysis of the countries in the same stratum

[2] Incidence in cases per 100,000 person-years

[3] CFR=Case fatality ratio

[4] Incidence in cases per 1,000 live births

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980 - 2019)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Pneumonia	Adedoyin 1987[56]	Ilorin, Nigeria 1985-1986	West	3	Hospital-based cross-sectional study	Referral teaching hospital	< 5 years	Clinical and radiologic pneumonia	Clinical & radiologic tuberculosis	Lung aspirate	2012	2014	22/95 (23.2%)[3]
	Forgie 1991[251]	Banjul, The Gambia 1986 - 1988	West	3	Hospital-based cross-sectional study	Secondary health facility	1 – 4 years	Cough, increased respiratory rate, indrawing	NR[4]	Blood Lung aspirate	1997	2009	1/64 (1.6%)[5] 3/29 (10.3%)[3]
	Adegbola 1994[53]	Banjul, The Gambia 1990-1992	West	3	Hospital-based cross-sectional study	Secondary health facility	3 months – 5 years	Clinical and radiologic pneumonia and	On malnutrition/ tuberculosis treatment	Blood Lung aspirate	1997	2009	1/ 119 (0.8%)[5] 2/119 (1.7%)[3]
	Fagbule 1994[54]	Ilorin, Nigeria 1988 – 1989	West	3	Population-based and hospital-based cross-sectional study	Referral teaching hospital	2 months – 5 years	Cough, increased respiratory rate, indrawing	Antibiotic use preceding two days	Blood Lung aspirate	2012	2014	25/73 (34.2%)[5] 33/73 (45.2%)[3] 0/35 (0.0%)[7]
	Johnson 2008[255]	Ibadan, Nigeria	West	3	Hospital-based cross-sectional study	Referral teaching hospital	2 weeks – 59 months	Clinical and radiologic pneumonia	NR[4]	Blood	2012	2014	22/205 (10.7%)[5]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2]Year of *Haemophilus influenzae* type b vaccine (Hib) and pneumococcal conjugate vaccine (PCV) introduction (*source:* http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3Jp ZD01NSZvdm *VybGF5a WQ9NA==*)

[3]Aetiolofic fraction for non-bacteraemic pneumonia

[4]NR=Not Reported

[5]Aetiologic fraction for bacteraemic pneumonia

[6]Case fatality ratio

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Pneumonia	Nantanda 2008[253]	Mulago, Uganda 2005 – 2006	East	3	Hospital-based cross-sectional study	District referral hospital	2 – 59 months	Cough, increased respiratory rate, indrawing	NR	Blood	2002	2013	9/157 (5.7%)[5]
	Siguaque 2009[247]	Manhica, Mozambique 2004-2006	South	3	Hospital-based study	Rural district referral hospital	< 5 years	Cough, increased respiratory rate, indrawing	NR	Blood	2009	2013	9/613 (1.5%)[5]
	El-Mdaghri 2012[256]	Casablanca, Morocco 2007 - 2008	North	2	Hospital-based study	Referral teaching hospital	< 5 years	WHO definition of CXR-confirmed pneumonia	NR	Blood	NR	2010	1/76 (1.3%)[7]
	Breiman 2015[246]	Gatwikera and Soweto West, Kenya 2007 - 2010	East	3	Population- and hospital-based study	Community healthcare centre	< 5 years	Cough, difficult breathing, indrawing, danger signs	NR	Blood	2001	2011	14/836 (1.7%)[5]
	Benet 2015[254]	Bamako, Mali 2011 – 2012	West	3	Case-control study	Referral teaching hospital	2 – 59 months	Cough, increased respiratory rate, radiologic pneumonia	Presence of wheeze	Blood	2007	2011	1/118 (0.9%)[5]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2]Year of *Haemophilus influenzae* type b vaccine (Hib) and pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3JpZD01NSZvdmVybGF 5aWQ9NA==)*

[4]NR=Not Reported; CSF=Cerebrospinal fluid

[5]Aetiologic fraction for bacteraemic pneumonia

[6]Case fatality ratio

[7]Aetiologic fraction

[8]Incidence

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Meningitis	Sow 2005[252]	Bamako, Mali 2002 - 2004	West	3	Population-based surveillance	Referral teaching hospital	< 5 years	Clinically suspected meningitis	Nil	CSF	2007	2011	77/100,000 person-years[8]
	Lagunju 2009[161]	Ibadan, Nigeria 2004 – 2007	West	3	Hospital-based cross-sectional study	Referral teaching hospital	2 – 59 months	Clinically suspected meningitis	NR	CSF	2012	2014	2/27 (7.4%)[7]
	Roca 2009[60]	Manhica, Mozambique 2006	South	3	Population-based surveillance	District referral hospital	< 5 years	Clinically suspected meningitis	No	CSF	2009	2013	172/100,000 person-years[8] 4/25 (16.0%)[7] 8/33 (24.2)[6*]
	Traore 2009[245]	Bobo-Dioulasso, Burkina Faso 2002–2005	West	3	Community- and hospital-based surveillance	Regional referral hospital	< 5 years	Clinically suspected meningitis	NR	CSF	NR	NR	80/100,000 person-years[4]
		Sotouboua, Togo 2003 – 2006	West	3		Regional referral hospital	< 5 years	Clinically suspected meningitis	NR	CSF	NR	NR	190/650 (29.2) [6*]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2] Year of *Haemophilus influenzae* type b vaccine (Hib) and Pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3JpZD01NSZvdmVybGF5aWQ9NA==)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation; IPSC= International Paediatric Sepsis Consensus

[6] Case fatality ratio

[7] Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Meningitis	Ba 2010[257]	Dakar, Senegal 2002 - 2006	West	2	Hospital-based cross-sectional study	National Hospital	< 5 years	Clinically suspected meningitis	NR	CSF	2005	2013	5/139 (3.6%)
Septicaemia (< 5 years)	Akpede 1993[269]	Benin, Nigeria 1988 - 1989	West	3	Hospital-based cross-sectional study	Referral teaching hospital	1 – 59 months	Fever (≥ 38.0°C)	Localising signs	Blood	2012	2014	28/68 (41.2%)[7]
	Brent 2006[46]	Kilifi, Kenya 2003	East	3	Population-based study	District referral hospital	< 5 years	All in- and out-patients	NR	Blood	2001	2011	105/100,000 person-years[8] 2/1,093 (0.2%)[7]
	Berkley 2005[6]	Kilifi, Kenya 1998 - 2002	East	3	Population-based study	District referral hospital	< 5 years	All admitted patients	Elective surgery or observation for minor accidents	Blood	2001	2011	27/100,000 person-years[8] 0.44/1,000 live births[8]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2]Year of *Haemophilus influenzae* type b vaccine (Hib) and pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3Jp ZD01NSZvdm VybGF5a WQ9NA==)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation

[6] Case fatality ratio

[7]Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Septicaemia (< 5 years)	Enwere 2006[8]	Basse, Gambia 2000 – 2004	West	3	Randomised controlled trial	Rural primary health care clinic	2 – 29 months	Signs suggestive of pneumonia, meningitis, musculoskeletal tenderness/swelling, fever 38°C	NR	Blood	1997	2009	1009/100,000 person-years[8]
	Sigauque 2009[45]	Manhica, Mozambique 2001-2006	South	3	Population-based study	District referral hospital	< 5 years	All in- and out-patients	NR	Blood	2009	2013[10]	118/100,000 person-years[8] 180/18,372 (1.0%)[7] 60/952 (6.3%)[9]
	Onipede 2009[23]	Ile-Ife, Nigeria 2005 - 2006	West	3	Hospital-based cross-sectional study	Referral teaching hospital	2 – 60 months	All admitted patients with signs of systemic infections. Fever – temp[4] < 36°C or ≥38°C	Neonates; children with no features suggestive of systemic infections	Blood	2012	2014	26/55 (47.7%)[7]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2] Year of *Haemophilus influenzae* type b vaccine (Hib) and Pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b 3JpZD01NSZvdmVybGF5aWQ)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation

[6] Case fatality ratio

[7] Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Septicaemia (< 5 years)	Nielsen 2012[51]	Agogo, Ghana 2007 - 2009	West	3	Population-based study	District referral hospital	< 5 years	All admitted patients	Dermatological or surgical conditions	Blood	2001	2012	630/100,000 person-years[8] 32/1196 (2.7%)[7]
	Thriemer 2012[241]	Pemba, Tanzania 2009-2010	East	2	Population-based study	District Referral hospital	< 5 years	Admitted patients, history of fever, tympanic temp[4] ≥37.5°C	NR	Blood	2009	2012	146/100,000 person-years[8] 2/637 (0.3%)[7]
	Isendahl 2014[260]	Bissau Guinea Bissau 2010	West	4	Hospital-based cross-sectional study	National Referral Hospital	< 5 years	Fever (axillary temp[4] ≥38.0°C) ± tachycardia	NR	Blood	2008	2015	26/48 (54.2%)[7]
	Kibuuka 2015[259]	Jinja, Uganda 2012	East	3	Hospital-based cross-sectional study	Regional Referral Hospital	6 – 60 months	Fever (axillary temp[4] > 37.5°C) or history of fever	Antibiotic use preceding 5 days	Blood	2002	2013	19/45 (42.2%)[7]
	Onubogu 2015[261]	Port Harcourt, Nigeria 2015	West	3	Hospital-based cross-sectional study	Referral teaching hospital	1 - 59 months	Axillary temp[4] ≥37.5°C	Antibiotic use preceding 3 days	Blood	2012	2014	18/32 (56.3%)[7]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2] Year of *Haemophilus influenzae* type b vaccine (Hib) and Pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3JpZD01NSZvdm VybGF5aWQ)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation

[6] Case fatality ratio

[7] Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Septicaemia (< 5 years)	Odutola 2019[242]	Basse, The Gambia 2008 - 2015	West	3	Population-based study	Rural primary health care clinic	< 5 years	Signs and symptoms suggestive of pneumonia, meningitis, septicaemia	NR	Blood	1997	2009	78/100,000 person-years[8] 3.5/1,000 live births[8] 198/18,372 (0.8%)[7] 28/198 (14.1%)[6] 84/2092 (4.0%)[9] 13/84 (15.5%)[6]
Neonatal Septicaemia	Owa 1998[263]	Ile-Ife, Nigeria 1986	West	3	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Presence of risk factors ± signs of sepsis	NR	Blood	2012	2014	8/31 (25.8%)[9]
	Antia-Obong 1992[248]	Calabar, Nigeria 1989	West	3	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Maternal risk factors, temp[4] ≤ 35.0^{o}C or ≥ 38.0^{o}C	NR	Blood	2012	2014	15/79 (19.0%)[8] 3/15 (20.0%)[7]
	Klingenberg 2003[249]	Moshi, Tanzania 1998 - 1999	East	2	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Presence of risk factors ± signs of sepsis	NR[4]	Blood	2009	2012	6/16 (37.5%)[8] 2/6 (33.3%)[6]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2] Year of *Haemophilus influenzae* type b vaccine (Hib) and Pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3Jp ZD01NSZvdm VybGF5a WQ9NA==)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation; IPSC= International Paediatric Sepsis Consensus

[6] Case fatality ratio

[7] Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Neonatal Septicaemia	Ojukwu 2006[250]	Enugu, Nigeria 2002- 2003	West	3	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Presence of risk factors ± signs of sepsis	NR	Blood	2012	2014	15/33 (45.5%)[9] 4/15 (26.7%)[6]
	Mugalu 2006[268]	Kampala Uganda 2002	East	3	Hospital-based cross-sectional study	National referral hospital	Neonates	Clinical sepsis by WHO[2] classification	Antibiotic use preceding 3 days	Blood	2002	2013	69/110 (62.7%)[9]
	Kayange 2010[264]	Bugando, Tanzania 2009	East	2	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Clinical sepsis by WHO[4] classification	NR[4]	Blood	2009	2012	32/149 (21.5%)[9]
	Mhada 2012[48]	Muhimbili, Tanzania 2009 - 2010	East	2	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Clinical diagnosis of septicaemia	NR	Blood	2009	2012	27/74 (36.5%)[9]
	Kiwanuka 2013[266]	Mbarara, Uganda 2010	East	3	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Presumed sepsis	Local infections other than sepsis Congenital malformations Birth trauma	Blood	2002	2013	16/26 (44.4%)[9]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2] Year of *Haemophilus influenzae* type b vaccine (Hib) and Pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3Jp ZD01NSZvdm VybGF5aWQ9NA==)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation; IPSC= International Paediatric Sepsis Consensus

[6] Case fatality ratio

[7] Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

Table 2.2: Characteristics of the included studies with data on *S. aureus* pneumonia, meningitis, and septicaemia in Africa (1980-2019) (continued)

Clinical Syndrome	Author/ Year	Location/ Study dates	Region	MS[1]	Study design	Hospital Type	Age population	Inclusion criteria	Exclusion criteria	Sample	Hib[2] vaccine	PCV[2]	Incidence/ AF[7]
Neonatal Septicaemia	West 2014	Port Harcourt, Nigeria 2007	West	3	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Presence of risk factors ± signs of sepsis	No prior antibiotic use	Blood	2012	2014	34/169 (20.0%)[9]
	Mkony 2014	Muhimbili, Tanzania 2012 – 2013	East	2	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Clinical sepsis by WHO[4] classification	Very sick neonates Severe congenital malformations	Blood	2009	2012	4/40 (10.0%)[9]
	John 2015[267]	Kidera, Uganda 2013	East	3	Hospital-based cross-sectional study	Level IV health facility	Neonates	Clinical sepsis by IPSC[4] criteria	Very sick neonates	Blood	2002	2013	12/38 (31.6%)[8]
	Ogundare 2016[49]	Ilesa, Nigeria 2008 – 2009	West	3	Hospital-based cross-sectional study	Referral teaching hospital	Neonates	Presence of risk factors ± signs of sepsis	Congenital heart diseases and metabolic disorders	Blood	2012	2014	53/75 (70.7%)[9] 9/53 (17.0%)[6]

[1] MS=Mortality Stratum; Countries were stratified according to the under-5 mortality rates into a) low (< 30 per 1,000 live births), b) medium (30 - < 75 per 1,000 live births), c) high (75 - < 150 per 1,000 live births), d) very high (> 150 per 1,000 live births)

[2] Year of *Haemophilus influenzae* type b vaccine (Hib) and Pneumococcal conjugate vaccine (PCV) introduction (*source: http://view-hub.org/viz/?YXBwaWQ9MSZpbmRpY2F0b3 JpZD01NSZvdmVybGF5aWQ9NA==)*

[4] NR=Not Reported; BW=birth weight; temp=temperature; WHO=World Health Organisation; IPSC= International Paediatric Sepsis Consensus

[6] Case fatality ratio

[7] Aetiologic fraction

[8] Incidence

[9] Neonatal aetiologic fraction

There was considerable heterogeneity in all the meta-analyses both by mortality stratum and country (I^2 range 0% - 99%) (Figure 2.5 and Appendix 3).

2.4.3 Antimicrobial resistance (AMR)

Eighteen studies reported on AMR of *S. aureus* isolates, but data from two studies that did not disaggregate results by either age[45] or clinical syndrome[250] were not utilised. All the studies used the disc diffusion laboratory method to determine antibiotic resistance. Eight studies each were from East and West Africa, and eight studies were conducted among neonates. All isolates were from blood samples of patients with community-acquired infections. Most of the tested isolates (n=364; 69.5%) were from West Africa.

Pre-treatment antibiotic use

Of the included studies, only 18 (41.9%) reported on antibiotic use before sampling. Four studies excluded patients who had received antibiotics before presentation,[53,259,261,268] while eight studies reported that between 5.6% and 67.0% of the study participants had used antibiotics before sampling.[6,23,51,56,60,161,251,254] The commonly used antibiotics before presentation were amoxicillin, cefuroxime, cloxacillin, cotrimoxazole, erythromycin, and flucloxacillin.[51,254]

Antimicrobial resistance

Oxacillin (n=225)[241, 23,242,249] susceptibility was reported by four studies. Of these isolates, 4.9% were resistant to oxacillin.,[241] The prevalence of MRSA (based on oxacillin testing) was 0% in East Africa[241,249] and 5.1% in West Africa.[23,242] One study reported the prevalence of

Table 2.3: Summary of antimicrobial resistance patterns in East and West Africa

Antibiotic	East Africa Number of resistant isolates/numbers tested (%)	Pooled prevalence[1]	West Africa Number of resistant isolates/numbers tested (%)	Pooled prevalence
Amoxicillin	56/69 (81)[268]	-	30/32 (95),[262] 39/53 (74)[49]	87.0 (80.0 – 94.0)
Ampicillin	1/69 (88),[268] 23/27 (85)[48]	88.0 (81.0 – 94.0)	3/8 (38),[263] 28/32 (88),[262] 12/20 (60)[23]	68.0 (53.0 – 84.0)
Amoxicillin-clavulanic acid	NR[2]	-	6/21 (29),[23] 8/53 (15)[49]	18.0 (9.0 – 26.0)
Cefotaxime	NR		0/26 (0),[260] 10/32 (31)[262]	-
Cefoxitin	NR	-	6/193 (3)[242]	-
Ceftriaxone	4/27 (15)[48]	-	1/8 (13),[23] 9/53 (17)[49]	20.0 (11.0 – 30.0)
Cefuroxime	4/27(15)[48]	-	8/32 (56),[262] (4/53 (8)[49]	14.0 (7.0 – 20.0)
Chloramphenicol	1/5 (20), 5/12 (42),[267] 51/69 (74),[268] 6/9 (67)[253]	64.0 (44.0 – 83.0)	0/26 (0),[260] 0/8 (0),[263] 29/31 (94),[51] 8/186 (4)[242]	44.0 (0.0 – 100.0)
Ciprofloxacin	5/32(16),[264]	-	0/26(0),[260] 3/32(9),[262] 10/31 (32),[51] 1/5 (20),[23] 7/53 (13)[49]	26.0 (9.0 – 44.0)
Clindamycin	14/32 (44)[264]	-	0/26 (0)[260]	-
Cloxacillin	9/32 (28), 22/27(82)[48] 0/12 (0),[267] 24/69 (35)[268]	48.0 (17.0 – 79.0)	0/26 (0),[260] 1/8 (13),[263] 28/32 (88),[262] 5/30 (17),[51] 23/25 (92)[270]	56.0 (25.0 – 86.0)
Cotrimoxazole	2/5 (40),[241] 19/32 (59)[264]	57.0 (41.0 – 73.0)	1/6 (17),[23], 0/26 (0),[260], 2/8 (25),[263] 11/24 (46),[51] 40/180 (22)[242]	30.0 (14.0 – 46.0)
Erythromycin	1/5 (20),[241] 21/32 (66),[264] 2/9 (22)[253]	54.0 (40.0 – 68.0)	0/26 (0),[260] 0/8 (0),[263] 10/31 (32),[51] 8/21 (38),[23] 21/53 (40),[49] 8/173 (5)[242]	28.0 (5.0 – 50.0)
Gentamicin	3/69 (4),[268] 15/27 (56),[48] 3/9 (33.3),[253] 0/6 (0)[249], 0/9 (0),[271] 0/12 (0)[267]	30.0 (7.0 – 68.0)	3/8 (38),[23] 0/26 (0),[260] 0/8 (0),[263] 19/32 (59),[262] 9/31 (29),[51] 29/53 (55),[49] 3/177 (2)[242]	37.0 (1.0 – 63.0)
Oxacillin	0/5 (0),[241] 0/6 (0),[249]	-	11/22 (50),[23] 0/194 (0)[242]	
Penicillin	4/5 (80),[241] 11/69 (16),[268] 29/32 (91),[264] 12/12 (100),[267]	87.0 (80.0 – 93.0)	6/8 (75),[263]16/31 (52),[51]	58.0 (42.0 – 73.0)

[1] Meta-analysis was not done if ≤ 1 study reported testing the antibiotic or if all studies reported no resistance to the antibiotic; [2] NR=Not Reported

MRSA using cefoxitin as a surrogate (3.1%).[242] The use of cefoxitin as a surrogate for MRSA is preferred because oxacillin disc testing is not reliable.[272] There was a higher resistance rate of *S. aureus* to ampicillin in East Africa compared to West Africa (88.0% versus 68.1%) as well as to penicillin (87.0% versus 58.0%) (Table 2.4). The resistance rate of *S. aureus* to amoxicillin was similar in the two regions (81.0% – 87.0%). *S. aureus* was still relatively susceptible to cloxacillin (48.0% - 56.0%), chloramphenicol (44.0% - 64.0%), erythromycin (28.0% – 54.0%), and gentamicin (30.0% – 37.0%) in East and West Africa (Table 2.3). There were no AMR data from Central or North Africa.

2.5 Disease burden results

2.5.1 The population size of African children

There were an estimated 186,884,000 children aged less than five years on the African continent in 2015, 31.7% of them in the West, 13.3% in Southern, 12.2% in the North, 13.0% in the Central and 29.8% in the East sub-region (Table 2.4). The country with the least population was Seychelles (n=8000; 0.004%) and that with the largest was Nigeria (n=31,109,000; 16.7%) (See Appendix 4). The proportion of countries in mortality stratum 1 or 2 by region was 22.2%, 42.9%, 85.7%, 40.0% and 13.3% for Central, East, North, Southern, and West, respectively.

2.5.2 Staphylococcal cases

In 2015, an estimated 392,066 cases of staphylococcal infection occurred among children aged less than five years in Africa (Table 2.4). Six countries (two from the West - Nigeria (17.3%) and Ghana (5.4%); one from Central Africa - Democratic Republic of Congo (7.6%),

two from the East – Ethiopia (7.7%) and Tanzania (4.9%) and one from the North - Egypt (7.1%)) accounted for 50.0% of cases staphylococcal diseases in Africa. Country-specific incidence varied widely from 124 cases per 100,000 children per annum in Kenya to 693 cases per 100,000 children in Ghana (Figure 2.6, Appendix 5). West Africa had the highest incidence and the largest number of cases, Nigeria being the main contributor.

In the sensitivity analysis, where data from hospital-based studies were used to derive the aetiologic fraction of each of the three clinical syndromes, the estimated number of cases was 1,032,218, with an incidence of 552 cases per 100,000 children (Table 2.5). Using aetiologic fraction data from the hospital-based studies, the total estimated number of cases was higher than from population-based studies.

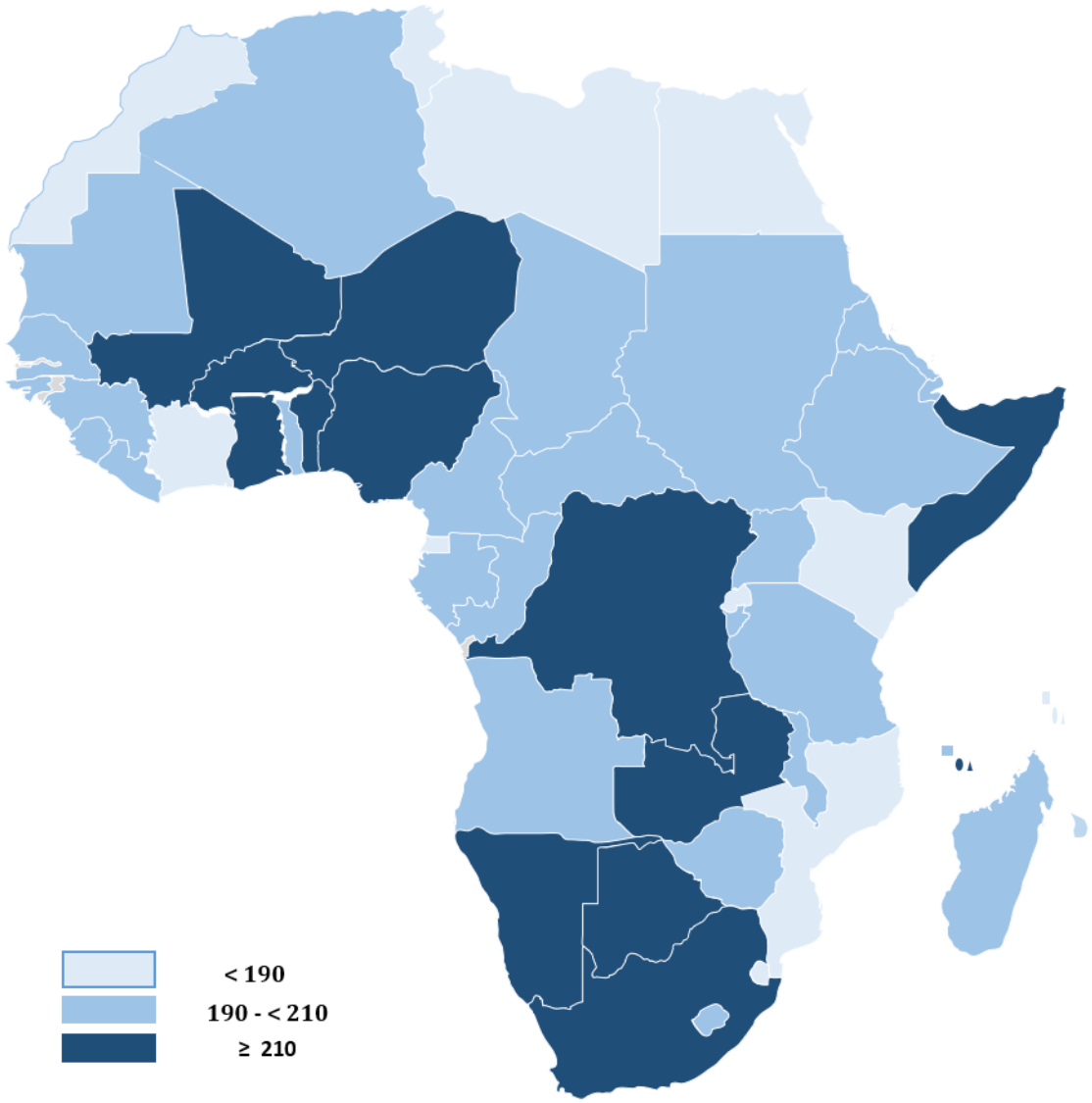


Figure 2.5: Incidence of staphylococcal infections in children aged less than five years in Africa (per 100,000 children)

Table 2.4: Estimated numbers of staphylococcal cases and deaths by sub-region (using population-based studies)[1]

	Africa	Central	East	North	Southern	West
Total						
Total deaths among children < 5 years in 2015 = 2,907,444[273]						
Children aged < 5 years	186,884,000	24,311,000	55,598,000	22,844,000	24,858,000	59,273,000
Incidence[2]	210	204	189	180	206	244
No. cases	392,066	50,179	104,911	40,994	51,313	144,669
Mortality rate[3]	25	26	22	19	26	29
Total no. deaths	46,467	6,297	12,098	4,425	6,559	17,087
Bacteraemic pneumonia						
Incidence[2]	61	66	59	39	57	70
No. cases	113,368	16,066	32,629	8,940	14,234	41,498
CFR[4]	7	9	6	3	15	8
Mortality rate[3]	4	6	4	1	4	6
No. deaths	8,083	1,402	1,917	252	1,065	3,430
Meningitis						
Incidence[2]	15	14	14	14	24	14
No. cases	27,973	3,310	7,570	3,110	5,990	7,992
CFR[4]	57	64	58	50	48	64
Mortality rate[3]	9	9	8	7	12	9
No. deaths	15,977	2,120	4,357	1,567	2,861	5,071

[1] Aetiologic fractions used to derive the number of cases were pooled estimates from population-based studies

[2] Incidence rates of pneumonia, meningitis and septicaemia are per 100,000 children, neonatal septicaemia per 1,000 live births in 2015

[3] Mortality rate=proportion of the under-five children (total live births) who died of *S. aureus* disease per 100,000 in 2015

[4] CFR=Case fatality ratio (%)

Table 2.4: Estimated numbers of staphylococcal cases and deaths by region (using population-based studies)[1] (continued)

	Africa	Central	East	North	Southern	West
Septicaemia						
Incidence[2]	134	127	116	127	125	161
No. cases	250,725	30,802	64,712	28,943	31,088	95,179
CFR[4]	9	9	9	9	9	9
Mortality rate[3]	12	11	11	11	11	15
No. deaths	22,407	2,772	5,824	2,605	2,626	8,580
Non-bacteraemic pneumonia						
Incidence[2]	948	1,033	909	612	913	1094
No. cases	1,771,730	251,036	505,619	139,691	226,981	648,405
CFR[4]	7	8	6	3	6	9
Mortality rate[3]	68	81	57	14	54	99
No. deaths	126,908	19,774	31,400	3,517	13,467	58,652
Neonatal Septicaemia						
Total neonatal deaths in 2015=1,112,623 [273]						
Live births	41,521,718	5,377,820	12,145,000	5,066,898	5,401,000	13,531,000
Incidence[2]	1.2	1.3	1.2	1.6	1.3	1.3
No. cases	51,215	6,776	14,014	6,384	6,805	17,235
CFR[4]	26	21	28	33	25	23
Mortality rate[3]	32	27	33	42	32	29
No. deaths	13,091	1,432	3,970	2,105	1,705	3,880

[1] Aetiologic fractions used to derive the number of cases were pooled estimates from population-based studies

[2] Incidence rates of pneumonia, meningitis and septicaemia are per 100,000 children, neonatal septicaemia per 1,000 live births in 2015

[3] Mortality rate=proportion of the under-five children population (total live births) who died of *S. aureus* disease per 100,000 in 2015

[4] CFR=Case fatality ratio (%)

Table 2.5: Estimated numbers of staphylococcal cases and deaths by region (sensitivity analysis - using hospital-based studies)[1]

	Africa	Central	East	North	Southern	West
Total						
Total deaths among children < 5 years in 2015 = 2,907,444 [273]						
Children aged < 5 years [235]	186,884,000	24,311,000	55,598,000	22,844,000	24,858,000	59,273,000
Incidence[2]	552	692	336	119	496	883
No. cases	1,032,218	168,295	186,981	27,062	123,360	523,519
Mortality rate[3]	54	69	34	15	47	86
Total no. deaths	101,105	16,781	18,783	3,336	11,557	50,648
Bacteraemic pneumonia						
Incidence[2]	210	246	120	36	176	360
No. cases	392,069	59,755	66,818	8,264	43,920	213,312
CFR[4]	8	9	6	3	8	9
Mortality rate[3]	17	22	7	1	15	31
No. deaths	31,568	5,240	3,982	280	3,680	18,387
Meningitis						
Incidence[2]	6	6	5	4	9	6
No. cases	10,816	1,522	2,617	796	2,259	3,623
CFR[4]	59	65	57	53	51	65
Mortality rate[3]	3	4	3	2	5	4
No. deaths	6,415	995	1,499	419	1,156	2,346

[1] Aetiologic fractions used to derive the number of cases were pooled estimates from both hospital-based studies

[2] Incidence rates of pneumonia, meningitis and septicaemia are per 100,000 children, neonatal septicaemia per 1,000 live births in 2015

[3] Mortality rate=proportion of the under-five children population (total live births) who died of *S. aureus* disease per 100,000 in 2015

[4] CFR=Case fatality ratio (%)

Table 2.5: Estimated numbers of staphylococcal cases and deaths by region (sensitivity analysis - using hospital-based studies)[1] (continued)

	Africa	Central	East	North	Southern	West
Septicaemia						
Incidence[2]	337	440	217	79	311	517
No. cases	629,334	107,018	120,547	18,002	77,182	306,585
CFR[4]	9	9	9	9	8	9
Mortality rate[3]	30	40	20	7	26	47
No. deaths	56,158	9,632	10,849	1,620	6,412	27,645
Neonatal Septicaemia						
Total neonatal deaths=1,112,623 [273]						
Live births	41,521,718	5,377,820	12,145,000	5,066,898	5,401,000	13,531,000
Incidence[2]	2.6	3.2	2.3	1.6	2.7	2.9
No. cases	107,056	17,089	27,653	8,133	14,354	39,826
CFR[4]	24	21	26	33	23	22
Mortality rate[3]	62	67	58	52	62	66
No. deaths	25,561	3,600	7,080	2,656	3,356	8,870

[1] Aetiologic fractions used to derive the number of cases were pooled estimates from hospital-based studies

[2] Incidence rates of pneumonia, meningitis and septicaemia are per 100,000 children, neonatal septicaemia per 1,000 live births in 2015

[3] Mortality rate=proportion of the under-five children population (total live births) who died of *S. aureus* disease per 100,000 in 2015

[4] CFR=Case fatality ratio (%)

The burden due to staphylococcal meningitis was higher using population-based than hospital-based studies. On the contrary, the burden due to staphylococcal bacteraemic pneumonia and septicaemia were higher using hospital-based studies.

2.5.3 Deaths due to *S. aureus* diseases

There were an estimated 46,467 deaths from staphylococcal diseases in children aged less than five years (Table 2.4) on the African continent. This represents 1.6% of all-cause deaths in this age group in 2015.[273] The highest burden of staphylococcal deaths occurred in West Africa (36.8%). Six countries (two from the West - Nigeria (18.3%) and Ghana (5.8%), one from Central Africa - Democratic Republic of Congo (7.6%), and two from the East – Ethiopia (7.8%), and Tanzania (4.3%), and one from the North - Egypt (5.0%)) accounted for 49.0% of staphylococcal deaths in Africa. The country-specific mortality rates are shown in Figure 2.7 (Appendix 6). The mortality rates were highest in West Africa. Ghana had the highest mortality rate due to staphylococcal diseases. Septicaemia accounted for 48.2% of staphylococcal deaths while pneumonia and meningitis accounted for 34.4% and 17.4% respectively.

Appendices 5 - 11 and 5a – 11a contain the country-specific number of cases and deaths due to staphylococcal diseases by clinical syndrome among these children from population-based and hospital-based studies, respectively.

2.5.4 Disease caused by *S. aureus*

Pneumonia

Bacteraemic pneumonia

Bacteraemic pneumonia was defined as cases of severe pneumonia (WHO definition of clinical pneumonia – cough, increased respiratory rate for age and lower chest wall in-drawing) who were *S. aureus*-blood culture positive. There was an estimated 113,368 cases among the children aged less than five years in Africa (Table 2.4; Appendix 7). This number accounted for 1.6% of all-cause severe pneumonia cases (7 million) in children aged less than five years in Africa.[274] The highest number of cases was in West Africa while the lowest in North Africa. Nigeria and Seychelles had the highest and lowest number of cases, respectively. The incidence of severe pneumonia with *S. aureus* bacteraemia was 61 cases per 100,000 children with the highest rates seen in West Africa (70 cases per 100,000 children) and lowest in North Africa (39 cases per 100,000 children) (Table 2.4).

Using data from hospital-based studies to derive the aetiologic fraction, the number of staphylococcal severe pneumonia cases increased to 392,069 with about half (50.1%) occurring in West Africa (Table 2.5). The estimated incidence of *S. aureus* pneumonia was 210 per 100,000 children.

Non-bacteraemic pneumonia

The number of non-bacteraemic pneumonias defined as cases who are *S. aureus*-culture positive from lung aspirate, but with negative blood culture, was estimated at 1,771,730 cases in 2015 (Table 2.4; Appendix 8). The highest number of such cases (36.0%) occurred in West Africa and the lowest (4.6%) in North Africa. The incidence of non-bacteraemic pneumonia was 948 annual cases per 100,000 children much greater than for the bacteraemic pneumonia definition (61 cases per 100,000 children). Incidence varied widely between the regions ranging from 612 per 100,000 children in North Africa to 1,094 per 100,000 children in West Africa (Table 2.4).

The majority of pneumonia deaths attributable to both bacteraemic and non-bacteraemic pneumonia classifications (43.2% and 46.2% respectively) occurred in West Africa.

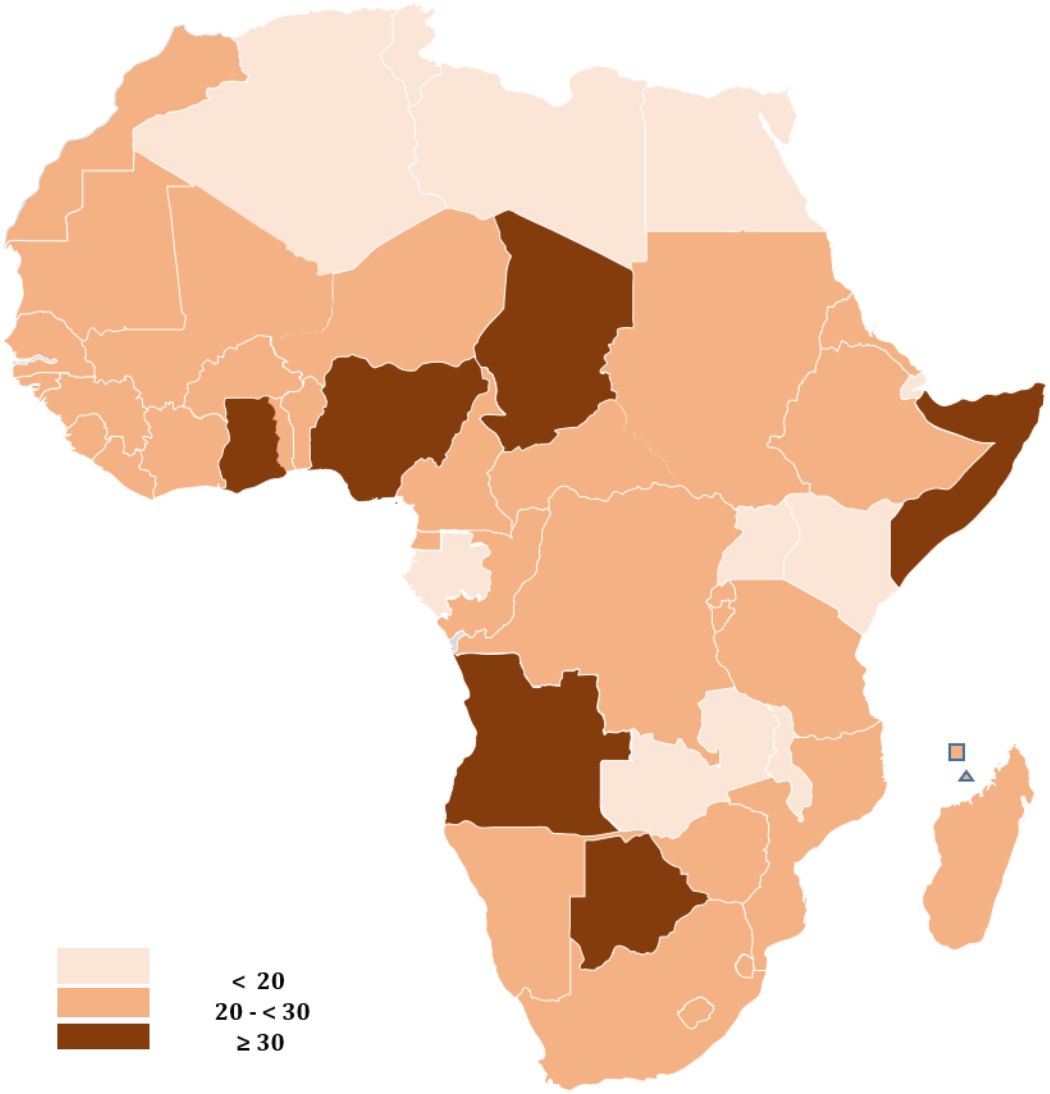


Figure 2.6: Staphylococcal mortality rates among children aged less than five years (per 100,000 children in 2015. (Mortality rates are the proportion of children aged less than five years who died of staphylococcal infections per 100,000 children in each country)

Meningitis

An estimated 27,973 *S. aureus* meningitis cases occurred among children aged less than five years in Africa, with about one-third (28.6%) occurring in West Africa and 11.8% in Central Africa (Appendix 9). The incidence was 15 per 100,000 children (Table 2.4). An estimated 15,977 *S. aureus* meningitis cases died, giving a mortality rate of 9 per 100,000 children and a CFR of 57% (Table 2.4). Using the aetiologic fraction derived from hospital-based studies, there was a lower number of estimated cases of staphylococcal meningitis – 10,699 - with an overall incidence of 6 cases per 100,000 children across the sub-regions (Table 2.5). An estimated 6,415 *S. aureus* meningitis cases died, giving a mortality rate of 3 per 100,000 children (Table 2.5).

Septicaemia

Children aged less than five years

The incidence of *S. aureus* septicaemia among children aged less than five years was 134 annual cases per 100,000 children with an estimated 250,725 cases (Appendix 10). Most cases occurred in West Africa (36.6%). *S. aureus* septicaemia incidence ranged from 116 per 100,000 children in East Africa to 261 per 100,000 children in West Africa (Table 2.4).

Total numbers of deaths due to *S. aureus* septicaemia followed the same pattern as the number of cases. An estimated 22,407 deaths due to *S. aureus* disease occurred in this age group giving a mortality rate of 12 per 100,000 children (Table 2.4).

Using data from hospital-based studies to derive the aetiologic fraction, the number of staphylococcal septicaemia cases increased to 629,334 with almost half of the cases (48.7%) occurring in West Africa (Table 2.4). The incidence of *S. aureus* septicaemia was 337 per

100,000 children ranging from the lowest (79 per 100,000 children) in North Africa to the highest in West Africa (517 per 100,000 children) (Table 2.5).

Neonates

Using data from population-based studies to derive the aetiologic fraction, 51,215 neonatal *S. aureus* septicaemia cases were estimated (Appendix 11) accounting for 20.4% of all the staphylococcal septicaemia cases in children less than five years of age. The highest number of cases was seen in West Africa and the lowest in North Africa. The incidence of neonatal septicaemia was 1.2 cases per 1,000 live births with a mortality rate of 0.32 per 1,000 live births. The estimated number of neonatal deaths due to staphylococcal septicaemia in Africa was 13,091, accounting for 58.4% of all deaths due to staphylococcal septicaemia. The mortality rate in neonatal septicaemia was higher than in other clinical syndromes.

Using data from hospital-based studies to derive the aetiologic fraction, the estimated number of cases was greater at 107,056 with 25,561 deaths due to neonatal staphylococcal septicaemia. The incidence of *S. aureus* neonatal septicaemia was 2.6 per 1,000 live births (Table 2.5).

2.6 Discussion

An estimated 392,066 staphylococcal cases and 46,467 deaths occurred among children aged less than five years in Africa in 2015. These deaths represented 1.6% of the total childhood deaths that occurred in 2015.[273] Among neonates, there were 51,215 cases and 13,091 deaths due to staphylococcal septicaemia. These neonatal deaths represented 1.2% of all-cause deaths among this age group in 2015.[273] In East and West Africa, *S. aureus* had

high resistance rates to ampicillin and amoxicillin, but greater susceptibility to cloxacillin and gentamicin.

Burden by sub-region and country

This review and analysis showed that there is sub-regional variation in the burden of *S. aureus* disease among children aged less than five years in Africa. For each of the clinical syndromes assessed, the number of cases and deaths, as well as the incidence and mortality rates, were lowest in North Africa (except for incidence and deaths due to septicaemia). This pattern is reflected in the lower overall under-five mortality rates (U5MR) and higher gross domestic product (GDP) per capita in these countries. With the exception of Mauritania with an U5MR of 85 per 1,000 live births, all the North African countries had an U5MR less than 75 per 1,000 live births.[238] The West African sub-region consistently had the highest burden of *S. aureus* cases and deaths. The reason for this is unclear but may be due to variation in pathogen virulence, host susceptibility, case definition, blood culture sampling practices, laboratory techniques and capacity. Additionally, the West African sub-region had the largest population, driven by Nigeria which accounted for 16.7% of the population of children aged less than five years in Africa.[235] In 2017, Nigeria ranked 17 on the African continent in terms of GDP, had the largest population, a high U5MR, and the most substantial staphylococcal disease burden on the continent. However, Ghana had the highest incidence of *S. aureus* disease, a high U5MR and ranked 18 on the African continent in terms of GDP. In general, the countries in the West African sub-region (13/15) had the highest overall mortality rates (≥ 75 per 1,000 live births).

For countries with a large population size like Nigeria, Ethiopia, Democratic Republic of Congo and Egypt interpretation of the estimates can be a challenge because of substantial

variations in the childhood mortality, access to health care, parent's health-seeking behaviour, and incidence of disease within each country.[275] Disaggregation of data by the states or provinces would have been ideal but was not possible due to the lack of necessary information such as childhood mortality or access to health care by these strata.

Pneumonia

Using the bacteraemic pneumonia definition, there was an estimated 113,368 cases of *S. aureus* pneumonia, accounting for 1.6% of all-cause severe pneumonia cases (7.0 million)[240] in children aged less than five years. This estimate is based on the aetiologic fraction from two population-based studies.[246,247] These studies are more likely to be representative of the true burden of *S. aureus* pneumonia in the community than hospital-based studies. This is not surprising because most severe cases are selectively referred to the hospital and *S. aureus* is associated with severe pneumonia.[274] Conversely, hospital-based studies are affected by factors such as parent's health-seeking behaviour, access to healthcare and the subjective decision by the physician to admit or not to admit the patient.

The assumption that the aetiologic fraction of pneumonia due to *S. aureus* is the same as the proportion of pneumonia deaths due to *S. aureus* was made because of the dearth of fatality data on *S. aureus* pneumonia; however, this assumption was also used by O'Brien and colleagues in their estimation of pneumococcal disease burden.[229] The assumption that the aetiologic fraction of pneumonia due to *S. aureus* is similar to the proportion of pneumonia deaths due to *S. aureus* might have under-estimated or over-estimated *S. aureus* pneumonia mortality. This assumption presumes that all bacterial pneumonia deaths would have had significant pathology, which would have been evident on chest X-ray (CXR) as alveolar consolidation (CXR positive) at the time of death. The other assumption was that the CFR of *S. aureus* CXR positive pneumonia was comparable to that of CXR positive pneumonia due

to other pathogens. In countries with good access to health care, the CFR of *S. aureus* CXR positive pneumonia is likely to be lower than CXR positive pneumonia due to other pathogens while in poor access to health care settings, the reverse is the case. In the latter health care settings, there is a tendency for patients to present late when the disease would have spread to affect other contiguous tissues resulting in empyema or purulent pericarditis. Consequently, this might have under-estimated the number of deaths due to *S. aureus* pneumonia for some countries and over-estimated for others, which highlights the importance of completeness in the reporting of studies and the fact that in Africa, *S. aureus* has not been considered a disease of public health importance.

The higher incidence of non-bacteraemic than bacteraemic pneumonia may be due to the better recovery of *S. aureus* from lung aspirates compared to blood cultures.[276] However, the rate of performing lung aspirations was low in the reported studies as the procedure was done only in cases who had presented to the hospital with evidence of consolidation on CXR. *S. aureus* is known to be responsible for more severe cases of pneumonia.[274,277] This could result in an over-estimation of the burden of disease, especially if patients with consolidation on CXR were selectively referred to hospitals with facilities for lung aspirates.

Identification of the aetiological cause of pneumonia can be technically challenging, especially in children. The body fluid samples used for pathogen identification in pneumonia cases include blood, sputum, bronchoalveolar lavage, nasopharyngeal aspirates or swabs and lung aspirates. Blood culture, the most commonly used method, is positive in only about 5% - 10% of cases, the yield of which may increase to 20% in severe cases.[158] Bronchoalveolar lavage gives a higher rate of pathogen identification (38%). However, it requires patient sedation and monitoring, and also runs the risk of contamination with upper airways pathogens during bronchoscopy.[278] Sputum and nasopharyngeal aspirates or

swabs have higher culture positivity rate (50% and 70% respectively) but are also often contaminated with the upper airway flora especially in children, and may therefore not always reflect the aetiology of pneumonia.[279] For this review and analysis, the definition of non-bacteraemic pneumonia included only those who had lung aspirate performed because of the low risk of contamination.

The yield of pathogens in lung aspirates in children is about 41%.[276] Lung aspirate is rarely done routinely, although investigators have demonstrated the safety of the procedure in children.[53,251,280] There is no gold standard test for the detection of pathogens in pneumonia. This review disaggregated pneumonia cases by the body fluid sample used for pathogen detection, that is bacteraemic, and non-bacteraemic pneumonia cases were analysed separately to avoid double-counting cases. Only four studies in two countries (Gambia and Nigeria) reported *S. aureus* data on lung aspirates in children aged less than five years.[53,54,56,251]

The number of cases of *S. aureus* bacteraemic pneumonia could have been under-estimated because the sensitivity of blood culture, which was used for pathogen identification is low.[158] The number of cases may also have been underestimated because severe cases often die before any investigation is done. Only two countries provided data on the aetiologic fraction of pneumonia which might have introduced bias if there is variation between countries in this parameter.

Meningitis

Meningitis was the most severe clinical form of *S. aureus* disease (in terms of case fatality ratio) and had the lowest number of cases. Although *S. aureus* rarely crosses the blood-brain

barrier,[281] it can do so particularly when bacterial loads are high, and fatality is usually high.[281]

There were only three prospective studies on the continent – from Mozambique, Mali, Burkina Faso and Togo - reporting the incidence of meningitis in children aged less than five years in a non-outbreak setting and using only conventional microbiology to isolate pathogens.[60] Prospective population-based data on CFR due to *S. aureus* meningitis in children in Africa were not available; therefore, estimates of CFR from the pre-antibiotic era or all-cause bacterial meningitis were the only available options. The use of more conservative estimates of CFR of all-cause bacterial meningitis in this analysis was preferred[229] because treatment may have affected survival.

Septicaemia

Septicaemia accounted for the majority of the cases of severe staphylococcal diseases. About a fifth of *S. aureus* septicaemia cases (20.4%) occurred during the neonatal period. Immature immune function,[282] rapid acquisition of *S. aureus* carriage shortly after delivery[101] and exposure to unhygienic practices increase the risk of *S. aureus* disease in newborns.

Mortality from neonatal septicaemia was higher than for other clinical syndromes due to *S. aureus,* which may be as a result of immature immune response and rapid progression of the disease or late presentation to the health facility. Strategies are required to reduce neonatal SAB and mortality rates on the continent.

There was considerable heterogeneity among the studies combined in the meta-analyses of incidence and aetiologic fraction estimates (I^2=0% - 99%). This may be attributable to the varying study designs used, varying levels of poverty, ignorance, overcrowding, poor

hygienic practices, poor access to healthcare, pre-treatment antibiotic use, public health programmes, and the burden of HIV infection in the countries. The burden of childhood HIV infection was not considered in this analysis because the prevalence was considered low.[236] Although this assumption might have underestimated the disease burden, but only to a negligible degree.

Antibiotic use and antimicrobial resistance

Most of the studies did not report on pre-treatment antibiotic use; however, some excluded patients who had received antibiotics in the preceding 2 – 7 days before presentation. None of the studies took a history of pre-treatment antibiotic use into account when interpreting data on culture results or antimicrobial susceptibility.

The use of antibiotics by patients can hinder the aetiologic diagnosis of all infectious disease syndromes.[158] The extent of the decrease in sensitivity is not known, but it is affected by the type of antibiotics, the dose, the duration of use, and possibly the pathogen involved. Data on study participants with a history of pre-treatment antibiotics should be reported separately to promote the comparability of data and possibly increase the yield of cultures.

In East and West Africa, resistance to ampicillin, amoxicillin and penicillin was high but lower for cloxacillin, chloramphenicol, and erythromycin. This is consistent with the findings of two recent systematic reviews on antimicrobial resistance among children in sub-Saharan Africa.[178,194] The high resistance rates of *S. aureus* to ampicillin, amoxicillin and penicillin may be a result of the strong selective pressure of these drugs in the sub-region. The prevalence of resistance to cefotaxime and ceftriaxone, third-generation cephalosporins is low in the two sub-regions likely because their use is more restricted since they are

administered parenterally and would require skilled persons for administration. Their use as second-line drugs for the treatment of *S. aureus* disease in childhood remains relevant.

Challenges in estimating the burden of disease

Data from population-based studies were only available from a small number of countries. Of note, no relevant population-based data were identified from North and Central African countries, although together they account for about a quarter of the population of under-fives on the continent. Moreover, there were no population-based studies on the NPNMNS clinical syndromes for consideration in this review.

Incomplete reporting of study findings on disease burden was also a challenge. It is plausible that studies on the aetiology of pneumonia and meningitis may not have considered *S. aureus* as a significant cause of fatality and therefore not reported this outcome. Furthermore, the full texts of 60 articles could not be found, despite substantial efforts highlighting the importance of open access publication.

The burden (incidence, cases, and deaths) of a disease can be estimated using population-based longitudinal or vaccine probe studies. In population-based studies where case ascertainment requires pathogen identification by cultures, the true burden of the disease is usually under-estimated. Reasons for this underestimation include low rates of hospital presentation and investigation, antibiotic use before investigation and the sensitivity of the method used for pathogen identification.

The vaccine probe approach, which uses the incidence of vaccine-preventable disease and the aetiologic fraction, remains the best method to estimate cases of disease attributable to a particular pathogen.[283] Vaccine probe studies are usually randomised trials of vaccines

with known efficacy; while vaccine-preventable disease incidence is the difference in the disease incidence between the unvaccinated and vaccinated groups, the aetiologic fraction is the ratio of vaccine efficacy for a clinical syndrome to that for aetiologically-confirmed cases.[283] However, currently, there is no effective vaccine against *S. aureus* disease. In the absence of vaccine probe studies, population-based studies reporting systematic blood culture collection usually describe a considerable disease burden.[6,241] All included studies reported investigating all or at least 80% of eligible patients.

There is a need for consensus with respect to case ascertainment and classification. The case definition of septicaemia and meningitis is widely accepted while there is a lack of consensus on the definition and classification of pneumonia, even by experts.[284] For this review, the case definition for severe pneumonia in all included studies was as the presence of cough, chest wall in-drawing with or without increased respiratory rate (WHO classification or similar). However, for studies in which lung aspirates were performed, the presence of consolidation on CXR as well as the signs mentioned earlier made up the case definition of severe pneumonia. When combining various data, case definition and methods need to be as similar as possible in order to reduce heterogeneity.

Given the fact that there were few relevant population-based studies in Africa; sensitivity analyses were done to compare the estimates of cases and deaths when using the aetiologic fraction from hospital-based studies to those from population-based studies. The use of aetiologic fraction data from hospital-based studies gave a higher estimated burden of invasive staphylococcal diseases due to bacteraemic pneumonia and septicaemia. This approach could have over-estimated the number of cases and therefore, the number of deaths due to the disease because of referral bias as severe or complicated cases are more likely to have presented to the hospitals. In contrast, the burden due to staphylococcal

meningitis was higher using population-based studies than hospital-based studies. This can be explained by the fact that the population-based study on meningitis included neonates while the hospital-based study did not. I chose not to combine the use of both population-based and hospital-based studies to determine aetiologic fraction because the estimates differ. This further highlights the need for increased investments in population-based research or routine collection of high-quality data through surveillance systems in Africa.

Strengths and limitations

To the best of my knowledge, this is the first attempt to estimate the burden of community-acquired *S. aureus* disease among children aged less than five years in Africa. This study has provided conservative estimates of the burden of diseases caused by *S. aureus* through a rigorous systematic review. These estimates will serve as a baseline, a crucial advocacy tool for policymakers, and increase awareness among other stakeholders, including clinicians and investigators.

Second, this review has highlighted a knowledge gap in information on *S. aureus* disease burden in Africa. Even though *S. aureus* is responsible for a wide range of clinical syndromes, there is a dearth of population-level aetiology data on most of the diseases caused by this pathogen.

However, this study has some limitations. A significant limitation is the relatively few country representative data from Africa. The lack of representative studies was pronounced in North and Central African countries. Given this dearth of data, imputation of data for countries without information was done which might have over-estimated the numbers for some countries and under-estimated for others. In addition to the lack of data on incidence, there was a scarcity of prospectively collected data on AMR in community-acquired

infections in Africa. However, the findings of this study are similar to those of two high-quality reviews on AMR in African children.[178,194] There was also a lack of data on the incidence of key clinical syndromes such as endocarditis, septic arthritis and osteomyelitis caused by *S. aureus*. Given the limited data on these syndromes, the true burden of *S. aureus* disease cannot be estimated. Third, even though immunocompromised patients such as those with malnutrition, haemoglobinopathy and HIV-infection are at increased risk of acquiring *S. aureus* disease due to repeated exposure to healthcare facility and recurring antibiotic use, the estimates were not disaggregated according to these sub-groups. It was assumed that these subgroups of patients were included in the estimation because individual studies did not screen for these patients. Lastly, uncertainty ranges were not determined because of the scarcity of eligible studies. The resampling methods used to determine uncertainty ranges require large sample sizes (studies) for more accurate estimation.

Conclusion

This study provides the first estimates of the burden of diseases caused by *S. aureus* in children aged less than five years in Africa. The burden is substantial, with an estimated 392,000 cases and 47,000 deaths. The burden is particularly high among neonates with about one-fifth of the septicaemia cases occurring among this age group. *S. aureus* isolates showed relatively high rates of susceptibility to the first-line drug, cloxacillin.

The findings of this review and analysis will inform policy decisions and prioritization in the treatment and prevention of *S. aureus* disease in Africa. There is a need for population-based research, especially in North and Central Africa. These data will also serve as a useful baseline to evaluate the impact of interventions. It is hoped that these findings will

encourage stakeholders to pursue the development of an effective vaccine and undertake clinical trials of such vaccines among African children.

Chapter 3: The burden of community-acquired *Staphylococcus aureus* bacteraemia in children aged less than five years in rural Gambia

Preamble

This chapter describes the burden (the incidence and case fatality ratio) of *S. aureus* bacteraemia (SAB) among children aged less than five years using data from a surveillance study carried out in the Basse Health and Demographic Surveillance System in The Gambia. Data collection covered the period before and six years after the introduction of pneumococcal conjugate vaccine (PCV) during the course of a study which evaluated the impact of PCV introduction on the incidence of SAB.

3.1 Overview

There is a lack of recent population-based data on the burden of *S. aureus* bacteraemia (SAB) among children in The Gambia. Most of the data available on *S. aureus* SAB in The Gambia come from studies investigating the overall aetiology of invasive bacterial infections (IBI).[7,47,53,285] Some of these studies were population-based[7] while others were hospital-based.[47,53,285,286] The results from these studies cannot be compared because of the different study settings and populations. Hospital-based studies have shown that the proportion of bacteraemia due to *S. aureus* seems to be increasing following the introduction of pneumococcal conjugate vaccine (PCV).[287,288] However, an increase in incidence post-PCV introduction has not been confirmed using population-based studies. Such studies are essential to assess disease burden because they are less prone to bias as everyone in a defined geographical area is under surveillance.

Before the introduction of the Hib vaccine in 1997, *S. aureus* was not considered a significant cause of IBI in The Gambia. Two hospital-based studies which assessed children aged less than five years with features of pneumonia reported that between 0.6% and 0.8% of all patients recruited had SAB, and *S. aureus* accounted for between 1.3% and 3.6% of all significant isolates recovered from these children between 1987 and 1992.[53,251] These two studies were restricted to children with clinical features suggestive of pneumonia. In a population-based study in rural Gambia conducted at a similar time (1989 - 1991), *S. aureus* was detected in 0.4% of all recruited patients with suspected IBI and accounted for 2.7% (5/187) of all isolates (Table 3.1).[7] Another hospital-based study conducted in 1990 – 1992 in the same area of The Gambia showed that *S. aureus* was the leading cause of IBI in children less than 91 days of age, representing 3.6% of all cases recruited and accounted for 35.4% (17/48) of all isolates.[47] Apart from the age difference of the children in these last two studies, the different findings may be because the former study recruited children from outpatient clinics in a rural community while the latter recruited patients from a secondary and a tertiary health care facilities in a semi-urban area. Children seen at the semi-urban hospitals might have presented to other primary healthcare facilities before presenting to these hospitals and therefore, be exposed to healthcare-associated pathogens such as *S. aureus*. In this era, *S. aureus* was not demonstrated to be a significant cause of IBI.

Following the introduction of the Hib vaccine and before the introduction of PCV, the proportion of bacteraemia due to *S. aureus* appeared to increase. A study assessing the aetiological agents of bacteraemia in all patients admitted to the MRC Gambia hospital between 2003 and 2005, reported *S. aureus* as the second leading cause of bacteraemia

Table 3.1: Summary of the studies reporting *S. aureus* bacteraemia in The Gambia (1980 - 2015)
S. aureus

Author/ Year	Study design /Clinical syndrome	Year of data collection	Study site	Population	Prevalence of *S. aureus* disease[1,2]
Before the introduction of Hib[3] vaccine and PCV[3]					
Forgie 1992[251]	Hospital-based, prospective Clinical diagnosis of severe pneumonia	1987 - 1988	MRC[4] Hospital, Fajara, WCR[5]	< 5 years	1/145[1] (0.7) 1/75[2] (1.3)
O'Dempsey 1994[7]	Population-based, prospective Suspected cases of pneumonia, meningitis, septicaemia	1989 - 1991	Basse, URR[5]	< 5 years	5/1162 [1] (0.4) 5/187 [2] (2.7)
Adegbola 1994[53]	Hospital-based, prospective Malnourished with pneumonia	1990 - 1992	MRC[4] Hospital, Fajara, WCR[5]	3 months – 5 years	1/159 [1] (0.6) 1/28 [2] (3.6)
	Malnourished no pneumonia				1/119 [1] (0.8) 1/5 [2] (20.0)
	Well-nourished with pneumonia				1/119 [1] (0.8) 1/42 [2] (2.4)

[1] Proportion of *S. aureus*-positive cases among all investigated cases
[2] Proportion of *S. aureus*-positive cases among true bacteraemia cases
[3] Hib=*Haemophilus influenzae* type b; PCV=pneumococcal conjugate vaccine
[4] MRC=Medical Research Council Unit The Gambia; RVH=Royal Victoria Hospital
[5] WCR=West Coast Region; URR=Upper River Region

Table 3.1: Summary of the studies reporting *S. aureus* bacteraemia in The Gambia (1980 - 2015) (continued) *S. aureus*

Author/ Year	Study design /Clinical syndrome	Year of data collection	Study site	Population	Prevalence of *S. aureus* disease[1,2]
Before the introduction of Hib[3] vaccine and PCV[3] (continued)					
Enwere 1998[286]	Hospital-based, prospective Confirmed cerebral malaria cases	1992 - 1994	RVH[4], Banjul, WCR[5]	1 - 9 years	6/276 [1] (2.2) 6/14 [2] (42.9)
Mulholland 1999[47]	Hospital-based, prospective Suspected cases of infection	1990 – 1991 1992	MRC[4] Hospital, Fajara, WCR[5] and RVH[4], Banjul, WCR[5]	< 91 days	17/476 [1] (3.6) 17/38 [2] (44.7)
After Hib[3] vaccine introduction and before PCV[3] introduction					
Hill 2007[285]	Hospital-based, prospective Suspected cases of infection	2003 - 2005	MRC[4] Hospital, Fajara, WCR[5]	2 months - 5 years	10/573 [1] (1.8) 10/65 [2] (15.4)
After PCV introduction					
Darboe 2019[287]	Laboratory-based retrospective Blood culture positive	2005 - 2015	MRC[4] Hospital, Fajara, WCR[5]	1 - 59 months	125/477 [2] (26.2)

[1] Proportion of *S. aureus*-positive cases among all investigated cases
[2] Proportion of *S. aureus*-positive cases among true bacteraemia cases
[3] Hib=*Haemophilus influenzae* type b; PCV=pneumococcal conjugate vaccine
[4] MRC=Medical Research Council Unit The Gambia; RVH=Royal Victoria Hospital
[5] WCR=West Coast Region; URR=Upper River Region

(after *S. pneumoniae*) representing 1.8% of all investigated children aged 2 months – 5 years; and accounting for 15.4% (10/65) of bacterial isolates recovered from these patients. Following the introduction of PCV, a retrospective study of microbiological data from children aged less than five years with features suggestive of bacteraemia admitted into the same hospital reported *S. aureus* as the leading cause (26.2%; 125/477) of IBI.[287] Though these were hospital-based studies in which the investigation of patients was not

standardised, the temporary increase in the prevalence of SAB raised the possibility that the incidence of SAB may also be increasing.

It is thought that PCV may affect the nasopharyngeal microbiome,[289,290] may impact on the prevalence of carriage and potentially the incidence of diseases caused by organisms other than *S. pneumoniae*. As discussed in chapter one, the introduction of PCV has led to a considerable reduction in carriage and disease due to vaccine-type *S. pneumoniae* thereby altering the balance of the organisms in the nasopharynx with, in many cases, an increase in carriage of non-vaccine types.[11,12] Therefore, there has been a growing concern about the association between the risk of *S. aureus* disease and changes in pneumococcal carriage following the introduction of PCV. Some investigators have reported an inverse association between *S. aureus* and *S. pneumoniae* carriage.[101,291] However, others have reported that the association is confounded by age.[25] Given that the vaccine did not substantially change the overall pneumococcal carriage, the replacement of *S. pneumoniae* by other respiratory pathogens following the introduction of PCV may be a fallacy.

Nonetheless, a study in The Netherlands reported an increase in the carriage of *S. aureus* after the administration of PCV,[292] even in the setting of a randomised controlled trial.[293] In the US, a study which compared the proportion of pathogens causing bacteraemia in children 3 - 36 months of age in the pre-PCV era to that in the post-PCV era reported an increase in *S. aureus.*[288] In The Gambia, a retrospective study of laboratory data suggested an increase in the prevalence of SAB after the introduction of PCV.[287]

It has been 23 years and 11 years since the introduction of Hib vaccine and PCV respectively in The Gambia, yet their effects on *S. aureus* have not been systematically evaluated. Given the paucity of recent population-based data on the epidemiology of SAB in The Gambia and sub-Saharan Africa, the incidence, clinical characteristics, case-fatality, and risk factors for

SAB in young children in rural Gambia have been studied. I also explored the association of SAB with the introduction of PCV.

Hypothesis

The incidence of *S. aureus* bacteraemia among children aged less than five years in Gambia did not increase after the introduction of PCV.

3.2 Objectives

The objectives of this study were to:

a. Estimate the incidence of and risk factors for SAB among children aged below five years in rural Gambia.
b. Identify risk factors for fatality from SAB.
c. Assess trends in the incidence of SAB before and after the introduction of PCV.

3.3 Methods

3.3.1 Fieldwork

Study site, study design and population

The study was conducted in the Basse Health Centre (BHC) (now known as Basse District Hospital) and the Basse Health and Demographic Surveillance System (BHDSS) in the south bank of the Upper River Region, and the Bansang Hospital and the Fuladu West Health and Demographic Surveillance System (FWHDSS) in the Central River Region of The Gambia (Figure 3.1). The BHDSS was established in 2007, comprising 225 villages with a population of ~179,000 in 2015 (19% of whom were children aged less than five years). The population

is enumerated every four months by updating records of all births, deaths, migration, pregnancies and marriages.[243] A resident in the BHDSS was defined as someone who had lived in the area for at least four uninterrupted months, and such a person was assigned a unique14-digit identifier.

The BHDSS is served by five satellite clinics (Demba Kunda, Fatoto, Gambisara, Garawol and Koina) and the BHC, a 58-bed primary/secondary healthcare facility with 25 paediatric beds. The health centre has an average of 3,600 and 2,000 admissions and deliveries per year, respectively. Severe cases that cannot be managed in BHC are referred to Bansang Hospital, about 60 km away.

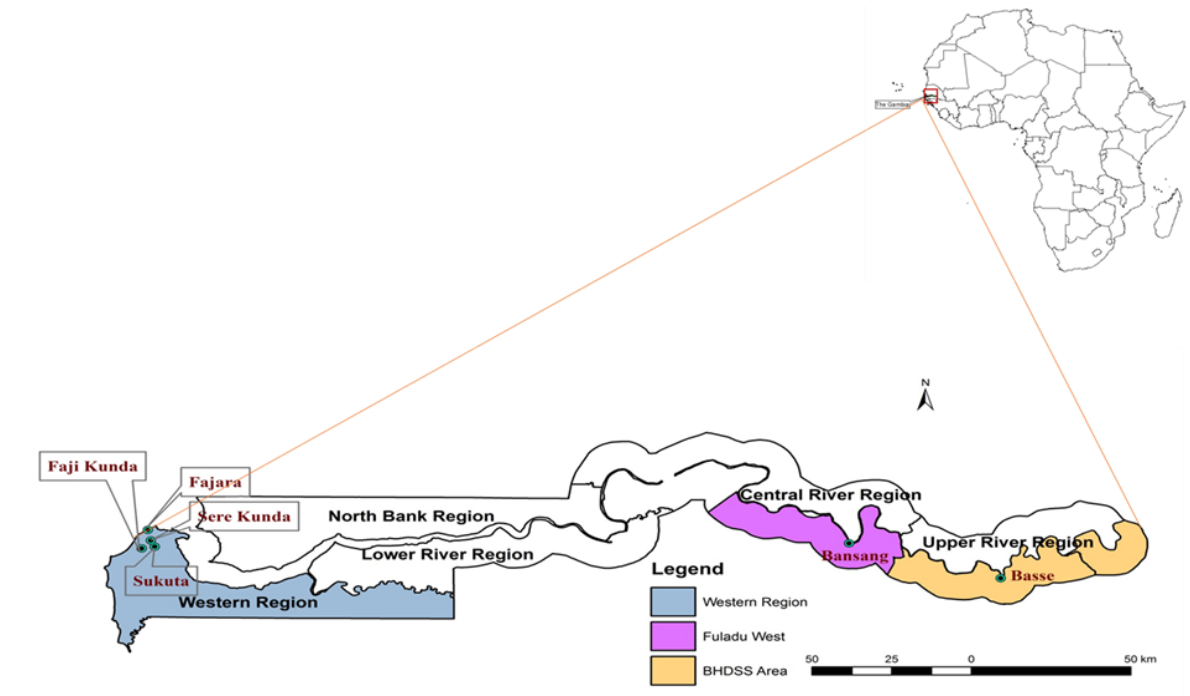


Figure 3.1: Map of The Gambia showing the health and demographic surveillance sites (Basse and Fuladu West)

Surveillance for pneumonia, meningitis and septicaemia was conducted among residents of the BHDSS aged two months and under five years from May 12, 2008, to December 31, 2015.

From January 1, 2011, until December 31, 2015, surveillance criteria were expanded in the BHDSS to include all children less than five years of age who were admitted with an acute medical problem from whom blood was collected for culture (admission surveillance). Similarly, the surveillance criteria were expanded to include all children aged 0 – 59 months admitted with an acute medical problem in the adjacent geographic area of the Fuladu West Health and Demographic Surveillance System (FWHDSS, Figure 3.1) from September 12, 2011, until December 31, 2014 (admission surveillance). The FWHDSS, with a population of 92,464 in 2014 (18% of which are children aged less than five years) was enumerated annually. The FWHDSS is served by Bansang hospital and two satellite clinics - Brikama Ba and Jakhaly. Bansang Hospital is a 160-bed secondary health facility with 84 paediatric beds; it has an average of 7,000 and 2,000 admissions and deliveries annually, respectively.

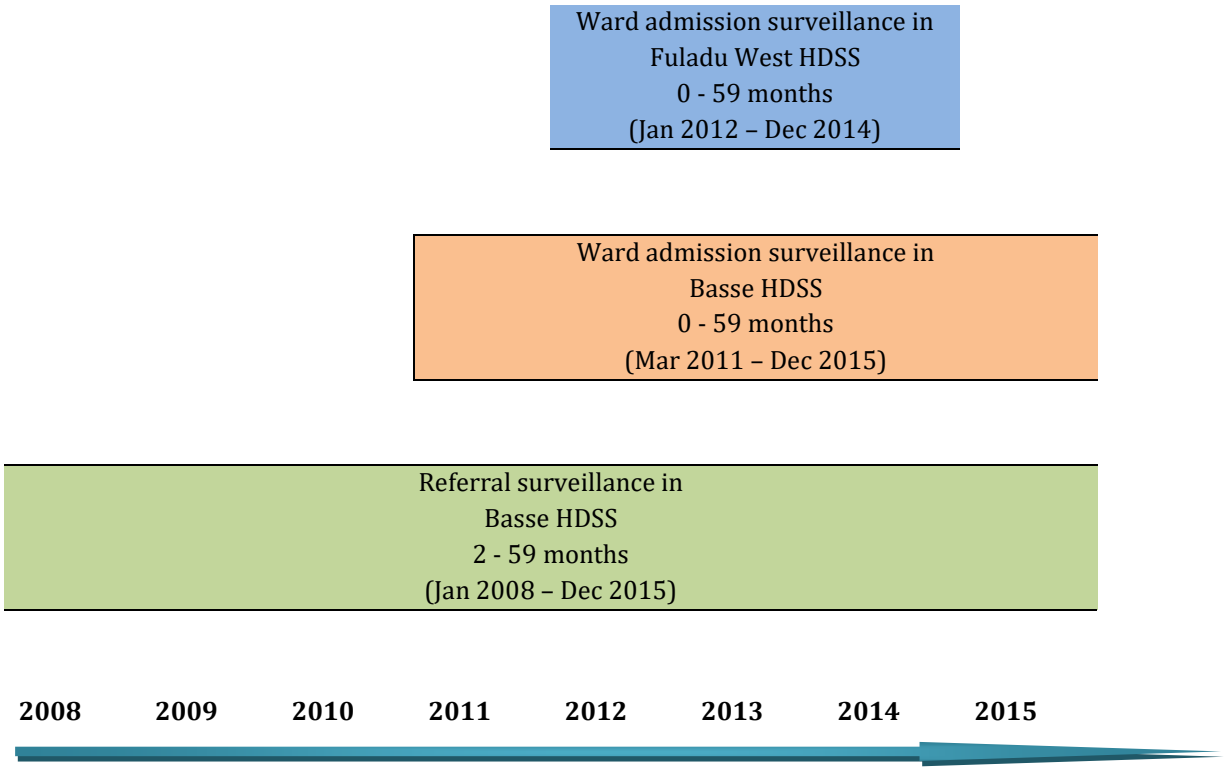


Figure 3.2: Diagram showing the type and different surveillance periods of observation in the Basse and Fuladu West HDSS

Conjugate vaccines against *H. influenzae* type b and pneumococcus were introduced into the Gambian National Programme on Immunization in 1997 and 2009, respectively. In 2011, the 13-valent vaccine (PCV13) replaced the seven-valent pneumococcal conjugate vaccine (PCV7). Vaccine coverage for the third dose of diphtheria-pertussis-tetanus vaccine in these regions was about 81.7% in 2012.[294] In The Gambia, the transmission of *Plasmodium falciparum* occurs mostly during the short rainy season between July and November.[295] In 2018, 40.6% of children under the age of five years slept under insecticide-treated nets.[296] The prevalence of HIV in women attending an antenatal clinic in The Gambia was 2.3% in 2018.[297]

Community sensitisation

After obtaining written approval to conduct the study from the director of the Regional Health Team, we undertook community sensitisation in stages. A series of meetings to share the details of the study were held with the village heads, members of the Village Development Committees, religious leaders, and all members of all the villages in the south bank of the Upper River Region. Also, the villagers had an opportunity to ask questions at the end of each meeting.

Training

At the beginning of, and whenever a new staff member joined the study, clinicians, nurses, laboratory, and radiology staff were trained on study-specific procedures. Retraining sessions and quality assurance processes were carried out regularly to standardise data collection.

Surveillance procedures

Surveillance was conducted between May 12, 2008 and December 31, 2015, except for four weeks in 2010 (October 5 – November 3, 2010) when there was flooding of the Medical Research Council (MRC) Basse field site. All children aged 2 - 59 months who had an HDSS unique identifier and presented to the BHC or any of the other five satellite clinics in the BHDSS were screened for pneumonia, meningitis and septicaemia by a study nurse using standardised criteria (referral surveillance) (Table 3.2).[243] These included children who were treated as either as outpatients or admitted.

Table 3.2: Screening criteria used by study nurses

Patients 2 – 59 months of age
The presence of one or more of the following:
• Bulging fontanelle
• History of convulsion
• History of cough or difficulty in breathing accompanied by raised respiratory rate[1]
• Impaired consciousness[2]
• Irrespective of age any child with suspected meningitis
• Local musculoskeletal swelling or tenderness
• Lower chest wall in-drawing, or nasal flaring or grunting
• Oxygen saturation less than 92%
• Prostration[3]
• Stiff neck
• Weight for age z-score ≤-3 for age

[1] Raised respiratory rate for age referred to above 60 breaths per minute for infants less than 2 months of age, above 50 breaths per minute for those between 2 and 12 months of age and above 40 breaths per minute for children more than 12 months of age but less than 5 years.

[2] Impaired consciousness was accessed using the Blantyre Coma Scale. *From Taylor, T. Caring for children with cerebral malaria: insights gleaned from 20 years on a research ward in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene 2009:103 (Suppl 1): S6-S10 DoI: 10.1016/j.trstmh.2008.10.049*

[3] Prostration is defined as inability to drink or sit in a child who had been able to do so before the illness started

Study nurses referred infants or children who were positive on screening to a clinician, who used standardised criteria for assessment and investigation (Table 3.3).[243] Data collected included age, sex, anthropometric measurements, presenting symptoms and signs, and suspected diagnosis.

UNICEF chronometers were used to count respiratory rates which were observed for one minute in an awake quiet child, while a digital pulse-oximeter was used to measure the pulse rate and oxygen saturation. Blood was collected for culture and depending on clinical presentation, cerebrospinal fluid, lung aspirates or pleural fluid samples were taken for conventional microbiology.[12] A rapid diagnostic test (RDT) for malaria (ICT Diagnostics, Cape Town, South Africa) was routinely done between August and December, and at other times at the discretion of the clinician. All SAB cases identified were linked to the DSS databases using the unique 14-digit identifier.

Anthropometry

Weight, assessed to the nearest 100 g, was measured using a digital weighing scale (TANITA, Arlington Height, IL, USA), and length or height and mid-upper arm circumference were recorded to the nearest millimetres with a ShorrBoard (Weigh and Measure, Olney, MD, USA) and measuring tapes, respectively.

Blood culture collection procedure

All the equipment required for the procedure were set in a tray, and the required sample bottles labelled. The mother carried the patient on her lap, and the phlebotomist identified the vein to use, tied a tourniquet above the selected vein, and cleaned the skin over the area

with an alcohol swab in a concentric circular motion and allowed this to dry. Meanwhile, the phlebotomist put on a pair of sterile gloves. After that, the vein was cannulated using a sterile

Table 3.3: Case definitions for suspected surveillance diagnoses used by clinicians

Patients 2 – 59 months of age
Pneumonia
Cough or difficulty breathing AND one or more of the following: • Raised respiratory rate for age[1] • Lower chest wall in-drawing, nasal flaring or grunting • Oxygen saturation < 92% • Focal chest sign (dull percussion notes, coarse crackles, bronchial breathing)
Meningitis
One or more of the following: • Convulsion • Impaired consciousness[2] • Neck stiffness with or without photophobia • Prostration • Bulging fontanelle
Septicaemia
One or more of the following: • Any patient with axillary temperature <36°C or ≥38°C • Any patient with severe clinical malnutrition • Clinician diagnosis of focal sepsis (including but not limited to cellulitis, endocarditis, liver abscess, osteomyelitis, peritonitis, septic arthritis, soft tissue abscess), or of generalised septicaemia

[1] Raised respiratory rate defined as above 60 breaths per minute for infants less than 2 months of age, above 50 breaths per minute for those between 2 and 12 months of age and above 40 breaths per minute for children more than 12 months of age but less than 5 years.

[2] Impaired consciousness is accessed using the Blantyre Coma Scale. *Taylor, T. Caring for children with cerebral malaria: insights gleaned from 20 years on a research ward in Malawi. Transactions of the Royal Society of Tropical Medicine and Hygiene 2009:103 (Suppl 1): S6-S10 DoI: 10.1016/j.trstmh.2008.10.049*

butterfly needle and one to three millilitres of blood drawn into a syringe. The needle was removed from the syringe and replaced with a sterile needle which was then used to inoculate the BD BACTEC™ Peds Plus™/F culture vial.

3.3.2 Laboratory methods

One to three millilitres of blood collected from patients with suspected pneumonia, meningitis, or septicaemia was inoculated into a paediatric BACTEC™ bottle and incubated in an automated BACTEC™ 9050 blood culture system for a maximum of five days. Standard methods were used to investigate other microbiological samples.[53] Positive cultures were subcultured on blood agar plates and isolates were confirmed as *S. aureus* by catalase and coagulase tests. Cultures that grew *Bacillus* spp, C*orynebacteria* spp, *Streptococcus viridans* and coagulase-negative *Staphylococcus* were classified as contaminated. Disc diffusion methods were used to determine antibiotic susceptibility according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[272] Cefoxitin was used as a surrogate for methicillin-resistance with a cut-off diameter of 21mm.[272] All laboratory processes were internally controlled. The laboratory submitted to external quality assurance by the WHO Reference Laboratory, Denmark, the Royal Australasian College of Pathologists, Sydney, Australia and United Kingdom National External Quality Assessment Service, Sheffield, UK.

3.3.3 Management of patients

Any child with an oxygen saturation of less than 92% had oxygen therapy. According to the Gambian national treatment guidelines[175] for treating suspected septicaemia, we commenced empiric antibiotics after sampling and before the results of blood cultures were available. For suspected mild and severe pneumonia, oral amoxicillin and intravenous penicillin and gentamicin were administered, respectively; for meningitis, intravenous chloramphenicol, and penicillin and for suspected septicaemia, intravenous ampicillin and gentamicin were given. Antibiotics were changed when indicated by the outcome of the culture.

Case definition

SAB cases were clinically suspected cases of pneumonia, meningitis, septicaemia, abscess, osteomyelitis, pyomyositis, or septic arthritis identified using standardised criteria[243] and from whom *S. aureus* was isolated only from the blood.

When the surveillance diagnosis included more than one clinical syndrome, the most clinically severe one was chosen as the diagnosis for the participant (meningitis>>septicaemia>> pneumonia>>focal sepsis).

3.3.4 Ethical considerations

Ethical approval for the study was granted by the Gambia Government/Medical Research Council Joint Ethics Committee, and the London School of Hygiene and Tropical Medicine Ethics Committee. Written informed consent was obtained from the parents or guardians of all patients.

3.3.5 Statistical methods

Surveillance data from two sources were used for statistical analyses:

a) standardised nursing referrals (referral surveillance) in the BHDSS from May 12, 2008 - December 31, 2015 restricted to children aged 2 - 59 months who met clinical criteria for investigation and, b) routine admissions (admission surveillance) in the BHDSS (2011 - 2015) and FWHDSS (2012 - 2014) which included children aged 0 - 59 months admitted with an acute medical problem (Figure 3.2). Each patient had a unique identifier irrespective of the dataset in which he or she appeared, so was not counted twice. The referral data were used to investigate trends in incidence because it covered a more extended period (2008 -

2015) than the admissions data (2011 – 2015). While the combined admission (2011 – 2015) and referral data (2011 – 2015) were used to estimate the age-specific incidence and case fatality ratio.

Incidence estimates were obtained by dividing the number of SAB cases by the number of person-years at risk, calculated from mid-point population estimates. To account for the shorter period of observation in 2008 (May 12 – December 31), person-years at risk were calculated as the mid-year population multiplied by 234/365. The incidence in neonates was calculated using two methods: firstly, as cases per 1,000 live births and secondly as cases per 100,000 person-years. Neonatal period was defined as the period between birth and 28 days of life.

Using the data from referral surveillance, trends in incidence over time and variation in incidence before and after the introduction of PCV were assessed using Poisson regression with robust error variance to allow for over-dispersion of the data. The era before PCV introduction (pre-PCV period) was defined as the interval between May 12, 2008 and May 11, 2010 while that after the introduction (PCV13 period) as the period between January 1, 2013 and December 31, 2015. To account for an increase in the rate of eligible patients requiring blood culture over time, the annual numbers of age-specific SAB cases were multiplied by a correction factor, the reciprocal of the ratio of the annual rate of eligibles enrolled/mean rate of enrolment of eligibles over the study period. The population denominators for the pre-PCV and PCV13 periods were averages of the mid-point populations in the BHDSS in the comparator periods. Categorical variables were compared using the Chi-square test. Surveillance diagnosis was categorised into mutually exclusive groups in the order of severity (meningitis>> septicaemia>>pneumonia).

The case fatality ratio (CFR) was defined as the ratio of the number of SAB cases who died before discharge to the total number of SAB cases. Potential risk factors for fatality before discharge were identified using logistic regression, although surveillance was not designed to assess risk factors. The risk factors assessed include age, sex, nutritional status, axillary temperature, pulse rate, respiratory rate, cough, difficult breathing, prostration, hypoxia, admission in the preceding two weeks before presentation and season. SAB death in relation to the total number of deaths in the population was determined by dividing the number of SAB deaths by those of the total number of deaths in both HDSS areas between 2011 and 2015.

Weight-for-age (WAZ) and weight-for-height (WHZ) z-scores were generated using 2006 WHO child growth standards. Children with WAZ and WHZ z-scores <-3 were considered severely underweight and wasted, respectively.

Analyses were done using Stata 14.0 (College Station, Texas 77845 USA). A p-value of <0.05 was used to indicate statistical significance.

3.4 Results

A total of 33,060 children met the criteria for investigation and 27,851 (84.2%) blood culture samples were collected at presentation at the health facility (Figure 3.3). Of the 5,209 children who did not have samples taken for blood culture, 487 (9.3%) had an unsuccessful venepuncture, 416 (8.0%) declined consent for venepuncture, 249 (4.8%) declined to join the study, and for 4057 (77.9%) no reason was given. Contaminants grew in 2,539 (9.1%) of blood cultures, and these children were excluded from the analysis as contamination may have masked a true SAB.

3.4.1 Characteristics of included patients

Using the combined admission and referral data, 8.3% (2,092/25,307) of all enrolled patients were neonates, 44.0% (11,138/25,307) were less than one year of age and 56.8% (14,369/25,307) were males. Of all patients, 20.0% (4,541/22,729) were severely underweight, 16.1% (3,661/22,729) severely wasted, and 18.3% (4,183/22,902) had been admitted in the two weeks before presentation. Antibiotic use in the week before presentation was uncommon (11.0%; 1,694/15,407). The majority of patients had pyrexia (≥37.5^{O}C) and tachypnoea (Table 3.4).

3.4.2 Bacteraemia

Bacteraemia were identified in 1,130 children aged 0 - 59 months (Figure 3.3). Nine hundred and thirty-two of these children (521 identified through referral surveillance and 418 through admission surveillance) had pathogens other than *S. aureus* isolated. These pathogens include *S. pneumoniae* (35.0%; n=326), *Salmonella* spp (15.1%; n=141) and *Escherichia* spp (10.7%; n=100). *S. aureus* was isolated from 198 (17.5%) of children with bacteraemia and was the most common cause of neonatal bacteraemia (46.4%; 84/181). Of the 198 *S. aureus* bacteraemia cases, 76 were identified through referral surveillance and 122 through admission surveillance. In seven children with bacteraemia, *S. aureus* and a second bacterial pathogen were isolated.

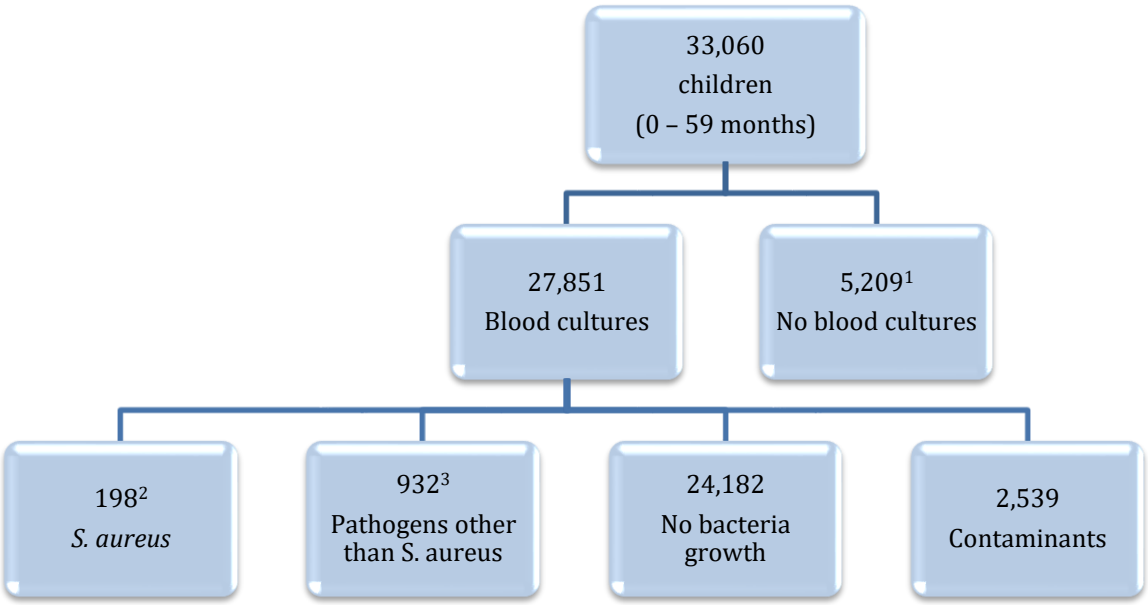


Figure 3.3: Study profile (2008 - 2015)

[1] Reasons for not having blood culture done include unsuccessful venepuncture 487, declined consent for venepuncture 416, declined consent to join study 249, reasons not given 4057

[2] 76 identified through referral surveillance and 122 admission surveillance

[3] Seven patients had polymicrobial (*S. aureus* and a second bacterial pathogen) bacteraemia, n=521 cases identified through referral surveillance and n=418 through admission surveillance

3.4.3 Clinical features of all patients identified through both surveillance systems

Patients with SAB were significantly younger than those with bacteraemia due to other pathogens and those without bacteraemia (p<0.001) (Table 3.4). Fewer SAB patients presented with a cough and difficult breathing than patients with bacteraemia caused by other pathogens and those without bacteraemia (Table 3.5). SAB patients were less likely to be severely wasted compared to patients with bacteraemia due to other pathogens and more likely to be severely wasted compared to patients without bacteraemia. More SAB patients presented with an altered level of consciousness compared to patients with bacteraemia due to other pathogens, while the reverse was the case for signs of meningism. SAB patients were more likely to have a diagnosis of suspected septicaemia or other focal sepsis and less likely

to have a diagnosis of suspected pneumonia compared with patients with bacteraemia caused by other pathogens and those without bacteraemia ($p<0.001$) (Table 3.5).

Among SAB cases, a diagnosis of suspected pneumonia, meningitis, or septicaemia was made in 28.3%, 6.7%, and 56.2% of cases, respectively (Table 3.5). The median (IQR) duration of hospital stay for SAB patients was 5 (2, 6) days (Table 3.4). SAB patients had significantly longer hospital stay compared to patients with other bacteraemia and those without bacteraemia (Table 3.4). One hundred and thirty-one SAB cases were tested for malaria, of whom 10.7% tested were positive.

Table 3.4: Characteristics of patients with *S. aureus* bacteraemia, bacteraemia other than due to *S. aureus*, and those with suspected pneumonia, meningitis, or septicaemia without bacteraemia, identified through referral and admission surveillance in the Basse and Fuladu West HDSS (2008 – 2015)

Patient characteristics	Patients with *S. aureus* bacteraemia (N=198) n/N (%)	Patients with bacteraemia other than *S. aureus* (N=932) n/N (%)	Patients without bacteraemia (N=24,182) n/N (%)	p-value[1]
Age				
<1 month	84/198 (42.4)	97/932 (10.4)	1911/24177 (7.9)	
1 – 11 months	61/198 (30.8)	310/932 (33.3)	8675/24177 (35.9)	
12 – 23 months	33/198 (16.7)	265/932 (28.4)	7505/24177 (31.0)	
24 – 59 months	20/198 (10.1)	260/932 (27.9)	6086/24177 (25.2)	<0.001
Sex				
Males	97/198 (49.0)	532/932 (57.1)	13740/24177 (56.8)	
Females	101/198 (51.0)	400/932 (42.9)	10437/24177 (43.2)	0.14
Severely wasted (weight for height <-3SD)[2]				
Yes	20/109 (18.3)	216/884 (24.4)	3425/21736 (10.5)	<0.001
Mid-upper arm circumference (cm)				
< 11	81/198 (40.9)	184/932 (19.7)	3080/24182 (12.7)	
≥ 11	117/198 (59.1)	748/932 (80.3)	21102/24182 (87.3)	<0.001
Admitted in the past two weeks				
Yes	31/162 (19.1)	157/843 (18.6)	3995/21897 (18.2)	0.93
Length of hospital stay (days)				
Median (IQR)	5 (2, 6)	4 (3, 6)	3 (2,4)	<0.001
Season[3]				
Dry	101/198 (51.0)	597/932 (64.1)	13836/24171 (57.2)	
Wet	97/198 (49.0)	335/932 (35.9)	10335/24171 (42.8)	<0.001
Outcome				
Recovered	170/198 (85.9)	771/932 (82.7)	23322/24182 (96.4)	
Died	28/198 (14.1)	161/932 (17.3)	860/24182 (3.6)	<0.001

SD=standard deviation. Weight-for-height was calculated in STATA using z-scores from the 2006 WHO child growth standards in STATA.

[1] p-value was calculated using Chi-square test; comparison is across patient groups

[2] Neonates were excluded

[3] Wet season = July-October; dry season=November-June

Table 3.5: Symptoms and signs of patients with *S. aureus* bacteraemia, bacteraemia other than due to *S. aureusS. aureus* , and those with suspected pneumonia, meningitis, or septicaemia without bacteraemia, identified through referral and admission surveillance in the Basse and Fuladu West HDSS (2008 – 2015)

Symptoms or signs	Patients with *S. aureus* bacteraemia (N=198) n/N (%)	Patients with bacteraemia other than *S. aureus* (N=932) n/N (%)	Patients without bacteraemia (N=24,182) n/N (%)	p-value[1]
Symptoms				
Cough	103/198 (52.0)	675/928 (72.7)	19523/24148 (80.9)	<0.001
Difficult breathing	89/197 (45.2)	535/927 (57.7)	14280/24102 (59.3)	0.003
Prostration	29/197 (14.7)	147/918 (16.0)	1602/23906 (6.7)	<0.001
Diarrhoea	38/190 (20.0)	271/861 (31.5)	5798/22772 (25.5)	<0.001
Convulsion	8/198 (4.0)	72/927 (7.8)	1174/24127 (4.9)	<0.001
Signs				
Lower chest wall in-drawing	164/198 (82.8)	732/927 (79.0)	17856/24129 (74.0)	<0.001
Meningism[1]	1/192 (0.5)	34/867 (3.9)	174/22841 (0.8)	<0.001
Altered level of consciousness	124/193 (64.2)	407/873 (46.6)	9590/23518 (40.8)	<0.001
Axillary Temperature				
<36.5°C	18/198 (9.1)	79/932 (8.5)	2405/24182 (10.0)	
36.5 – 37.5°C	40/198 (20.2)	147/932 (15.8)	6819/24182 (28.2)	
>37.5°C	140/198 (70.7)	706/932 (75.7)	14958/24182 (61.8)	<0.001
Pulse Rate (/min)[2]				
Abnormal for age	84/198 (42.4)	621/932 (66.6)	15107/24182 (62.5)	<0.001
Respiratory Rate[3] (/min)				
Abnormal for age	128/198 (64.6)	682/932 (73.2)	17157/24177 (71.0)	0.07
Oxygen saturation (%)				
≤92%	33/198 (16.7)	116/932 (12.4)	2140/24182 (8.9)	<0.001
Suspected diagnosis[4]				
Pneumonia	55/194 (28.3)	347/896 (38.8)	13244/23068 (57.4)	
Meningitis	13/194 (6.7)	96/896 (10.7)	718/23068 (3.1)	
Septicemia	109/194 (56.2)	434/896 (48.4)	8549/23068 (37.1)	
Other focal sepsis	17/194 (8.8)	19/896 (2.1)	557/23068 (2.4)	<0.001
Malaria positivity[5]				
Yes	14/131 (10.7)	84/723 (11.6)	3276/21626 (15.2)	0.01

[1] Meningism is defined as the clinical syndrome of headache, neckstiffness and photophobia. *Solomon T., Manji H. Neurologic Diseases: In Hunter's Tropical Medicine and Emerging Infectious Disease (9th Edition) Saunders Elsevier2013, Pages 84-97*

[2] Definition of normal pulse rate: Less than 1 month old: 70 to 190 beats per minute; 1 to 11 months old: 80 to 160 beats per minute; 1 to 2 years old: 80 to 130 beats per minute; 3 to 4 years old: 80 to 120 beats per minute; *Bernstein D. Evaluation of the cardiovascular system: History and physical evaluation. In: Kliegman RM, Stanton BF, St. Geme JW III, et al., eds. Nelson Textbook of Pediatrics. 19th ed. Philadelphia, PA: Elsevier Saunders; 2011: chap 416.*

[3] Abnormal respiratory rates: >60 breaths per minute for children less than two months of age, >50 breaths per minute for children between 2 months and 12 months of age, >40 breaths per minute for children between >12 months and five years of age (Handbook of IMCI).

[4] Surveillance diagnosis was categorised into mutually exclusive groups in order of severity (meningitis>>septicemia>>pneumonia).

[5] Malaria was tested using rapid diagnostic test (ICT Malaria P.f. Antigen, ICT Diagnostics, http://www.ictdiagnostics.co.za).

3.4.4 Incidence and risk factors for *S. aureus* bacteraemia

Using the combined referral and admission data (2011 - 2015 in BDHSS and 2012 - 2014 in FWHDSS), the incidence of SAB was 78 cases per 100,000 person-years (95% CI 67 - 91 cases per 100,000 person-years) in children aged 0 - 59 months. The incidence was highest in the neonatal period (2,080 cases per 100,000 person-years; 3.5 cases per 1,000 live births) and decreased in older age groups (Figure 3.4). The incidence of SAB in the 1 - 11 month age group was 133 cases per 100,000 person-years (95% CI 99 – 174 cases per 100,000 person-years) and in the 1 - 4 year age group the incidence was 27 cases per 100,000 person-years (95% CI 20 – 36 cases per 100,000 person-years). Among the 84 neonatal SAB cases, 13 (15.5%) and 35 (41.6%) presented in the first and second weeks of life, respectively (Figure 3.5). The incidence of SAB was higher in the wet season than in the dry season (Table 3.6).

Table 3.6: Factors associated with *S. aureus* bacteraemia in Basse and Fuladu West HDSS (2011 - 2015)

Variables	Cases/Person-years at risk	Rate/100,000 person-years	Rate ratios (95% CI)	p-value
Age				
24-59 months	18/128994	14.0	1	
12-23 months	29/44433	65.3	4.7 (2.6-8.4)	
1–11 months	53/39969	132.6	9.5 (5.6-16.2)	
< 1 month	70/3367	2079.6	149.0 (88.8 – 250.1)	<0.001
Sex				
Males	82/107515	76.3	1	
Females	88/109248	80.6	1.06 (0.8-1.4)	0.72
Season				
Dry	85/144508	58.8	1	
Wet	85/72255	117.6	2.00 (1.5-2.7)	<0.001

CI=Confidence Interval

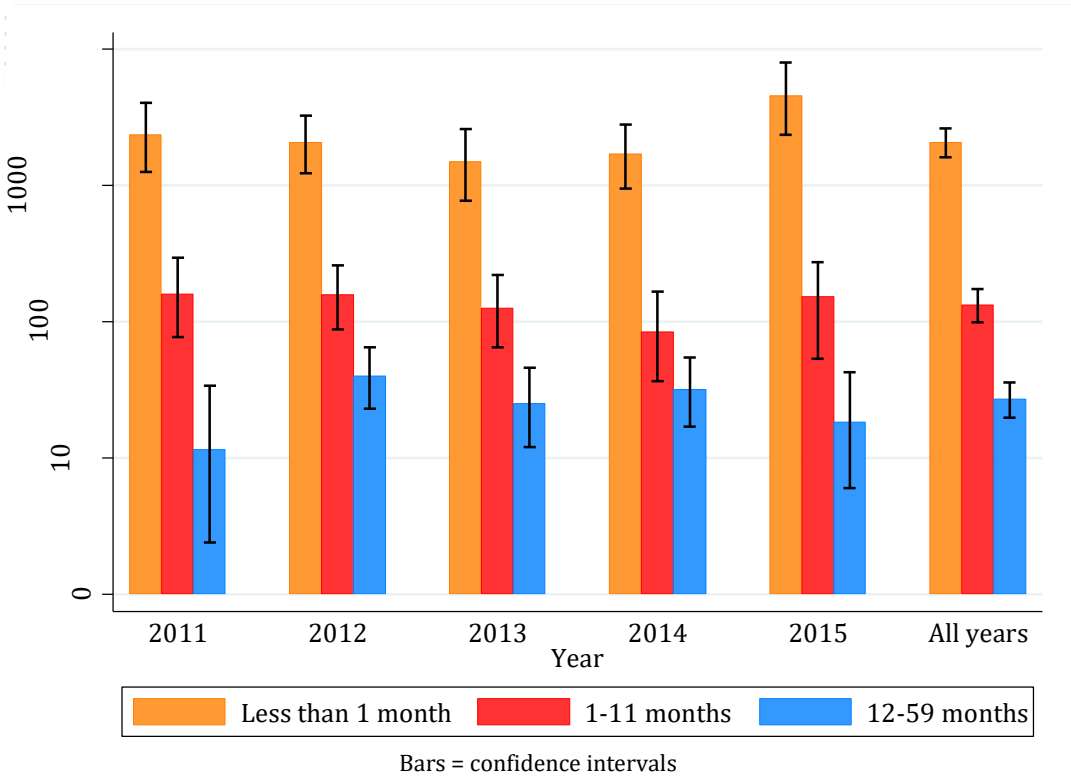


Figure 3.4: Age-specific incidence of *S. aureus* bacteraemia (cases identified through combined referral and admission surveillance) in children aged 0-59 months in the Basse and Fuladu West HDSS in 2011 - 2015.

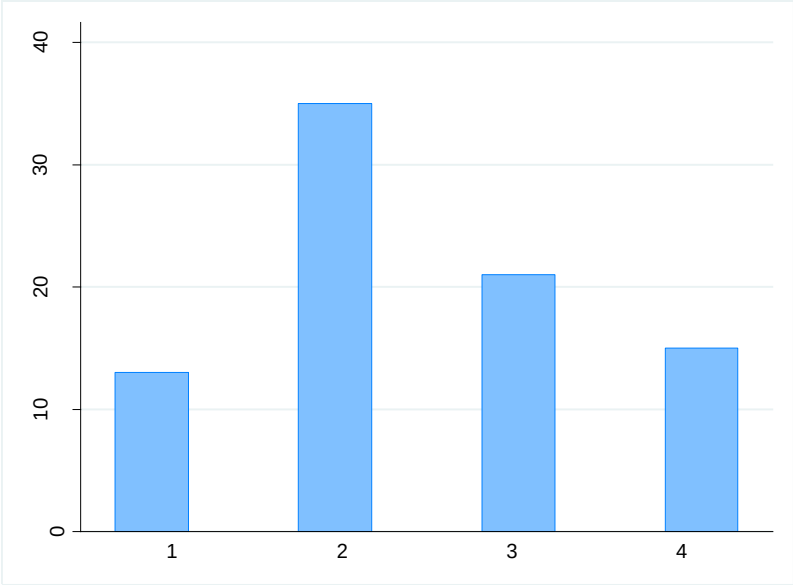


Figure 3.5: Frequency of cases by week during the neonatal period

3.4.5 Trends in the incidence of SAB over time

Based on referral surveillance conducted between 2008 and 2015 in the BHDSS, the mean annual incidence of SAB in children aged 2 - 59 months was 22.3 cases per 100,000 person-years (95% CI 16.7 – 29.2). There was no change in incidence over this period (p-value for trend=0.28) although PCV vaccination coverage increased during the period (Figure 3.6).

Using referral data, among children aged 2 - 59 months there were nine observed and ten adjusted SAB cases in the pre-PCV period (May 12, 2008 – May 11, 2010) and 26 observed and 23 adjusted SAB cases in the PCV13 period (January 1, 2013 - December 31, 2015). After adjusting for increased enrolment of eligible children over time, there was no significant increase in SAB incidence from the pre-PCV (18 cases per 100,000 person-years) to the PCV13 period (23 cases per 100,000 person-years) (IRR=1.3, 95% CI 0.6 – 2.7; p=0.49).

3.4.6 Case fatality ratios and risk factors associated with fatality

There were 28 deaths before discharge among 198 SAB cases (CFR=14.1% 95% CI 9.4 – 20.4). In comparison, the CFR in patients without bacteraemia was 3.6% (95% CI 3.3 – 3.8) and 17.2% (95% CI 14.7 – 20.1) in patients with bacteraemia due to other pathogens. The SAB CFR did not vary by year (p-value for trend=0.75) or with age (p-value for trend=0.99). Deaths associated with SAB were most common on the day of admission (71.4%; 20/28). Of the fatality that occurred in neonates, 15.0% (2/13) died in the first week of life while 85.0% (11/13) occurred in the last three weeks of the neonatal period. SAB deaths accounted for 7.6% (13/171; 0.7 per 1,000 live births) and 4.2% (28/662; 1.4 per 1,000 live births) of all deaths in neonates and children aged less than five years, respectively between 2011 and 2015. About 5.5% (3/55), 38.5% (5/13), and 17.4% (19/109) of patients with pneumonia,

meningitis, and septicaemia, respectively died. A risk factor for fatality was clinical prostration at presentation (Table 3.7).

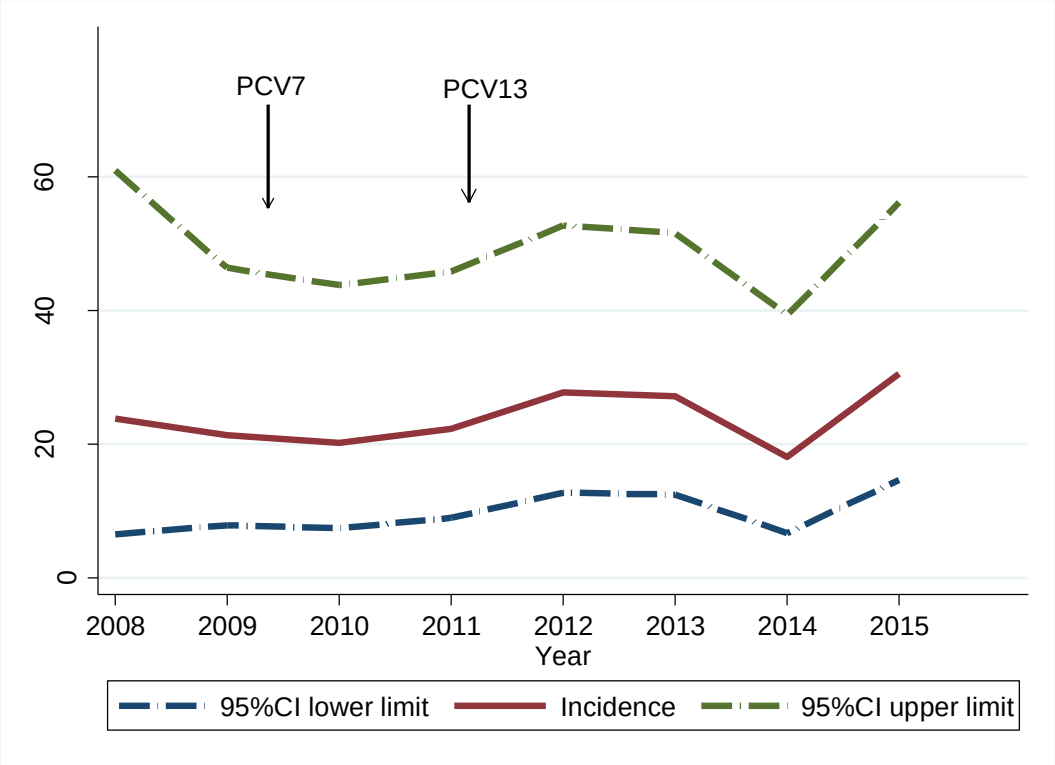


Figure 3.6: Annual incidence of *S. aureus* bacteraemia per 100,000 person-years in children aged 2 - 59 months (cases identified through referral surveillance) in the BHDSS.

Table 3.7: Case fatality associated with *S. aureus* bacteraemia*S. aureus* and socio-demographic, clinical parameters

Variables	Deaths/Persons at risk (%)	Unadjusted OR (95% CI)	p-value
Age			
< 1 month	13/84 (15.5)	1	
1 – 11 months	8/61 (13.1)	0.8 (0.3 – 2.1)	
12 - 23 months	4/33 (12.1)	0.8 (0.2 – 2.5)	
24 - 59 months	3/20 (15.0)	1.0 (0.3 – 3.8)	0.96[1]
Sex			
Males	16/97 (16.5)	1	
Females	12/101 (11.9)	0.7 (0.3 – 1.5)	0.35
Severely wasted (weight-for-height <-3SD)[2]			
No	19/149 (12.8)	1	
Yes	4/40 (10.0)	0.8 (0.2 – 2.4)	0.63
Axillary temperature			
36.5 – 37.5°C	4/18 (22.2)	1	
<36.5°C	4/40 (10.0)	0.4 (0.1 – 1.8)	
>37.5°C	20/140 (14.3)	0.6 (0.2 – 2.0)	0.48
Pulse Rate (/min)			
Normal for age	13/114 (11.4)	1	
Abnormal for age	15/84 (17.9)	1.7 (0.8 – 3.8)	0.20
Respiratory Rate (/min)			
Normal for age	8/70 (11.4)	1	
Abnormal for age	20/128 (15.6)	1.4 (0.6-3.5)	0.41
Need for oxygen supplementation			
No	21/165 (12.7)	1	
Yes	7/33 (21.2)	1.9 (0.7 – 4.8)	0.22
Season			
Dry	18/101 (17.8)	1	
Wet	10/97 (10.3)	0.5 (0.2 – 1.2)	0.13
Cough			
No	13/95 (13.7)	1	
Yes	15/103 (14.6)	1.1 (0.5- 2.4)	0.86
Difficult breathing			
No	14/108 (13.0)	1	
Yes	14/89 (15.7)	1.3 (0.6 – 2.8)	%)0.58
Prostration			%
No	17/168 (10.1)	1	
Yes	11/29 (37.9)	5.4 (2.2 – 13.4)	0.0004
Admitted in the past two weeks			
No	20/131 (15.3)	1	
Yes	2/31 (6.5)	0.4 (0.1- 1.7)	0.16

OR=Odds Ratio; CI=Confidence Interval; SD=Standard Deviation.

[1] p-value for trend

[2] Weight-for-height were calculated using z-scores from the 2006 WHO child growth standards in STATA.

3.4.7 Treatment and susceptibility of isolates

Among SAB cases identified through referral surveillance, 17.1% (13/76) received initial empiric therapy with cloxacillin, 23.7% (18/76) empiric ampicillin and 31.6% (24/76) empiric penicillin while 50.0% (38/76) received empiric gentamicin. Fifty (65.8%) patients received more than one antibiotic while 26 (34.2%) had monotherapy. Only 53 (69.7%) patients had appropriate antibiotics. Appropriate antibiotics are those that were considered effective for treatment of diseases caused by *S. aureus.* There was no difference in mortality according to the empiric treatment received. Among the SAB cases, 3.1% of the 193 isolates tested were methicillin-resistant using cefoxitin as a surrogate (Table 3.8). At least 80% of the isolates were sensitive to all the antibiotics tested except cotrimoxazole and penicillin.

Table 3.8: Antibiotic susceptibility of *S. aureus* isolates*S. aureus*

Antibiotic	Number of isolates tested	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Cefoxitin	193	187 (96.9)	0 (0.0)	6 (3.1)
Chloramphenicol	186	176 (94.7)	2 (1.0)	8 (4.3)
Cotrimoxazole	180	119 (66.1)	21 (11.7)	40 (22.2)
Erythromycin	173	141 (81.5)	24 (13.9)	8 (4.6)
Gentamicin	177	174 (98.3)	0 (0.0)	3 (1.7)
Oxacillin	194	170 (87.6)	24 (12.4)	0 (0.0)
Penicillin	101	7 (7.0)	0 (0.0)	94 (93.0)

Cefoxitin was used as a surrogate for methicillin-resistance according to CLSI

3.5 Discussion

The incidence and case fatality ratio of *S. aureus* bacteraemia (SAB) in rural Gambia were estimated using surveillance data over five years and trends in incidence over 8 years were also evaluated. The incidence was high, particularly in neonates (3.5 cases per 1,000 live births) but did not increase with time or following the introduction of PCV (Figure 3.6). The case fatality ratio (14.1%) was substantial (Table 3.6) and did not vary with age.

Incidence of SAB

The observed incidence of SAB in children aged 0 - 59 months (78 cases per 100,000 person-years) was higher than that reported in some studies conducted in high-income countries (6.5 – 42.0 cases per 100,000 person-years),[26,70] and in some African[6] and Asian[44,298] studies but lower than in others.[45] An incidence of SAB of 10.9 cases per 100,000 person-years was reported among children less than 18 years of age in Iceland.[70] This was a retrospective study in which both clinical and microbiological data were obtained from medical records in the two tertiary hospitals serving the area. Additionally, other secondary healthcare facilities in the area sent their blood cultures to one of the tertiary hospitals. In Australia and New Zealand, a study that prospectively reviewed cases of SAB in 33 hospitals reported an incidence of 8.3 cases per 100,000 person-years and 14.4 cases per 100,000 person-years, respectively in children less than 15 years of age.[79] However, the reported incidence in both studies might have been under-estimated because some cases may have presented to hospitals other than those used in the studies. The difference in our estimate and those of these studies may be related to the age of the population studied.

In Thailand, a study of bacteraemia which reviewed national hospital-based data reported a SAB incidence of 83.5 cases per 100,000 person-years in children less than 12 months old.[44] A Bangladeshi population-based study which focused on children aged 1 - 59 months with respiratory symptoms reported an incidence of 9.9 cases per 100,000 person-years.[298]

In South Africa, a study that reviewed the records of SAB patients aged less than 13 years in the only public hospital serving the study area reported an incidence of 26 cases per 100,000 person-years.[71] The difference in incidence reported in the South African and Asian studies may be due to the high prevalence of HIV in the community. *S. aureus* diseases have higher incidence rates in HIV-infected compared to HIV-uninfected children.[71,82] Other reasons for the difference include the age of the population and the clinical syndrome studied. A Kenyan study reported a SAB incidence of 27 cases per 100,000 person-years in children less than five years of age[6] while an incidence of 118 cases per 100,000 person-years was observed in children aged less than five years in Mozambique.[45] Even though the Kenyan and Mozambican studies were population-based, the difference in reported incidence may be attributable to the prevalence of HIV in the two countries. The prevalence of HIV in pregnant women attending the antenatal clinic in Kenya was 9.8%[299] while that in Mozambique was 23.4%.[300] Other differences in incidence between these studies including the current study are likely to be related to the selection criteria employed by the various studies or related to the high levels of antibiotic use without a prescription, especially in Asia.[301]

There was no trend over time in the incidence of SAB between 2008 and 2015. Researchers in the United States and The Gambia have reported an increase in the proportion of all bacteraemia caused by *S. aureus* following the introduction of PCV.[287,288] Both of these studies were retrospective reviews of medical records. However, our data do not support an association between SAB incidence and the introduction of PCV. Nevertheless, this study

needs to be repeated in other settings to further ascertain the relationship between SAB and PCV introduction.

Neonatal SAB

S. aureus was the most common cause of bacteraemia in neonates, and the incidence of SAB was highest in neonates, eight times greater than that reported in Kenya.[6] This finding is similar to other studies in Africa,[45,48,250,302] where *S. aureus* was responsible for between 39.0% and 56.2% of isolates recovered from neonates.

S. aureus carriage, which is a prerequisite for disease, is also highest during the neonatal period, higher than the carriage of *S. pneumoniae* and *H. influenzae.*[100] In addition to high carriage rates of *S. aureus* in the neonatal period, other reasons for the high risk of SAB may include an immature innate immune system.

The incidence of neonatal SAB (3.5 cases per 1,000 live births) was higher than the other pathogens responsible for neonatal bacteraemia. In Africa, the pooled incidence of neonatal bacteraemia caused by group B *streptococcus* (GBS) was 1.12 cases per 1,000 live births,[303] while the incidence of *E. coli* bacteraemia ranged between 0.8 cases per 1,000 live births[6] and 1.7 cases per 1,000 live births.[250]

In this study, only 16% and 42% of the neonatal SAB cases presented within the first and second weeks of life respectively unlike neonatal GBS disease where about 80% of patients present within this period. Reasons for the difference in timing of presentation may relate to the age at, and source of *S. aureus* acquisition. Unlike neonatal GBS disease where the source of transmission is from the mother's birth canal during delivery, the source of

acquisition may be different in neonatal SAB. Acquisition of *S. aureus* during the neonatal period peaks by the end of the first week of life.[25,100,101] In Tanzania, a study on the aetiology of neonatal infections reported that SAB*S. aureus* was more common in babies after the first week of life (which is similar to the finding in this study) and that *S. aureus* was the most frequent cause of pustules and omphalitis throughout the neonatal period.[48] This finding demonstrates that the source of *S. aureus* infection may be from handling by healthcare workers or family members or contact with hospital surfaces and fomites. A Ugandan study which aimed to correlate the pathogens recovered from neonates with sepsis with those recovered from their mothers' vagina demonstrated no concordance between the pair.[266] This shows that transmission to neonates may be from a different source. Further studies are needed to clarify how neonates acquire their infection.

Risk factors for *S. aureus* bacteraemia

S. aureus bacteraemia was found to be more common during the wet season. This seasonal variation may relate to *S. aureus* carriage (a prerequisite for disease), the prevalence of which is highest during the wet season[304] or seasonal differences in the incidence of viral infections which are known to disrupt the mucosal epithelium, thereby encouraging *S. aureus* invasion.[305] In a study of US adults,[306] the peak incidence of *S. aureus* infection occurred during the winter months and coincided with the peak incidence of viral infections. In Africa, the incidence of viral infections usually peaks during the wet season,[246] coinciding with the peak SAB incidence. Of note, this surveillance was not designed to assess risk factors.

Diagnosis

Blood culture was the diagnostic method used in this study because it is the gold standard and affords the opportunity for antibiotic sensitivity testing and comparability to other studies. However, blood culture has a low sensitivity which might have led to an underestimation of the incidence. The yield could have been increased from about 65% to between 80% – 95% by taking two or more blood cultures per patient. Most authorities recommend collection of multiple sets of blood culture samples. However, such an approach is not the norm in epidemiological studies.

The administration of antibiotics before sampling inhibits the growth of the pathogen leading to an under-estimation of disease incidence. In this study, apart from the fact that the use of antibiotics before sampling was uncommon, the utilisation of BACTEC culture media with antimicrobial-binding agents such as resins and charcoal should have reversed the inhibitory effects of the antibiotics.[307] This is particularly true for staphylococci.[307]

Prior antibiotic use, treatment, and antimicrobial resistance

Use of antibiotics in the week before presentation or sampling for cultures is known to delay the median time to grow or outrightly mask the yield of pathogens. In this study, the use of antibiotics in the week before presentation was uncommon and would have had a negligible influence on the incidence of SAB.

Only 70% of SAB cases received appropriate antibiotics in this study. However, there was no difference in mortality by treatment groups. About 66% of the patients received more

than one antibiotic concurrently. As per the National guidelines, two antibiotics are recommended for the treatment of suspected septicaemia and severe pneumonia.

About 12% of the *S. aureus* isolates were resistant to oxacillin. This is similar to the reports from some studies from Tanzania and Gambia[249,285] and different from other studies from Nigeria.[48,250] In this study, only a few isolates (2%) were resistant to gentamicin. This is comparable to reports from some studies[45,249,285] and contrary to reports from other studies where about 50% of their isolates were resistant.[48] Diverse patterns of antibiotic use may explain this disparity in antimicrobial susceptibility patterns in different settings and varied forms of referral from primary to secondary health facilities.[195] The prevalence of methicillin-resistance is low in this setting and similar to some African studies[50,241] but lower than in a South African study.[71] This difference may be due to the high prevalence of HIV in the South African population because HIV infected patients are exposed to repeated hospital visits,[71] and recurrent antibiotics use.[308]

Contamination

The contamination rate in this study is high (9.1%). This finding is similar to the reports from Iceland and South Africa[70,72] and less than the reports from Kenya, Mozambique and Guinea Bissau.[6,45,46,260] The differences may be due to the method of sample collection (peripheral venepuncture or use of an intravenous catheter for collection), mode of asepsis before sample collection, type of antiseptic, type of gloves, competency of staff and the number of pathogens considered to be contaminants. However, most of these studies did not discuss the process of blood culture sample collection in detail. According to CLSI and the British Department of Health, contamination rate should be less than 3%;[166,309] however,

the National Health Service (NHS) Trusts in the UK state that the rate can be as high as 10%.[309]

Outcomes

Length of hospital stay

The duration of hospital stay in our study population was shorter compared to reports from South Africa[71] and other high-income countries.[200] The differences in the prevalence of HIV and co-morbidities in the populations studied may explain this difference in length of hospital stay. Additionally, according to the Gambian national treatment guidelines, duration of therapy for uncomplicated *S. aureus* disease is five to seven days,[175] shorter than in high-income countries. South Africa has a higher HIV prevalence than The Gambia. HIV patients are more likely to have drug-resistant strains of bacteria[71] and therefore have an extended hospital stay. In this study, we did not perform HIV tests routinely on the study participants as HIV prevalence in The Gambia is low.[297]

Case fatality ratio

Studies from high-income countries reported CFR ranging from 1.5% to 2.6%.[27,70,79] In LMIC, investigators have reported a CFR of between 6.0% and 71.7%.[49,71,247] The CFR observed in the current study (14.1%) is, therefore, within the range of what is expected for a LMIC study. The considerable variation in CFR between studies may be explained by the differences between the study populations regarding age, quality of and access to healthcare, the focus of infection, antibiotic resistance rates, the severity of SAB cases and presence of co-morbidities. In our study, 71.4% of deaths occurred on the day of admission,

which may reflect the severity of disease at presentation. Even though age is strongly associated with SAB-related deaths,[27] in this study, CFR did not vary with age.

Mortality

SAB death in relation to the total number of deaths in the population is a measure of its contribution to neonatal and under-five mortality. In this study, SAB deaths accounted for 7.6% and 4.2% of neonates and children less than five years who died in the population between 2011 and 2015, respectively. These figures are underestimated as some patients might have died before presentation to the hospital. Therefore, SAB makes a considerable contribution to neonatal and child mortality in this setting.

Risk factors for fatality

Even though age is strongly associated with SAB-related death in HIC,[27,310] in our study, CFR did not vary with age. This variation of fatality with age maybe because the majority of the SAB cases seen in high-income countries are hospital-acquired, especially in neonates and infants due to the use of central venous catheters, parenteral nutrition and high survival rates of premature babies.[70] Hospital-acquired infections have the worse prognosis. In this present study, all cases were community-acquired. Other risk factors associated with fatality include the organisation of the health systems, the presence of co-morbidities such as congenital malformations, musculoskeletal diseases, and digestive diseases, among others.

Strengths and limitations

The strengths of this study were that the surveillance was population-based and uninterrupted. Data collection spanned from the pre-PCV era to the post-PCV era, and over 84% of eligible patients had blood culture performed. However, the study also had some limitations. First, the incidence may have been underestimated as some SAB cases never present to the hospital, and the sensitivity of blood culture is less than 100%. Second, an increasing rate of enrolment and investigation overtime required adjustment of annual case counts. Third, prior consumption of antibiotics, although uncommon in this study area, may reduce the detection of SAB by blood culture. Lastly, this study was not explicitly designed to evaluate risk factors for SAB such as *S. aureus* nasal carriage, hospitalisation in the past six months and HIV status.

Conclusion

The incidence of SAB is high in rural Gambia, especially in neonates and infants. Priority should be given to diagnosis, treatment, and prevention of SAB in this and similar settings. Clearly, strategies are urgently needed to reduce the burden of SAB and should target children in the first month of life. Further research into strategies to reduce the disease burden in this age group will be required to decrease neonatal and childhood mortality in The Gambia and similar settings.

Chapter 4: Acquisition of *Staphylococcus aureus* in neonates in rural Gambia: a longitudinal study

Preamble

Chapter three showed that neonates have the highest burden of *S. aureus* bacteremia. *S. aureus* carriage is a significant risk factor for *S. aureus* disease. Therefore, in this chapter, I have explored the risk factors for neonatal *S. aureus* carriage at birth, and those for acquisition at one week of age. Carriage data from a newborn's mother, caregiver, household children and midwife were used to identify potential sources of acquisition.

4.1 Overview

Carriage of *S. aureus* is a significant risk factor for *S. aureus* disease. As discussed in chapter 1, this is evidenced by the fact that patients who develop *S. aureus* disease usually carry the same strain of bacteria as the one causing disease.[24,89,90] Reducing the acquisition of *S. aureus* carriage in newborns is, therefore, an important step in reducing the disease burden among neonates.

As discussed in chapters 2 and 3, neonates have a higher risk of *S. aureus* disease compared to older children, possibly due to the high prevalence of *S. aureus* carriage and poorly developed innate immunity. Studies in low- and middle-income countries (LMIC) have shown that neonatal carriage peaks in the first week of life at between 50% to 60% (See section 1.1.2).[101,103] However, the source of, and the risk factors for acquisition of *S. aureus* in neonates in LMIC are poorly documented. In high-income countries (HIC), longitudinal studies applying both conventional microbiology and molecular methods have identified the

mother as the primary source of neonatal and infantile carriage acquisition either through vertical or horizontal transmission.[94,100,105]

Only two African studies – one in Gabon and the other in The Gambia - have evaluated the source of *S. aureus* carriage acquisition in infants.[104,107] These two studies produced significantly different estimates of the proportion of neonatal carriage that was attributable to maternal carriage: the Gabonese study estimated the figure to be 5.6% while the Gambian study estimated it to be 21.0%. The disparity between these estimates might be due to the differences between the studies in terms of the timing of screening, the anatomical screening sites used, the use of antibiotics and the method of *S. aureus* identification (the Gabonese study used molecular methods and the Gambia study used conventional microbiology). Although, molecular methods are more sensitive than cultures at detecting *S. aureus*,[311] the participants in the Gabonese study used antibiotics in the immediate post-partum period.

Despite the differences, these African studies suggest that newborns frequently acquire *S. aureus* carriage from sources other than the mother. Other potential sources could include healthcare workers (HCW), hospital surfaces and equipment, other caregivers, siblings, or household members.

While risk factors for *S. aureus* carriage in infants have been studied,[100,107] there are few data regarding carriage at birth or carriage acquisition in the early neonatal period. Risk factors for *S. aureus* carriage among infants include maternal carriage,[100,107] breastfeeding,[100] increasing number of older siblings, and ethnicity.[107] It is unclear if these risk factors apply equally to carriage at birth and carriage acquisition shortly thereafter.

Furthermore, a significant pathway for the emergence and spread of resistant strains of bacteria is the development of resistance first in carriage isolate due to exposure to

antibiotics during carriage and then transfer of these genes horizontally to pathogenic strains.[312] In The Gambia, few studies have reported on the pattern of antibiotic susceptibility of *S. aureus* isolates among healthcare workers or in the community.[98,313] Given the genetic similarities between carriage and clinical strains in patients with disease,[24,314] knowledge of antibiotic susceptibility profiles in the community can inform the choice of empirical therapy in community-acquired *S. aureus* disease. Accordingly, this study was conducted to close these gaps in knowledge.

Hypothesis

Risk of *S. aureus* carriage acquisition in a newborn in the first week of life after exposure to maternal sources is higher than the risk after exposure to non-maternal sources

4.2 Objectives

The study objectives were to:

a. Determine the incidence of carriage acquisition on the seventh day of life and assess the associated risk factors
b. Determine the prevalence of *S. aureus* carriage at birth and assess the associated risk factors
c. Describe the antibiotic susceptibility pattern of *S. aureus* carriage isolates obtained from study participants

Outcomes: Microbiological isolation of *S. aureus* from the nose or umbilicus of the newborn at birth and on the seventh day of life.

Exposures: Potential sources of exposure of the newborn were investigated by assessment of the carriage of *S. aureus* in the nasal, vaginal or breastmilk samples obtained from the mother, or in nasal samples obtained from the midwife or TBA and household contacts, and isolation of *S. aureus* from delivery-related hospital surfaces. In addition, a questionnaire was used to collect information on sociodemographic data and maternal factors such as age at delivery, occupation, educational status, parity, hospitalisation in the preceding six months, antibiotic use in the preceding six months, current skin disease, nose-picking and degree of contact with animals. The questionnaire was also used to collect data on perinatal and postnatal factors such as premature rupture of membranes, dysuria, foul-smelling vaginal discharge, mode and place of delivery, maternal history of fever in the week preceding delivery, umbilical cord care, breastfeeding, hand washing, and the sharing of fomites with the newborn.

4.3 Methods

4.3.1 Fieldwork

Study site, design, and population

The study was conducted in the Basse Health Centre (BHC) and villages located in the Upper River Region of The Gambia between February and June 2017. The study site is described in section 3.1.1 and includes villages that are part of the Basse Health and Demographic Surveillance System. The labour ward in the BHC has three delivery couches separated by plastic drapes, three beds for women in active labour, a resuscitaire, a sink, an autoclave and a nurses' station. The postnatal ward, directly opposite the labour ward, has six beds with a cot next to each bed. Visitors are allowed in the postnatal ward but not in the labour ward.

Annually, about 2,000 deliveries take place at the BHC, which is the main referral hospital in the region. Box 4.1 highlights the prevailing infection control practices in the labour ward during the study period. The study recruited mothers and their healthy infants delivered either at home or at the labour ward of the BHC and the midwife or traditional birth attendant (TBA) conducting the delivery. In addition, two children under five years of age resident in the same household as the newborn and a caregiver other than the mother (usually the maternal grandmother) were recruited.

Recruitment of participants

In The Gambia, 98% of pregnant women attend an antenatal clinic (ANC) at least once before delivery.[315] We approached consecutive pregnant women in their third trimester of pregnancy (as assessed by trained staff), and those who met the eligibility criteria (Table 4.1) were invited to participate in the study after the study procedures were explained to

Box 4.1

Infection prevention and control practices in the labour ward during the study period.

Hand hygiene

- Handwashing with liquid soap and water
- Hand hygiene with alcohol-based hand rubs
- Dress code – short-sleeve tops or dresses exposing elbows
- Nurse-to-patient ratio 1:4 during the day and 1:9 at night

Environmental cleaning and decontamination

- Daily cleaning of the floor with water, soap, and disinfectant
- Daily disposal of hospital waste
- Decontamination of delivery couch and resuscitaire after every delivery
- Plastic drapes only cleaned and disinfected if visibly stained with blood
- Terminal cleaning and disinfecting of patient's environment

them and they had provided written informed consent. We excluded high-risk pregnancies (as defined in Table 4.1) because the underlying risks for newborn acquisition of *S. aureus* in high-risk pregnancies are likely to be different compared to healthy pregnancies. Additionally, mothers with high-risk pregnancy were likely to have eventful deliveries and therefore require antibiotics postpartum.

Pre-delivery visit

A member of the study field staff visited the pregnant woman's household to further explain the details of the study to household members and reinforce the previously written consent. This process was completed within two weeks of obtaining written informed consent.

Table 4.1: Eligibility criteria

	Inclusion criteria	Exclusion criteria
Mother	All pregnant women (between 30- and 36-weeks' gestation) aged 15-49 years attending the antenatal clinic at Basse Health Centre	High-risk pregnancy[1]
	No plans to travel out of the study area in the week after delivery	Maternal use of antibiotics within one month of enrolment
Newborn	Alive and apparently healthy infants born to women who had provided consent to participate in the study	Acute or chronic illness or obvious congenital abnormality
		Birth weight less than 2.0kg

[1]High risk pregnancy is defined as one that threatens the health or life of the mother or her foetus for example, teenage pregnancy, antepartum haemorrhage, postpartum haemorrhage, eclampsia, diabetes, hypertension, premature labour or delivery, oligohydramnios, polyhydramnios.

At birth and follow-up visits

Willingness to continue participation in the study was verbally confirmed from the mother when she presented in labour. Pre-delivery, while the mother was in labour, we obtained vaginal and nasal samples from the mother, nasal, and hand swabs from the attending

midwife- or TBA-in-charge of the delivery and swabbed the delivery couch and resuscitaire (or improvised delivery couch and resuscitaire if the delivery took place at home).

Within the first two hours, post-delivery, we obtained nasal and umbilical samples from the newborn. The questionnaire was used to obtain information on potential risk factors during pregnancy, labour, and delivery from the mother postpartum (see the section on exposure). At discharge from the hospital, participants were visited in their homes where further information on potential risk factors was documented, and nasal swabs were collected from caregivers and two children under the age of five years living in the household (randomly selected for the BDHSS list). The caregiver was defined as the person, as identified by the mother, who provided the most care for the newborn other than the mother (usually the maternal grandmother).

Table 4.2: Study procedures

Time/Study Procedure	Pre-delivery	Birth, Day 0 (Visit 1)	1 week post-delivery, Day 7 (Visit 2)
Inform HHH[1]	√		√
Written consent	√		
Administer questionnaire		√	√
Maternal			
Vaginal swab		√	
Nasal swab		√	√
Breast milk			√
Newborn			
Nasal swab		√	√
Umbilical swab		√	√
One other caregiver			
Nasal swab		√	√
Midwife/TBA[1]			
Nasal swab		√	
Hand swab		√	
Delivery equipment			
Resuscitaire		√	
Delivery bed		√	
Other HH[1] children (age <5years)			
Nasal swab		√	√

[1] HHH= Household head, HH=Household; TBA=Traditional Birth Attendant
Each visit had a window period of ±2 days

nasal samples from the mother, baby, caregiver and the two other children already selected for sampling. An umbilical swab was obtained from the newborn, and breast milk was collected from the mother (Table 4.2). The field assistant or nurse recorded the date and time of sample collection. A questionnaire was administered to obtain information on umbilical cord care, breastfeeding, as well as the sharing of bed and fomites between other family members and the newborn. For mothers who opted to deliver at home, the TBA notified the study team that the woman was in labour. The study team visited the home of the woman, obtained samples, and administered questionnaire as described above.

The study team did not provide medicines. Instead, mothers were encouraged to report at the clinic or call the study team if they or their newborn were unwell or had concerns about their health or that of their newborn. Participants were free to withdraw from the study at any time and were not obliged to provide a reason.

4.3.2 Sample size calculation

The sample size calculation was based on the expectation that 63% of newborns would acquire *S. aureus* in the first week of life,[107] and that the prevalence of exposure (e.g. carriage in adults and children in the community) would be 25%.[98] Based on these parameters, it was estimated that a minimum of 216 mother-newborn-contact pairs would be required to detect a risk ratio of at least 1.3 between exposed (for example, exposure to carrier non-maternal sources) and unexposed groups with 80% power at the 5% significance level.

4.3.3 Samples and laboratory assessments

Sample collection and handling

Nasal Swab

Nasal swabs were collected by brushing the walls of both nostrils with nylon flocked swabs FLOQSwabs™ (Copan, Brescia, Italy).

Vaginal Swabs

We obtained low vaginal swabs by inserting a cotton swab (Sterlin Ltd, Newport, UK®) 2-3 cm into the vagina, sweeping the mucosa of the vagina and rotating the swab.

Breast milk samples

Mothers were asked to express breast milk manually after disinfecting the nipple and areola with 0.02% chlorhexidine. After discarding the initial 0.5 mL, 1 - 2 mL of breast milk was expressed directly into a sterile Bijoux bottle (Thermo Fischer Scientific, Loughborough, UK®).

Umbilical swab

Umbilical swabs were collected by brushing a cotton swab (Sterlin Ltd, Newport, UK®) moistened with normal saline and applying the wet swab around the umbilical stump in a clockwise fashion.

Hand swab

Shortly before delivery, a moistened cotton swab (Sterlin Ltd, Newport, UK®) was rubbed on both hands and between the digits, nail beds, and ring area of the midwife or TBA, paying attention to the clefts between the digits.

Environmental swab

A moistened cotton swab (Sterlin Ltd, Newport, UK®) was used to brush a marked 10 cm by 10 cm area on the delivery couch and resuscitaire. The swab was wiped horizontally and then vertically over the area. Sampling was done immediately before delivery and not after cleaning of the surfaces.

All swabs were immediately inoculated into skim-milk-tryptone-glucose-glycerol (STGG)-containing vials placed in a cold box maintained at between 2^0C – 8^0C and transported to the MRC laboratory, at the Basse Field Station within 8 hours of collection. At the laboratory, samples were stored at -70^0C until they were analysed. Each sample had a unique identification number which was also placed on the laboratory request form.

4.3.4 Laboratory methods

Standard microbiology

Stored frozen samples were thawed to room temperature and vortexed for 20 seconds to give a homogenised suspension. An aliquot of the suspension was added to an enrichment broth (tryptone soy) to increase the yield of *S. aureus*[316] and incubated overnight. A 10 µl aliquot of the suspension was plated on mannitol salt agar (MSA) and incubated aerobically at 37^0C overnight. The golden yellowish raised colonies on the MSA plate were subcultured on blood agar (BA) plates and incubated at 37^0C for 24 hours to obtain pure colonies. Presumptive isolates were confirmed as *S. aureus* by performing catalase and coagulase tests using the Staphaurex latex agglutination test (Remel, Lenexa, KS).

Antibiotic Susceptibility screen

The Kirby-Bauer disc diffusion method was used to determine antibiotic susceptibility in accordance with CLSI guidelines.[272] Isolated colonies of *S. aureus* were suspended in normal saline and turbidity adjusted to a 0.5 McFarland standard. The vortexed suspension was used to inoculate a dried Mueller-Hinton Agar plate. Antibiotic discs (Oxoid, UK) were placed on plates and incubated aerobically at 35°C - 37°C for 24 hours. The discs contained cefoxitin (30 µg), clindamycin (2 µg), cloxacillin (5 µg), cotrimoxazole (25/125 µg), erythromycin (15 µg), and penicillin (10 µg). After incubation, the zones of inhibition were measured using a transparent ruler under white light. Diameters of inhibition (in millimetres) were recorded and interpreted as sensitive, resistant or intermediate using the CLSI standards.[166] Cefoxitin was used as a surrogate for methicillin-resistance with a cut-off diameter of 21 mm.[272] The laboratory subscribed to external quality assurance schemes with the World Health Organization Reference Laboratory, Denmark, and United Kingdom National External Quality Assessment Service, Sheffield, UK and Oneworld Accuracy, Canada.

4.3.5 Data collection

Training of staff

The study team comprised five field assistants, two nurses, a scientific officer, a laboratory technician, and a data manager. Staff were trained on the study rationale and objectives. An introductory session was followed by practice sessions on sample collection and labelling, administering questionnaires and sample transport. The investigator developed working documents on study-specific procedures for use by study team members. A separate training session was conducted for the laboratory staff on sample processing. The team met every week to discuss progress, and further periodic training sessions were conducted when

required. Additionally, laboratory staff were trained on the laboratory processes using the study-specific procedure.

Quality assurance for data collection

Trained field assistants and nurses used a questionnaire for data collection. Field assistants reviewed their forms before submission to a supervisor who in turn reviewed the forms every week. In addition, the principal investigator reviewed a randomly selected subset of forms (10%) collected each week before submission for data entry. Field assistants corrected missing data or errors during the next visit or by a phone call. Similarly, the laboratory staff did a self-assessment of their forms before submission for data entry. Concerns related to data quality were discussed at the weekly team meetings.

The principal investigator was present at all enrolment and follow-up visits for the first two months of data collection to ensure proper collection and storage of samples.

Data entry, verification, and cleaning

Data were double entered into a Microsoft Access database by trained data entry clerks. The field supervisor resolved inconsistencies using the forms, phone calls to the participants or by visiting the participant at home. The investigator did consistency checks in Stata and inconsistencies were resolved by re-visiting the completed questionnaire.

4.3.6 Ethical consideration

Ethical approval for the study was given by the London School of Hygiene & Tropical

Medicine (LSHTM) ethics committee (Ref: 11677) (Appendix 13) and the Gambia Government/Medical Research Council Joint ethics committee (SCC 1510) (Appendix 14). Each participant gave written informed consent and participation was voluntary. Appendix 15 appendix shows a sample consent form.

4.3.7 Statistical methods

The prevalence of *S. aureus* carriage in neonates, mothers, caregivers, and siblings was estimated at day 0 (delivery) and day 7 and that of midwives on day 0 only. Newborns were considered carriers if the nasal or umbilical or both swabs were positive while mothers were considered carriers if nasal or vaginal swabs or breastmilk were positive for *S. aureus*. Midwives or TBAs were considered positive if either nasal or hand or both swabs were positive for *S. aureus.* The presence or absence of *S. aureus* was determined by conventional microbiology. Carriage acquisition in newborns was defined as carriage on day 7 but not at birth, and persistent carriage was defined as being *S. aureus* positive at both time points. *S. aureus*-carrying babies at birth were considered to have acquired carriage before delivery; therefore, were only included in the analysis of carriage at birth but not in the acquisition analysis.

Risk factors for newborn carriage at birth and acquisition were evaluated using logistic regression (see section 4.3.1). Factors (carriage status of all contacts) whose association reached $p<0.05$ in the univariate analysis were adjusted for maternal age, maternal educational status, and ethnicity in the multivariate analysis. The *punaf* command in STATA was used to compute the population attributable fractions (PAF) of the acquisition associated with specific exposures. The formula for the calculation of PAF is:

$$PAF = \frac{Risk_p - Risk_e}{Risk_p}$$

where $Risk_p$ is the risk in the population and $Risk_e$ is the risk in the exposed group.

4.4 Results

Four hundred and forty-five eligible pregnant women were approached to join the study, and 231 (51.9%) were enrolled. Of the 214 women who did not join the study, 32 (15.0%) declined, and 182 (85.0%) travelled out of the study area during a period of political instability (Figure 4.1). After excluding two newborns because of missing carriage data at one-week post-delivery and two early neonatal deaths, the analysis dataset consisted of 227 (51.0%) newborn-contact pairs. All visits scheduled for the day of delivery (day 0) occurred as planned while visit 2 (one-week after delivery) occurred on day 7 for 147 (64.8%) participants, on day 6 for 35 (15.4%) and day 8 for 45 (19.8%) participants.

4.4.1 Characteristics of participants

Mothers

The median (IQR) age of mothers was 27 years (24, 30). Most of them were housewives and about a quarter had primary school education or above. All the women were married,

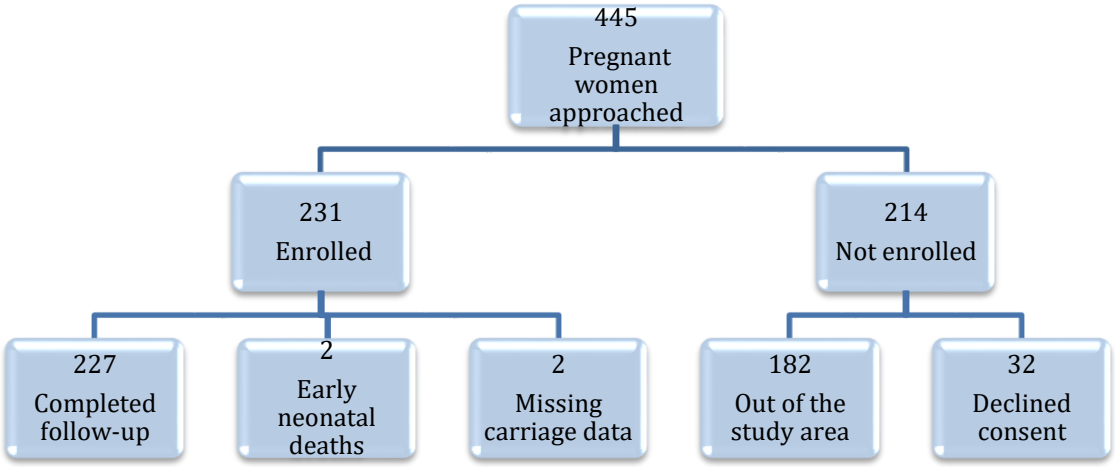


Figure 4.1: Study profile

and one-fifth had more than five living children. About half (53.3%) alluded to nose-picking, and 64.6% had daily contacts with animals (Supplemental Table 4.1 – Appendix 16).

Forty-one deliveries (18.1%) took place at home; all pregnant women delivered vaginally at term, and none of them had a history of prolonged rupture of membranes. Forty-two per cent of the mothers reported a history of fever one week before delivery (Supplemental Table 4.2 – Appendix 16) for which 12 had antibiotics (Amoxicillin), 61 had an antipyretic (paracetamol), and others were not treated.

Newborns

Of the 227 newborns, 122 (53.7%) were boys, and the mean (SD) birth weight was 3.1kg (0.4). For the majority of the newborns, the umbilical cord was cut using a new blade (96.9%) and tied with a piece of thread (62.0%). At home, mothers applied substances such as shea butter, ash, baby powder and cow dung to the umbilical stump of 166 (73.1%) of the newborns. Table 4.3 shows the characteristics of the newborns.

Midwives or Traditional birth attendants

Twenty-eight traditional birth attendants and 25 midwives were recruited; four midwives and one TBA were males. Supplemental Table 4.3 (Appendix 16) shows the characteristics of the deliveries by midwives and TBAs. The midwives were significantly younger than the TBAs (median age: 36 years versus 55 years). There was no difference between TBAs and midwives in the reporting rates of having a cold and cough, nose-picking and antibiotic use in the past six months between TBAs and midwives. In 12.2% and 2.7% of the deliveries facilitated by TBAs and midwives, respectively, they had reported not washing their hands before taking the delivery (Supplemental Table 4.3 – Appendix 16). The reasons given for not washing their hands included: no water, no soap, time pressure, had washed their hands before taking the last delivery and wearing gloves. The majority of TBAs or midwives delivered one (22/53; 41.5%) or two (14/53; 26.4%) babies.

Household children

Four hundred and fifty-four household children were recruited. The median (IQR) age of household children was 38 months (29, 50), and about half were males (53.2%). Bed-sharing with the newborn and antibiotic use were uncommon (Supplemental Table 4.4 – Appendix 16).

Table 4.3: Characteristics of the newborns

Characteristics	n/N (%)
Sex	
Male	122/227 (53.7)
Female	105/227 (46.3)
Birth weight (kg)	
Mean (SD)	3.1 (0.4)
GA by Ballard score[1]	
Median (IQR)	38 (36, 39)
Mode of resuscitation	
None	122/227 (53.7)
Airway suctioning	89/227 (39.2)
Others[2]	16/227 (7.1)
Instrument used to cut the umbilical cord	
New razor blade	220/227 (96.9)
Non-sterile material	7/227 (3.1)
Material used to tie the cord	
Cord clamp	86/226 (38.0)
Piece of thread	140/226 (62.0)
Substance applied to the cord	
Methylated spirit	18/227 (7.9)
Cow dung	19/227 (8.4)
Baby powder	16/227 (7.0)
Shea butter	110/227 (48.5)
Others[3]	39/227 (17.2)
Nothing	25/227 (11.0)
Bed shared with baby (mother)	
Yes	196/227 (86.3)
Number of people who shared bed with baby	
1	88/227 (38.8)
>1	139/227 (61.2)
Length of hospital stay after birth	
< 6 hours	101/212 (47.6)
≥ 6 hours	111/212 (52.4)

[1] GA=Gestational Age by Ballard score; SD=Standard deviation; IQR=Interquartile range

[2] Ambu bagging, endotracheal intubation

[3] Ash, local herbs, soil

Caregivers

The caregivers sampled were either fathers (4.1%) or grandmothers (71.2%). The median (IQR) age of the caregivers was 54.5 (44, 65) years. About half of them lived in the same

house as the newborn; only 3.8% of them shared a bed with the newborn (Supplemental Table 4.5 – Appendix 16).

Medications

Apart from a few caregivers (3.3%) and household children (2.3%), none of the participants used medications during the study period. However, 5.3% of mothers, 29% of midwives and 14.7% of TBA reported the use of antibiotics in the preceding six months.

Number of swabs

A total of 3,987 swabs were collected, representing 97.6% of the expected number of samples (Table 4.4). Nasal and umbilical swabs were obtained for 189 (83.3%) and 31 (13.7%) newborns within the first 2 and 2 - 6 hours of life, respectively.

Table 4.4: Number of swabs per person group and time

Type of swab	Day 0 (Visit 1) n/N (%)	Day 7 (Visit 2) n/N (%)	Total n/N (%)
Newborn Nasal	227/227 (100.0)	227/227 (100.0)	454/454 (100.0)
Newborn Umbilical	226/227 (99.6)	227/227 (100.0)	453/454 (99.8)
Mothers Nasal	227/227 (100.0)	227/227 (100.0)	454/454 (100.0)
Mother Vaginal	227/227 (100.0)	NA[1]	227/227 (100.0)
Breast milk	NA	227/227 (100.0)	227/227 (100.0)
Midwives/TBAs[1] Nasal	198/227 (87.2)	NA	198/227 (87.2)
Midwives/TBAs Hand	222/227 (97.8)	NA	222/227 (97.8)
Delivery bed	227/227 (100.0)	NA	227/227 (100.0)
Resuscitaire	226/227 (99.6)	NA	226/227 (99.6)
Household children	440/454 (96.9)	423/454 (93.2)	863/908 (95.0)
Caregiver	226/227 (99.6)	210/227 (92.5)	436/454 (96.0)
Total	**2446/2497 (98.0)**	**1541/1589 (97.0)**	**3987/4086 (97.6)**

[1] TBAs=Traditional birth attendants; NA=Not applicable

Newborns

Twenty-two newborns (9.7%) were *S. aureus* carriers at birth; more than half of which (59.1%) were already carriers within two hours of age. The prevalence of umbilical carriage at birth was higher than nasal carriage (8.4% versus 2.2%; p=0.002). One week post-delivery, 179 (78.9%) babies were carriers. The prevalence of nasal carriage was similar to that of umbilical carriage at one-week post-delivery (63.9% versus 66.5%; p=0.45). One week post-delivery, the prevalence of nasal and umbilical carriage (78.9%) was higher than at birth (9.7%) (Table 4.5). Persistent nasal and umbilical carriage occurred in one (20.0%) and 11 (57.9%) of the carrier newborns, respectively.

Table 4.5: Prevalence of *S. aureus* carriage by participant group and time

Type of swab	At birth (day 0) n/N (%)	One-week post-delivery (day 7) n/N (%)	p-value[1]
Newborn Nasal	5/227 (2.2)	145/227 (63.9)	< 0.001
Newborn Umbilical	19/227 (8.4)	151/227 (66.5)	< 0.001
Mothers Nasal	39/227 (17.2)	59/227 (26.0)	0.03
Mother Vaginal	23/227 (10.1)	NA[b]	-
Breast milk	NA	44/227 (19.4)	-
Midwives/TBAs[b] Nasal	15/198 (7.6)	NA	-
Midwives/TBAs Hand	1/222 (0.5)	NA	-
Delivery bed	4/182 (2.2)	NA	-
Resuscitaire	4/181 (2.2)	NA	-
Household children	37/442 (8.4)	47/423 (11.1)	0.23
Caregiver	16/219 (7.3)	21/210 (10.0)	0.25
Total	**163/2350 (6.9)**	**467/1541 (30.3)**	

[1] p-value was calculated using McNemar's test; the comparison is across visits

[2] TBAs=Traditional birth attendants; NA=Not applicable

Mothers

Fifty-eight women (25.6%) were carriers at delivery (19 in the vagina, 35 in the nares and 4 in both sites) and 91 (40.1%) at one-week post-delivery (47 in the nares, 32 in the breast

milk and 12 in both) corresponding to an increase of 34% of nasal carriage (p=0.008). More mothers were nasal carriers one-week post-delivery than at birth (26.0% versus 17.2%; p=0.03). Nasal carriage at delivery was significantly higher than vaginal carriage (17.2% versus 10.1%; p=0.001); and nasal carriage at one-week post-delivery was higher than in breast milk (26.0% versus 19.4%; p<0.001) (Table 4.5). Fourteen of the *S. aureus* positive mothers (35.9%) had persistent nasal carriage.

Household children

Table 4.5 shows that there was no difference in the prevalence of *S. aureus* carriage among the household children at delivery (8.4%) compared to one-week post-delivery (11.1%) (p=0.23). Persistent carriage occurred in nine of the *S. aureus* positive household children (24.3%) at one-week post-delivery. There was no difference in *S. aureus* carriage between males or females either at day 0 (p=0.28) or at day 7 (p=0.34).

Caregivers

Among the caregivers, the prevalence of *S. aureus* carriage was similar at day 0 (7.3%) and day 7 (10.0%) (p=0.25) (Table 4.5). Only four of the *S. aureus* positive (25.0%) caregivers were persistent carriers.

Midwives or Traditional Birth Attendants

Of the 227 deliveries, sixteen (7.1%) were conducted by a midwife or TBA who was a carrier. Among the deliveries where the midwife or TBA tested *S. aureus* positive, 15 were carriers in the nares while only one on the hand.

Delivery surfaces

Eight (1.8%) of the delivery surfaces were contaminated. The rate of contamination was similar in the deliveries that occurred at home and those that occurred at the BHC (2.4% versus 1.6%).

4.4.3 Risk factors for *S. aureus* carriage at birth

The prevalence of *S. aureus* carriage at birth was 9.7% (22/227). *S. aureus* carriage at birth was associated with the midwife's or TBA's report of not washing hands before delivery (Table 4.9). However, it was not associated with maternal *S. aureus* carriage; neither was it associated with carriage in the midwives, TBAs, household children or caregivers (Table 4.6).

Table 4.6: Site-specific carriage or contamination as potential risk factors for *S. aureus* carriage at birth

S. aureus carriage /contamination	Cases/Persons at risk n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
Mother					
Nasal at birth					
No	18/188 (9.6)	1			
Yes	4/39 (10.3)	1.08 (0.34 – 3.39)	0.90		
Vaginal					
No	20/204 (9.8)	1			
Yes	2/23 (8.7)	0.88 (0.19 – 4.01)	0.86		
Any site at birth (Nasal or Vaginal)					
No	17/169 (10.1)	1			
Yes	5/58 (8.6)	0.84 (0.30 – 2.40)	0.75		
Midwife/TBA[2]					
Nasal or Hands					
No	20/211 (9.5)	1			
Yes	2/16 (12.5)	1.36 (0.29 – 6.44)	0.71		
Household children					
Nasal at birth					
No	21/193 (10.9)	1			
Yes	1/34 (2.9)	0.25 (0.03 – 1.91)	0.10		
Caregiver					
Nasal at birth					
No	19/203 (9.4)	1			
Yes	3/16 (18.6)	2.23 (0.58 – 8.55)	0.27		
Delivery surfaces					
No	20/219 (9.1)	1			
Yes	2/8 (25.0)	0.30 (0.06 – 1.59)	0.20		

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] TBA=Traditional birth attendant

Table 4.7: Maternal factors potentially associated with *S. aureus* carriage at birth

Characteristics	Cases/Persons at risk n/N (%)	Unadjusted Odds ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
Age (years)					
17-24	5/64 (7.8)	1			
25-30	12/109 (11.0)	1.46 (0.49 – 4.35)			
>31	5/54 (9.3)	1.20 (0.33 – 4.40)	0.78		
Ethnic group					
Sarahule	7/105 (6.7)	1			
Fula	7/57 (12.3)	1.96 (0.65 – 5.90)			
Other[2]	8/65 (12.3)	1.97 (0.68 – 5.70)	0.35		
Educational level					
None	10/80 (12.5)	1			
Arabic school	6/94 (6.4)	0.48 (0.17 – 1.38)			
Primary and above	6/53 (11.3)	0.89 (0.30 – 2.62)	0.34		
Occupation					
Unemployed	19/200 (9.5)	1			
Employed[3]	3/27 (11.1)	1.19 (0.33 – 4.33)	0.79		
Number of siblings					
0-3	6/101 (5.9)	1		1	
4-5	14/87 (16.1)	3.04 (1.11 – 8.29)		3.39 (0.86 – 11.48)	
>5	2/39 (5.1)	0.86 (0.17 – 4.43)	0.04	0.95 (0.13 – 6.55)	0.08
Antibiotic use within the previous six months					
Yes	1/12 (8.3)				
No	21/205 (10.2)				
Unsure	0/10 (0.0)	-	-	-	-
Nose picking					
No	7/106 (6.6)	1			
Yes	15/121 (12.4)	2.00 (0.78 – 5.11)	0.14		
Current skin disease, e.g. dermatitis or skin ulcers					
No	20/217 (9.2)	1			
Yes	2/10 (20.0)	2.46 (0.48 – 13.4)	0.31		
Daily contact with animals					
No	4/80 (5.0)	1			
Yes	18/147 (12.2)	2.65 (0.87 – 8.12)	0.06		

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] Other ethnic groups include Mandinka, Wolof, Jola

[3] Teacher, farmer, childcare attendant, accountant

Table 4.8: Newborn factors potentially associated with *S. aureus* carriage at birth

Characteristics	Cases/Persons at risk n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
Sex					
Male	15/122 (12.3)	1			
Female	7/105 (6.7)	1.96 (0.77 – 5.01)	0.15		
Mode of resuscitation					
None	12/122 (9.8)	-	-	-	-
Airway suctioning	10/89 (11.2)				
Other[2]	0/16 (0.0)				
Instrument used to cut the umbilical cord					
New razor blade	22/220 (10.0)	-	-	-	-
Non-sterile material	0/7 (0.0)				
Material used to tie the cord					
Cord clamp	5/86 (5.8)	1			
Piece of thread	17/140 (12.1)	2.24 (0.80 – 6.30)	0.11		
Substance applied to the cord					
Methylated spirit	3/18 (16.7)	1			
Other[3]	17/184 (9.2)	0.51 (0.13 – 1.94)			
Nothing	2/25 (8.0)	0.44 (0.06 – 2.92)	0.62		

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] Ambu bagging, endotracheal intubation

[3] Ash, local herbs, soil

Table 4.9: Characteristics of midwives or traditional birth attendants potentially associated with *S. aureus* carriage at birth in newborns

Characteristics	Cases/Persons at risk n/N (%)	Unadjusted Odds Ratios (95% CI)	p-value	Adjusted Odds Ratios [1] (95% CI)	p-value
Hand wash before taking delivery					
No	3/10 (30.0)	1		1	
Yes	19/217 (8.8)	0.22 (0.05 – 0.94)	0.04	0.19 (0.04 – 0.87)	0.04
Nose picking					
No	9/82 (11.0)	1			
Yes	13/145 (9.0)	0.80 (0.32 – 1.96)	0.62		
Cough or cold since the last visit					
No	17/165 (10.3)	1			
Yes	5/62 (8.1)	0.76 (0.27 – 2.17)	0.61		
Travelled out of the village in the past two weeks					
No	18/173 (10.4)	1			
Yes	4/54 (7.4)	0.69 (0.22 – 2.14)	0.52		
Antibiotic use since the last visit					
No	22/226	-	-	-	-
Yes	0/1				

[1] Adjusted for maternal age, maternal educational status, and ethnicity

4.4.4 Risk factors for *S. aureus* carriage acquisition at one week of age

In the unadjusted analysis, maternal nasal carriage at one week, breast milk colonisation at one week (Table 4.10), household child nasal carriage at one week (Table 4.10), maternal history of dysuria or UTI in the week preceding delivery (Table 4.11), duration of hospital stay (Table 4.12) and midwife or TBA report of use of antibiotics in the preceding six months (Table 4.13) were associated with *S. aureus* carriage acquisition at one week of age. In the adjusted analysis, *S. aureus* carriage acquisition at one week of age was positively associated with maternal nasal carriage at one week, breast milk colonisation at one week, household child nasal carriage at one week, midwife or TBA report of the use of antibiotics in the preceding six months and negatively associated with maternal history of dysuria or urinary tract infection one week before delivery,

Table 4.10: Site-specific carriage or contamination as potential risk factors for *S. aureus* carriage acquisition between birth and one-week post-delivery in neonates

S. aureus carriage/ contamination	Carriage acquisition one-week post-delivery n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
Mother carriage					
Nasal at birth					
No	135/188 (71.8)	1		1	
Yes	28/39 (71.8)	1.00 (0.47 – 2.15)	1.00	1.06 (0.49 – 2.32)	0.88
Vaginal at delivery					
No	145/204 (71.1)	1		1	
Yes	18/23 (78.3)	1.47 (0.52 – 4.13)	0.46	1.47 (0.51 – 4.23)	0.46
Nasal at one week					
No	112/168 (66.7)	1		1	
Yes	51/59 (86.4)	3.19 (1.42 – 7.18)	0.002	3.18 (1.40 – 7.22)	0.003
Breast milk at one week					
No	124/183 (67.8)	1		1	
Yes	39/44 (88.6)	3.71 (1.39 – 9.90)	0.003	3.81 (1.38 – 10.49)	0.004
Any site at birth (Nasal or Vaginal)					
No	119/169 (70.4)	1		1	
Yes	44/58 (75.9)	1.32 (0.67 – 2.62)	0.42	1.37 (0.68 – 2.74)	0.35
Any site at one week (Nasal or Breast milk)					
No	83/136 (61.0)	1		1	
Yes	80/91 (87.9)	4.64 (2.26 – 9.52)	<0.001	4.76 (2.29 – 9.89)	<0.001
Midwife/TBA[2] carriage					
Nasal at delivery					
No	147/207 (71.0)	1		1	
Yes	13/15 (86.7)	2.65 (0.58 – 12.11)	0.16	2.75 (0.59 – 12.74)	0.15
Nasal or Hands at delivery					
No	149/211 (70.6)	1		1	
Yes	14/16 (87.5)	2.92 (0.64 – 13.20)	0.12	2.96 (0.68 – 13.62)	0.12
Household children carriage					
Nasal at birth					
No	137/193 (71.0)	1		1	
Yes	26/34 (76.5)	1.33 (0.67– 3.11)	0.51	1.36 (0.57 – 3.21)	0.48
Nasal at one week					
No	127/184 (69.0)	1		1	
Yes	36/43 (83.7)	2.31 (0.97 – 5.50)	0.04	2.37 (0.97 – 5.74)	0.04

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] TBA=Traditional birth attendant

Table 4.10: Site-specific carriage or contamination as potential risk factors for *S. aureus* carriage acquisition between birth and one-week post-delivery in neonates (continued)

***S. aureus* carriage/ contamination**	**Carriage acquisition one-week post-delivery n/N (%)**	**Unadjusted Odds Ratio (95% CI)**	**p-value**	**Adjusted Odds Ratio[1] (95%CI)**	**p-value**
Caregiver carriage					
Nasal at birth					
No	147/203 (72.4)	1		1	
Yes	8/16 (50.0)	0.38 (0.14 – 1.06)	0.07	0.44 (0.15 – 1.27)	0.14
Nasal at one week					
No	140/194 (72.2)	1		1	
Yes	16/21 (76.2)	1.23 (0.43 –3.54)	0.69	1.15 (0.39 – 3.36)	0.80
Contaminated delivery surfaces					
No	159/219 (72.6)	1			
Yes	4/8 (50.0)	0.38 (0.09 – 1.56)	0.19	-	-

[1] Adjusted for maternal age, maternal educational status, and ethnicity

Table 4.11: Obstetric factors potentially associated with *S. aureus* carriage acquisition between birth and one-week post-delivery

Characteristics	Carriage acquisition one-week post-delivery n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
History of dysuria or UTI[2] one week before delivery					
No	158/215 (73.5)	1		1	
Yes	5/12 (41.7)	0.26 (0.08 – 0.84)	0.03	0.27 (0.08 – 0.91)	0.03
History of vaginal discharge one week before delivery					
No	155/215 (72.1)	1		1	
Yes	8/12 (66.7)	0.77 (0.22 – 2.67)	0.69	0.79 (0.23 – 2.79)	0.75
History of vaginal bleeding one week before delivery					
No	155/213 (72.8)	1		1	
Yes	8/14 (57.1)	0.50 (0.17 – 1.50)	0.23	0.48 (0.16 – 1.48)	0.21
Peripartum fever within one week of delivery					
No	94/132 (71.2)	1		1	
Yes	69/95 (72.6)	1.07 (0.60 – 1.93)	0.81	1.06 (0.52 – 1.92)	0.86
Foul-smelling liquor					
No	138/192 (71.9)	1		1	
Yes	25/35 (71.4)	0.98 (0.44 – 2.17)	0.96	0.94 (0.41 – 2.16)	0.88
Place of delivery					
Home	28/41 (68.3)	1		1	
Health Centre	135/186 (72.6)	1.23 (0.59 – 2.56)	0.58	1.28 (0.59 – 2.65)	0.57
Health care talks on cord care					
No	95/137 (69.3)	1		1	
Yes	68/90 (75.6)	1.37 (0.75 – 2.50)	0.31	1.30 (0.70 – 2.42)	0.41
Admitted during this pregnancy					
No	152/213 (71.4)	1		1	
Yes	11/14 (78.6)	1.47 (0.40- 5.46)	0.55	1.62 (0.42 – 6.19)	0.47

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] UTI=Urinary tract infection; TBA=traditional birth attendant

Table 4.11: Obstetric factors potentially associated with *S. aureus* carriage acquisition between birth and one-week post-delivery (continued)

Characteristics	Carriage acquisition one-week post-delivery n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
Nurse/TBA[2] washed hands before taking delivery					
No	6/11 (54.6)	1		1	
Yes	144/198 (72.7)	2.22 (0.65 – 7.58)		2.52 (0.70 – 8.99)	
Don't know	13/18 (72.2)	2.17 (0.45– 10.40)	0.46	2.92 (0.56 – 15.37)	0.36
Nurse/TBA wore gloves before taking delivery					
No	12/21 (57.1)	1		1	
Yes	149/203 (73.4)	2.07 (0.83 – 5.19)		2.24 (0.87 – 5.)	
Don't know	2/3 (66.7)	1.52 (0.12 – 19.2)	0.31	2.12 (0.16 - 29.07)	0.26

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] UTI=Urinary tract infection; TBA=traditional birth attendant

Table 4.12: Newborn factors associated with *S. aureus* carriage acquisition between birth and one-week

Characteristics	Carriage acquisition one-week post-delivery n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio [1] (95% CI)	p-value
Sex					
Male	88/122 (72.1)	1		1	
Female	75/105 (71.4)	1.04 (0.58 – 1.85)	0.91	1.08 (0.60 - 1.95)	0.88
Mode of resuscitation					
None	84/122 (68.9)	1		1	
Airway suctioning	64/89 (71.9)	1.16 (0.64 – 2.11)		1.26 (0.67 – 2.38)	
Other[2]	15/16 (93.8)	6.79 (0.87 – 53.3)	0.06	7.18 (0.90 – 57.22)	0.06
Instrument used to cut the umbilical cord					
New razor blade	157/220 (71.4)	1		1	
Non-sterile material	6/7 (85.7)	2.41 (0.28 – 20.4)	0.38	3.09 (0.35 – 27.48)	0.26
Material used to tie the cord					
Cord clamp	61/86 (70.9)	1		1-	
Piece of thread	101/140 (72.1)	1.06 (0.59 – 1.92)	0.84	1.14 (0.61 – 2.14)	0.68
Substance applied to the cord					
Methylated spirit	9/18 (50.0)	1		1	-
Other[3]	136/184 (73.9)	1.43 (1.06 – 7.56)		2.63 (0.95 – 7.23)	
Nothing	18/25 (72.0)	1.42 (0.72 – 9.17)	0.12	2.53 (0.69 – 9.24)	0.18
Bed shared with the newborn (mother)					
No	140/196 (71.4)	1		1	
Yes	23/31 (74.2)	0.87 (0.37 – 2.06)	0.75	0.92 (0.38 – 2.26)	0.86
Number of people who shared a bed with a newborn					
1	54/88 (61.4)	1		1	
>1	109/139 (78.4)	2.29 (1.27 – 4.13)	0.006	2.50 (1.36 – 4.57)	0.003
Duration of hospital stay after birth					
< 6 hours	78/101 (77.2)	1		1	
≥ 6 hours	72/111 (64.9)	0.54 (0.30 – 1.00)	0.05	0.57 (0.30 – 1.09)	0.09

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] Ambu bagging, endotracheal intubation

[3] Ash, local herbs, soil

Table 4.13: Midwife or traditional birth attendant factors potentially associated with *S. aureus* acquisition between birth and one-week post-delivery

Characteristics	Carriage acquisition one-week post-delivery n/N (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio [1] (95% CI)	p-value
Hand wash before taking delivery					
No	6/10 (60.0)	1		1	
Yes	157/217 (72.4)	1.74 (0.47 – 6.42)	0.40	1.93 (0.50 – 7.37)	0.35
Nose picking					
No	63/82 (76.8)	1		1	
Yes	100/145 (69.0)	0.67 (0.36 – 1.25)	0.21	0.66 (0.35 – 1.25)	0.20
Cough or cold since the last visit					
No	117/165 (70.9)	1		1	
Yes	46/62 (74.2)	1.18 (0.06 – 2.29)	0.63	1.12 (0.56 – 2.23)	0.75
Travelled out of the village in the past two weeks					
No	13/64 (20.3)	1		1	
Yes	41/163 (25.2)	1.30 (0.65 – 2.67)	0.44	1.27 (0.62 – 2.62)	0.51
Antibiotic use in the past six months					
No	114/167 (68.3)	1		1	
Yes	49/60 (81.7)	2.07 (1.00 – 4.31)	0.05	2.04 (1.00 – 4.42)	0.05

[1] Adjusted for maternal age, maternal educational status, and ethnicity

4.4.5 Population attributable fraction

At birth, 2.2% and 6.8% of *S. aureus* carriage was attributable to exposure to carriage in midwife or TBA and caregivers, respectively (Table 4.14). At one week post-delivery, 15.5%, 5.2%, and 1.9% of *S. aureus* carriage acquisition was attributable to maternal carriage and carriage in household children, and the midwife or TBA, respectively (Table 4.14).

Table 4.14: Proportion of carriage at birth and carriage acquisition at one week attributable to different sources

Contacts	Carriage at birth PAF % (95%CI)	Acquisition at one week PAF % (95%CI)
Maternal carriage		
At birth	-3.8 (-28.9 – 16.4) [1]	1.9 (-2.8 - 6.5) [2]
At one week	NA[3]	15.0 (8.3 – 21.2)
Birth or one week	NA	15.5 (6.0 – 23.5)
Midwife/TBA carriage	2.2 (-10.7 – 13.6)	1.9 (0.3 – 7.8)
Household children carriage[4]		
At birth	-12.3 (-22.8 - -2.6)	1.1 (-2.2 – 4.3)
At one week	NA	3.8 (0.3 – 7.3)
Birth or one week	NA	5.2 (1.0 – 10.0)
Caregiver carriage[5]		
At birth	6.8 (-8.4 – 19.9)	-2.3 (-5.0 – 0.3)
At one week	NA	0.5 (-2.1 – 3.1)
Birth or one week	NA	-1.2 (-4.7 – 2.2)
Non-maternal carriage[6]		
At birth	5.6 (-23.7 – 28.0)	-1.1 (-6.3 – 4.0)
At one week	NA	2.4 (1.1 – 9.4)
Birth or one week	NA	4.2 (-0.3 – 8.5)

[1] Maternal nasal and vaginal carriage at birth
[2] Maternal nasal and vaginal carriage at birth and nasal and breastmilk carriage at one week
[3] NA=Outcome (carriage at birth) occurred before exposure (contacts at one week); PAF=Population Attributable Fraction
[4] Nasal carriage by household children at birth and one week
[5] Nasal carriage by caregiver at birth and one week
[6] Carriage by all contacts except the mother

4.4.6 Susceptibility of isolates

Of the isolates tested, 2.2% and 9.2% were resistant to cloxacillin and erythromycin, respectively, and 3.3% were methicillin-resistant using cefoxitin as a surrogate measure (Table 4.15). Resistance to penicillin was high (90.1%) but to all other antibiotics tested, it was less than 10%. The pattern of antimicrobial resistance was similar across the study participant groups and time points (Table 4.15).

Table 4.15: Antibiotic resistance pattern of the *S. aureus* isolates by study participant groups

Antibiotics	Newborn		Mother		Midwife/ Traditional birth attendants	Delivery surfaces	Household children		Caregiver		Total
Visit	Birth	One week	Birth	One week	Birth	Birth	Birth	One week	Birth	One week	
	n/N[1] (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Cefoxitin	0/22 (0.0)	10/295 (3.4)	2/62 (3.2)	7/96 (7.3)	0/16 (0.0)	0/8 (0.0)	0/28 (0.0)	0/43 (0.0)	1/13 (7.7)	0/21 (0.0)	20/604 (3.3)
Clindamycin	0/22 (0.0)	3/295 (1.0)	0/62 (0.0)	0/96 (0.0)	0/16 (0.0)	0/8 (0.0)	0/28 (0.0)	1/43 (2.3)	0/13 (0.0)	0/21 (0.0)	4/604 (0.7)
Cloxacillin	1/22 (4.5)	6/293 (2.1)	1/61 (1.6)	1/96 (1.0)	0/16 (0.0)	0/8 (0.0)	1/28 (3.6)	2/42 (4.8)	1/13 (7.7)	0/21 (0.0)	13/600 (2.2)
Cotrimoxazole	1/22 (4.5)	10/295 (3.4)	3/62 (4.8)	4/96 (4.2)	0/16 (0.0)	0/8 (0.0)	2/28 (7.1)	3/43 (7.0)	0/13 (0.0)	0/21 (0.0)	22/604 (3.6)
Erythromycin	0/22 (0.0)	32/295 (10.9)	7/61 (11.5)	4/94 (4.3)	0/16 (0.0)	1/6 (16.7)	2/28 (7.1)	2/42 (4.8)	4/13 (30.8)	3/21 (14.3)	55/598 (9.2)
Penicillin	18/22 (81.8)	269/292 (92.1)	57/61 (93.4)	86/96 (89.6)	14/15 (93.3)	5/8 (62.5)	26/28 (92.9)	31/42 (73.8)	13/13 (100.0)	20/21 (95.2)	539/598 (90.1)

[1] Number of resistant isolates/Number of isolates tested (Percentage of resistant isolates)

4.5 Discussion

This study assessed the rate of and risk factors for *S. aureus* carriage at birth and carriage acquisition in the first week of life. The carriage rate at birth was low (9.7%) and was negatively associated with handwashing by the midwife or TBA. The risk of carriage acquisition within the first week of life was high (79.5%) and was associated with maternal nasal and breast milk carriage at one week, household child nasal carriage at one week, and healthcare worker antibiotic use in the preceding six months and negatively associated with maternal history of dysuria or urinary tract infection one week before delivery.

Prevalence of *S. aureus* carriage

Newborn

S. aureus has a broad predilection of body sites for carriage, occurring most frequently in the nares followed by the umbilicus in newborns.[94,104,105] Other sites include the nasopharynx, ears, rectum and gastrum.[106,107] In this study, more newborns carried *S. aureus* in the umbilicus than in the nares, which is contrary to the findings of Maayan-Metzger and colleagues[317] who reported higher carriage in the nares. The reason for this difference is unclear but may be related to umbilical cord care at birth and in the first few days of life. In this study, although the midwives or TBA used a sterile blade to cut the cord in most of the newborns, post-ligation cord care involved the use of substances such as shea butter, cow dung, baby powder and ash, which may have been contaminated with *S. aureus.*[318]

Though the prevalence of carriage was low at birth, the acquisition risk by day 7 was high. The immaturity of the immune system could influence the high risk of acquisition in

newborns. Secretory IgA antibodies are required for protection on the mucosal surfaces against *S. aureus* and may reduce the risk of its acquisition. These antibodies are not produced in significant quantities by the newborn to prevent acquisition; neither are they transferred to the newborn through the placenta.[319] Furthermore, maternal IgG antibodies transferred to the newborn during pregnancy do not protect the newborn against *S. aureus* carriage.[133] Breastfeeding leads to a substantial increase in IgA levels and may be part of the reason for declining carriage with time in infants. Other factors that might influence the high incidence of *S. aureus* carriage acquisition include close contact with a carrier mother, colonised breast milk, contaminated hands of the midwife during delivery and the large household sizes that are common in The Gambia.

Mother

The prevalence of vaginal *S. aureus* carriage in this cohort was similar to the estimates from other studies in The Gambia,[107] China,[320] Israel,[105] France[106] and Taiwan[94] (5.0% - 13.5%). In keeping with the findings from the Gabonese[104] and Gambian[107] studies, maternal nasal carriage was higher one-week post-delivery than at birth. The reason for this is unclear, but it is possible that the mothers acquire *S. aureus* from their babies during this period. Schaumburg and colleagues[104] established infant-to-mother transmission in 2.1% of mother-infant pairs in their study. Another reason may be that the mother acquires *S. aureus* from the health facility, including its staff. The health facility is a known reservoir for *S. aureus* and associated with increased risk of transmission.

The occurrence of *S. aureus* in breast milk is consistent with an earlier study in The Gambia.[107] Although human breast milk was thought to be sterile, recent studies have demonstrated that it frequently contains bacteria (96%), *S. aureus* inclusive (32% -

42%).[107,321] The origin of bacteria in breast milk is unclear. One possibility is that bacteria could reach the breast milk by retrograde flow from the newborn's mouth to the mother's lactiferous ducts during breastfeeding[322] or from the mother's clothing. Another possibility is the migration of bacteria from the maternal intestine through an endogenous cellular route to the mammary gland and then into the breast milk (entero-mammary pathway) during late pregnancy and lactation.[323] The latter option may explain why pre-colostrum secreted before the commencement of breastfeeding contains bacteria.

Household children

The prevalence of *S. aureus* carriage among household children in this study was similar to that reported in Israel[96] but lower than reports from another study in The Gambia[98] and a study in Ghana.[324] These disparities may be due to the differences in the profile of study participants. The Gambian study included newborns, while the Ghanaian study was among children admitted to a hospital where there is an increased risk of *S. aureus* acquisition. Secondly, the studies were conducted in different seasons: this study was conducted during the dry season, while the Gambian and Ghanaian studies took place over at least one whole year. There are seasonal variations in *S. aureus* carriage with higher prevalence during the wet season.[304]

Midwives and traditional birth attendants

The prevalence of *S. aureus* carriage among midwives in this study was similar to reports from the Democratic Republic of Congo (16%)[325] and Ethiopia (12%).[326] The prevalence of *S. aureus* carriage was higher among midwives than among TBAs, even though more

midwives used antibiotics in the six months preceding screening. This may be due to higher levels of exposure to patients who are potential *S. aureus* carriers and an environment that is more likely to be contaminated with *S. aureus*. Training of midwives and TBAs on infection prevention and control measures, improving hand hygiene compliance, regularly cleaning, and disinfecting of the health facility and strengthening the other infection control measures may help to reduce carriage in midwives and TBAs.

Caregivers

The prevalence of *S. aureus* carriage in caregivers should reflect the prevalence in adults in the community. The prevalence of *S. aureus* carriage among caregivers in this study was similar to the findings from the adult population in Ghana,[327] and lower than the reports from Gabon.[328] The reason for the difference, maybe because of the number of body sites screened or the characteristics of the population studied.

Increasing the number of body sites screened results in a cumulative increase in the prevalence of carriage in each of the study participant groups.[95] Additionally, the variations in *S. aureus* carriage in the participant groups in this study and other studies may be due to geographical and seasonal variations, characteristics of the study participants, and laboratory techniques used to isolate *S. aureus*.

Risk factors associated with *S. aureus* carriage at birth

Two African studies have examined the source of *S. aureus* carriage in infancy;[94,106] but, none have assessed risk factors associated with carriage at birth. In this study, I examined 27 risk

factors, but none except hand washing (midwife or TBA) was associated with *S. aureus* carriage at birth in the newborn.

The TBAs reported not washing hands significantly more than midwives (12.2% versus 2.7%). The reasons midwives gave for not washing their hands before attending a delivery were time pressure and wearing gloves while TBAs said not having water or soap, time pressure, wearing gloves and having washed before the last delivery. A lack of hand hygiene agents both at home and in health facilities and lack of knowledge and compliance with infection prevention and control measures are common in Africa.[217,318] In Uganda, provision of hand hygiene agents (including alcohol-based hand rub) especially at the point-of-care, and training and re-training of HCWs in paediatric wards on infection control measures including hand hygiene reduced transmission of bacteria to the children.[329] In the UK with a high level of hygiene and compliance with infection prevention and control guidelines, Price and colleagues[111] demonstrated that in the high-dependency and intensive care units, HCWs infrequently transmitted *S. aureus* to patients. Hand hygiene, though simple, remains the most effective intervention for the prevention of the spread of infection both at the home and hospital setting.[218]

Interestingly, handwashing by the midwife or TBA was associated with reduced carriage at birth, even though *S. aureus* positivity was not associated with increased carriage at birth. A possible explanation for this discrepancy is that the bacterial load obtained from the midwife or TBA hands was insufficient for culture. Another possibility is that the midwife or TBA were transient carriers[330] or carried the bacteria in other body sites not screened (for example, the throat) acting as vectors of transmission to the newborn.

Contrary to reports from other studies,[94,106] maternal carriage of *S. aureus* at the time of delivery was not associated with *S. aureus* carriage at birth in this study. The French[106] and Taiwanese[94] studies reported higher carriage rates in babies born vaginally compared to those delivered by caesarean section. However, vaginal carriage and maternal carriage (both nasal and vaginal) at birth in the two studies were similar to the estimates in the current study. The reason for this difference is unclear.

Neonatal *S. aureus* carriage acquisition one-week post-delivery and associated risk factors

The incidence of *S. aureus* carriage acquisition in this study (79.5% in the first week of life) was higher than that seen in a previous study in The Gambia[107] and much higher than the reports from Israel[105] where only 12.5% of neonates acquired *S. aureus* in the first 72 – 100 hours of life. This disparity may be related to the differences in the levels of hygiene compliance and the timing of screening the newborn. The current study and the Gambian study took place in primary health centres while the Israeli study was undertaken in a tertiary hospital. The primary care facility in rural Gambia is likely to have lower barriers to transmission compared to the tertiary hospital in Israel. Secondly, newborns were screened at 72 - 100 hours of life in the Israeli study, and therefore had less time to acquire carriage than newborns in The Gambia who were screened at one week of age. Additionally, cord care practices were different in the three studies.

In this study, maternal nasal and breast milk *S. aureus* carriage at one week were associated with neonatal carriage acquisition suggesting postpartum transmission to the newborn from the mother or vice versa. This finding is in keeping with the report from Israel[105] where maternal *S. aureus* carriage (vagina and nasal carriage) was the only risk factor associated with carriage acquisition in newborns. Leshem and colleagues[105] also observed similar rates

of acquisition among babies born vaginally and those born by caesarean section suggesting postpartum transmission. Using molecular methods, they also showed that 80% of the mother-newborn pairs who were carriers during the study carried genetically identical strains and 90% of the maternal strains were from the nares which further supports the importance of postpartum transmission to the newborn.

In this study, maternal vaginal carriage was not associated with *S. aureus* carriage acquisition in the newborn either at birth or one week of age. It is possible that intrapartum transmission to the newborn could have been missed because of low inoculum in the newborn. It is also possible that most transmissions from the mother occurred postpartum. This hypothesis is supported by the fact that less than 10% of them were carriers within the first 24 hours of life, and carriage acquisition increased to 79.5% by the end of one week. A similar age profile of carriage was observed in the Israeli study[105] which reported an association between maternal nasal carriage and carriage acquisition in the newborn but not vaginal carriage. The prevalence of maternal vaginal carriage was similar in this study to that found in the Israeli study. By contrast, a previous Gambian study[107] found a positive association between maternal vaginal carriage and carriage in the newborn. The reason for this difference in findings is unclear but may be related to the larger sample size and the seemingly higher prevalence of vaginal carriage reported in the previous Gambian study (17%).

In this study, 15.5% of *S. aureus* acquisition at one week of age was attributable to maternal carriage at the time of birth or one week later. This finding is similar to a previous report from The Gambia[107] but higher than that from Gabon.[104] This difference could be because the Gabonese study infrequently sampled the nares and throat over the first year of life or due to antibiotic use by mother and newborn in the immediate postpartum period. During

the first year of life, the infant would have acquired other bacteria competing for the niche, and the prevalence of *S. aureus* in the infant would have fallen considerably. Besides, the infant would have been in contact with other members of the household who could have been the source of transmission at that time. Despite these differences, the contribution of the mother to carriage acquisitions in the newborn is small, suggesting the unattributed acquisitions are from other contacts not sampled in this study or misclassification of carriage in the newborn or mother. Nevertheless, decolonisation of the mother using intrapartum antibiotics has been shown to reduce *S. aureus* carriage both in the mother and newborn up to 28 days post-delivery.[107]

The history of maternal urinary tract infection was negatively associated with carriage acquisition. This is surprising because maternal infections, including urinary tract infections, are usually risk factors for neonatal bacterial carriage and infections.[331] This negative association may be due to antibiotic use by the mother to treat the infection.

Healthcare workers are known vectors for the transmission of infection in healthcare facilities.[111] Only a negligible proportion of *S. aureus* acquisition by the newborn could be attributed to the midwife or TBA (2.2%). The proportion may be small because the healthcare workers improved their handwashing frequency and technique because they knew they were being observed (Hawthorne's effect). It is also possible that the proportion was small due to the short duration of the contact with the newborn as most women were discharged from the hospital within 24 hours. Furthermore, the midwife or TBA reported the use of antibiotics in the preceding six months in about one-third of deliveries (Supplemental Table 4.3). This lends support to the fact that reinforcement of handwashing with or without decolonisation will contribute to improved infection control strategies.

Children are known to transmit carriage and disease in the household[332]; however, only a small proportion of *S. aureus* acquisition by the newborn could be attributed to the household children (5.2%). This finding may be specific to the Gambian setting, where limited contact takes place between the newborn and the household children in the first few weeks of life.

Despite the poor compliance with infection control measures in the hospital and home settings in this study, we were unable to demonstrate an attributable source of carriage acquisition from the health facility. Low bacterial loads (not detectable by culture) from the mother, midwives, household children or caregivers may be the cause of the unattributable carriage acquisitions. Molecular methods (PCR) are more sensitive at detecting *S. aureus* both in neonates and adults.[311,333] These methods might have increased the yield of *S. aureus* in the study participants. It is also possible that the bacteria in the contacts were not culturable because either they were located intracellularly in macrophages or monocytes or existed in biofilms,[152,334] and rapid replication of the bacteria in the newborn with new acquisition. Although the inoculum from the contact to the newborn might have been low, rapid replication of the bacteria in the new immunologic environment may be responsible for the high carriage acquisition rate in the newborn. Further research is required to test this hypothesis.

Antimicrobial resistance

The prevalence of antimicrobial resistance to all tested antibiotics was low. The prevalence of MRSA among *S. aureus* isolates was low (3.3%) and similar to previous estimates from The Gambia.[98,313] Taken together the results from these studies suggest that the prevalence of MRSA among carriage isolates in The Gambia has been stable over the last decade.

Though the prevalence of MRSA has been steady, there is still the need for continuous MRSA surveillance and establishment of policies to prevent an increase in the prevalence or the spread of MRSA in the community and hospital given its grave consequences. Continuous MRSA surveillance will aid the identification of temporal trends, implementation of effective IPC and evaluation of the intervention.

The prevalence of resistance to cloxacillin, which is the first-line drug for the treatment of *S. aureus* infections in rural Gambia (2.2%) was higher than previous reports from the Gambia[98] but not sufficiently high to warrant a revision of the national guidelines for the treatment of *S. aureus* infections in the country. Resistance to erythromycin was also low. It is, therefore, worth retaining as a second-line medication against *S. aureus,* especially in those with an allergy to penicillin and penicillin-like drugs. In keeping with reports from Ethiopia[335] and Ghana,[324,327] resistance to clindamycin was negligible. Different rates of unrestricted antibiotic use in humans and livestock may explain the variations in antimicrobial susceptibility in different studies.

Strengths and limitations

The inclusion of different participants groups in the study was a significant strength because it allowed the exploration of the different sources of newborn acquisition of *S. aureus* and for comparison of carriage and AMR among the groups. It also allowed the comparison of AMR among community and hospital carriage strains. Second, detection of *S. aureus* carriage was enhanced by screening two body sites for the mother, baby, and healthcare worker. Third, the use of enrichment broth before plating enhanced the sensitivity of detection by culture. Fourth, the study design allowed the evaluation of the temporal association between contacts and acquisition by the newborn. Screening of all contacts took place before the

assessment of the outcome (carriage acquisition in the newborn). Lastly, real-time collection of information on perinatal history circumvented recall bias.

However, this study has some limitations. First, only 51.9% of women asked to join the study were enrolled due to migration during a period of political instability. Second, a significant proportion of the carriage acquisitions among the newborns were not attributable to a particular source, which may be because other sources of transmission were not sampled. Possible sources that were not sampled include midwives' throat, mother's skin, throat and hands, baby cot, baby's swaddling clothes, hospital bed, taps, doorknobs, air samples and other members of the household. However, even in a study where intensive sampling was done and molecular methods used, the investigators could link a source to only 26% of acquisitions events.[111] This may be because not all possible sources can be screened.

Conclusion

The prevalence of *S. aureus* carriage in newborns in rural Gambia was low at birth, but with a high acquisition rate in the first week of life. Only a modest proportion of the carriage acquisition was attributable to a particular source. This implies that there are other sources of acquisition that were not sampled or that the laboratory methods were not sensitive enough to detect the relatively small inoculum carried by screened individuals. Nevertheless, given that carriage often precedes disease, the high rate of *S. aureus* carriage acquisition in the first one week of life calls for strengthening of existing strategies to prevent transmission of *S. aureus* as well as the development of new tools to reduce the risk of *S. aureus* transmission to newborns.

Chapter 5: Implications for policy, practice, and future research

Preamble

This thesis describes the burden of *S. aureus* disease in children aged less than five years in Africa, provides updates on the burden of *S. aureus* bacteraemia (SAB) in The Gambia and explores the risk factors for *S. aureus* carriage at birth as well as those for carriage acquisition in newborns. This chapter summarises the key findings of the thesis, discusses the implications of the results for practice and policy and highlights areas for future research.

5.1 Key findings and contribution to the body of knowledge

5.1.1 The burden of severe *S. aureus* disease in Africa

The analyses described in Chapter 2 provided an estimate of 392,066 cases and 46,467 deaths due to severe *S. aureus* diseases among children aged less than five years in 2015 in Africa (Box 5.1). The burden is probably higher than these estimates because of the lack of data on the clinical syndromes caused by *S. aureus*. The burden of *S. aureus* disease showed substantial variability by region and country. However, the disease burden was consistently highest among neonates; therefore, tools are needed to reduce the burden of staphylococcal diseases in this population and hence contribute to a reduction in neonatal mortality in line with the targets of SDG 3.2.

5.1.2 The burden of *S. aureus* bacteraemia in The Gambia

Data on the epidemiological features of *S. aureus* disease from population-based studies are rare in Africa. As described in Chapter 3, the incidence of *S. aureus* bacteraemia in young

children in The Gambia was estimated to be 78 per 100,000 person-years (Box 5.1). This study found that the rate in neonates (3.5 per 1,000 live births) was higher than the global and African estimates of the incidence of neonatal GBS.[303] The case fatality ratio (CFR) among young children in this study was substantial (14.1%). The neonatal CFR was similar to the African estimates of the neonatal GBS case fatality ratio. Therefore, interventions are required to prevent *S. aureus* disease in children, especially neonates in rural Gambia and similar settings.

This study, therefore, provides valuable data on the burden of SAB in African children. Additionally, data collection spanned the period before and after PCV introduction and allowed assessment of its impact on SAB. I found no evidence that the introduction of PCV influenced the incidence of SAB.

5.1.3 Carriage acquisition of *S. aureus* in newborns in The Gambia

The risk of *S. aureus* carriage acquisition in the first week of life was high (79.5%). Potential sources of *S. aureus* carriage acquisition in newborns were identified, but the proportion of carriage acquisition associated with these sources was low at 22%, perhaps due to the diagnostic methods used (culture). Neonatal carriage acquisition was associated with maternal and household child carriage at one week after delivery, and bed share with the newborn and negatively associated with maternal history of dysuria or urinary tract infection one week before delivery. Molecular methods may help to identify the source of *S. aureus* in Gambian newborns, but the use of these methods was beyond the scope of this study.

The prevalence of antibiotic resistance was similar in carriage isolates from healthcare and community sources.

Box 5.1

Summary of main findings from the studies undertaken for this thesis

Background

- Preventable infections are still responsible for about 40% of the childhood deaths in sub-Saharan Africa
- *S. aureus* is the most common cause of IBI in newborns and a significant cause in childhood.
- *S. aureus* carriage is an important risk factor for *S. aureus* disease.
- The prevalence of carriage is higher among newborns than other age group, peaking at the end of the first week of life.

The burden of *S. aureus* disease in Africa

- An estimated 392,066 staphylococcal cases and 46,467 deaths occurred among children aged < 5 years in 2015.
- One-fifth and over half of the *S. aureus* septicaemia cases and deaths occurred among neonates, respectively.
- *S. aureus* isolates were frequently resistant to ampicillin and amoxicillin, but rarely resistant to oxacillin or gentamicin.
- There are limited data on the features of *S. aureus* disease incidence in Africa.

The burden of *S. aureus* bacteraemia in The Gambia

- The incidence of *S. aureus* bacteraemia in children aged less than five years was high, with the highest rates seen among neonates (3.5 per 1,000 live births).
- The incidence of *S. aureus* bacteraemia did not change after the introduction of PCV.
- The case fatality ratio was substantial (14.1%) and did not vary with age.

Neonatal acquisition of *S. aureus* in Gambia

- The prevalence of carriage at birth was low (9.7%)
- Carriage at birth was negatively associated with handwashing by midwife or TBAs.
- The rate of *S. aureus* carriage acquisition in the first week of life was high (79.5%) and associated with maternal carriage at one week, and household child carriage at one week and bed share with the newborn.

5.2 Implications of study findings for policy, practice and research

5.2.1 Policy and practice

Short term

The work in this thesis highlights some of the factors contributing to the high morbidity and mortality of *S. aureus* disease, especially among neonates. High mortality was associated

with late presentation. Factors responsible for this late presentation may include the inability of parents to identify clinical danger signs, poor healthcare-seeking behaviour of the parents, undue financial hardship, lack of social support or inaccessible healthcare facilities. Inadequate knowledge of mothers on newborn danger signs delays healthcare-seeking. Additionally, a number of poor practices were reported including cutting of the cord with a non-sterile blade (3.1%), ligation of the cord with a piece of thread (62.0%) and application of unhygienic substances to the cord (81.1%); these are likely to have contributed to the high rate of umbilical carriage acquisition of *S. aureus* observed in The Gambia. Maternal antenatal education has been demonstrated to improve knowledge of essential newborn care.[336] Therefore, information, education and communication strategies are required to increase awareness among mothers on essential newborn care especially the identification of newborn danger signs and effective cord care (for example, daily application of chlorhexidine for seven days) and the use of clean delivery kits. The WHO recommends the use of chlorhexidine for cord care in settings with high neonatal mortality like The Gambia. Periodic monitoring and evaluation of the programme is necessary as well.

In Africa, although institutional deliveries are encouraged,[337] hygienic practices are often sub-optimal because a parallel improvement in the health systems or the hospital capacity has not increased proportionately, thus increasing the risk of infections. In The Gambia, handwashing by the midwife or TBA was negatively associated with newborn *S. aureus* carriage. Clean birth practices, including using hand hygiene and delivery kits, can reduce neonatal infections and deaths at home by 15% and in the healthcare facility by 40%.[338] Therefore, low-cost interventions should be introduced or scaled up with regular refresher training to improve infection prevention and control (IPC) knowledge and practices.

Furthermore, monitoring and evaluation of compliance at the workplace are essential to identify gaps to be addressed promptly.

In The Gambia, modification of national guidelines for the treatment of disease is based on the prevailing antibiotic susceptibility pattern. Based on the findings of the current study, the existing Gambian National guidelines which recommend the use of ampicillin and gentamicin for treatment of neonatal sepsis[175] requires revision to cloxacillin and gentamicin. Susceptibility of *S. aureus* to oxacillin in both studies of disease (chapter 3) and carriage (chapter 4) in The Gambia ranged between 88% and 98%, to gentamicin it was 98% while that to penicillin was less than 10% in both studies. The guidelines also recommend the use of flucloxacillin in older children if *S. aureus* is suspected.[175] Given the low prevalence of resistance to oxacillin observed in both the carriage and bacteraemia studies, this recommendation is still valid. In Africa, there are few estimates of the prevalence of resistance of *S. aureus* to amoxicillin. The few studies (including a recent review in sub-Saharan Africa) that have reported data suggest that the prevalence is high (>70%).[19,49,262,268] Therefore, the WHO recommendation of amoxicillin for the treatment of childhood septicaemia when referral is not feasible or acceptable by parents,[174] likely requires revision.

Long term

A major finding of the studies conducted for this thesis is the scarcity of incidence data on community-acquired *S. aureus* disease in Africa even though it is likely to be responsible for a substantial burden of disease. The WHO sentinel surveillance for IBI through national and regional laboratories should be expanded to include *S. aureus* to increase awareness of stakeholders to this significant contributor to neonatal and childhood mortality (Box 5.2).

National governments should establish *S. aureus* disease surveillance programmes, including all hospitals and laboratories where confirmed cases are reported to a central database. Annual review of the database is required to inform the modification of the existing interventions and to guide the design of new ones if these are necessary.

Policies on antibiotic use in Africa must balance the effective treatment of *S. aureus* and other infections with overuse of these drugs. Pre-treatment antibiotic use in children is common,[50,161] because antibiotics can be procured over-the-counter or from community drug sellers without prescription in many countries. In addition, antibiotics are overprescribed by healthcare professionals who often treat without a microbiology laboratory result either due to lack of expertise, laboratory facility or due to the cost of the test. Consequently, governments should establish well-equipped laboratories with adequate staffing and make possible diagnostic tests at affordable prices. Guidelines for the proper collection of samples should be developed nationally and used at facility level since optimal sample collection is key to the interpretation of the results. Regular monitoring and evaluation of adherence to the guidelines are required. Regular external quality assessments of the laboratory processes are required to ensure reliability and comparability of results. Additionally, laws should be enacted and enforced to deter the purchase of antibiotics that have not been prescribed.

Governments in Africa should be committed to the establishment of autonomous national IPC teams with clearly defined goals, activities, and functions. These teams should have the ability to make decisions on IPC-related issues and have a budget to implement their recommendations. Furthermore, the national IPC teams should have the responsibility to ascertain the training of all healthcare personnel using the team- and task-based strategies

on locally adapted guidelines, and the provision of necessary infrastructure and supplies to safeguard implementation and compliance with the guidelines.

Only six of the 47 countries in the WHO African Region achieved MDG target 4A of reducing under-five mortality by two thirds.[339] Despite not meeting the MDG, the world has adopted the SDGs with even more ambitious health targets. For instance, the newborn mortality in the African Region (27 per 1,000 live births) falls significantly short of the interim goals for 2020 (less than 15 per 1,000 live births). This thesis has identified SAB as a potentially significant contributor to this (1.6 cases per 1,000 live births) and enumerated some short- and long-term interventions at various levels of the health systems to mitigate against this disease and other bacterial diseases.

5.2.2 Implications for future research

The work undertaken for this thesis has identified a number of research priorities related to finding ways for improved management of *S. aureus* infections in LMIC like The Gambia.

Disease surveillance

Large scale epidemiological studies to describe the burden and evaluate the risk factors associated with *S. aureus* disease are required to fill the gap in the significant lack of surveillance data in Africa. Understanding the role of risk factors in the development of *S. aureus* disease is critical to formulating interventions geared towards the reduction of the disease. Although such studies are expensive and time-consuming, they are necessary. The least expensive approach will be to set up continuous national surveillance to include all hospitals and laboratories, harmonise methods for data comparability and directed at young children.

Box 5.2

Summary for the implications of the study findings for policy and practice

Interventions to reduce *S. aureus* disease

- Strategies to increase mothers' awareness to identify early warning signs of deteriorating health of newborns and older children
- Provision and scaling up of chlorhexidine at health posts and postnatal clinics free of charge
- Scale up hand hygiene programmes among HCW and TBAs
- Modification of national guidelines to change drug of choice for the treatment of neonatal sepsis to cloxacillin and gentamicin in The Gambia
- WHO to include *S. aureus* among the pathogens under IBI sentinel surveillance
- National commitment to surveillance of *S. aureus* disease and AMR
- Review of guidelines for the treatment of suspected sepsis at the community level
- Controlled access to antibiotics in Africa
- Establishment of national and local infection prevention and control (IPC) teams

Tools and Technologies

- Guidelines for the proper collection of samples to harmonise methods
- Government to develop and equip regional laboratories
- Creation of national and local databases to monitor *S. aureus* diseases and antimicrobial resistance

Adequate reporting of prior antibiotic use and antimicrobial susceptibility patterns should be encouraged for studies describing the burden of bacterial diseases. These will lend support to the development and establishment of interventions to reduce the disease burden and lead to well-adapted guidelines on drug therapy for infection.

Intrapartum Antibiotics Prophylaxis

In the carriage study, maternal carriage was the most significant risk factor for neonatal carriage acquisition. Consequently, reducing *S. aureus* carriage in the mother during the perinatal period should reduce carriage acquisition in the newborn. A potential way of achieving this objective is by administering intrapartum antibiotics prophylaxis (IAP) to the

mother to clear *S. aureus* carriage, which has been shown to reduce both maternal and newborn *S. aureus* carriage by 60% in The Gambia.[103] Available strategies are to either administer IAP to all pregnant women (non-screening approach) or to screen for *S. aureus* between the 35th and 37th week of pregnancy and treat positive women (screening approach). The latter will be similar to the universal screening of pregnant women for GBS to prevent disease in their newborns. This approach may not be feasible in Africa because of the scarcity of well-equipped and adequately staffed laboratories.

Given that not all babies born to *S. aureus*-carrying mothers become infected, another strategy would be to reserve IAP for pregnancies with a high risk of infection, especially preterm deliveries (risk-based approach). The use of IAP in Africa would need careful consideration, in terms of the timing and practicability of screening, cost-effectiveness and the emergence of antibiotic resistance. Therefore, a study is required to compare the effectiveness of the two (screening and risk-based) approaches in preventing neonatal *S. aureus* carriage and disease. Another research to assess and compare the development of antibiotic resistance and the cost-effectiveness of the universal antibiotic administration to the screening approach is necessary before a policy can be formulated. If the Gambian government were to implement a programme of IAP use, then monitoring for antibiotic resistance would be essential.

Cord care

Chlorhexidine cord cleansing for seven consecutive days post-delivery reduced the risk of bacterial carriage, omphalitis and neonatal mortality in South Asia.[340] There is no data on the effect of chlorhexidine on neonatal mortality in Africa, therefore such trials are required in resource-poor settings. However, daily application of chlorhexidine has been associated with delayed cord separation, and the risk of poor adherence increases with time, thus

increasing the risk of infection. Additionally, it is an easier programmatically intervention to implement compared to seven days application. Therefore, RCTs are required to test the non-inferiority of the effect of the application of chlorhexidine to the cord once immediately post-partum compared to seven days application in Africa.

Evidence of transmission

Newborns of carrier mothers have similar rates of acquiring both maternal and non-maternal strains in the early neonatal period.[104,105] Therefore, molecular methods to determine the genetic relatedness of strains are required to detect the source of transmission of *S. aureus* between contacts and newborns. *Spa* typing will be a preferred method because it is highly discriminatory, reproducible, and not as expensive as other sequencing methods. Determination of the source of transmission to the newborn will aid the development of interventions to reduce carriage acquisition by the newborn.

Vaccine development

While IPC measures are necessary, the development of a vaccine against *S. aureus* is an attractive strategy given the success of other vaccines in the reduction of many childhood infections as well as the multiplicity of potential sources of the infection. As discussed in chapter 1, vaccines candidates have progressed into clinical trials but failed to demonstrate

Box 5.3

Research Priorities

The Gambia

- Qualitative studies into the health-seeking behaviour of mothers and cord care practices
- Effectiveness of chlorhexidine on neonatal mortality
- Assessment of the efficacy of single use of chlorhexidine for cord care compared to seven days
- Assessment of the effectiveness of maternal screening- and risk-based approaches in preventing neonatal *S. aureus* carriage and disease
- Assessment of knowledge gaps on IPC among HCW

The African Continent

- Prospective surveillance studies on the burden of *S. aureus* disease
- Clinical development of a vaccine against *S. aureus*
- Development of diagnostic POC tools with the ability to detect multiple pathogens at once

efficacy against disease.[226] It appears that the key barrier to vaccine development is the lack of understanding of the mechanisms of protective immunity to *S. aureus*.[226] Therefore, further research is required in this area. Another barrier is that previous vaccine candidates have focused on the use of either single or multiple proteins.[226] A way forward may be the use of the reverse vaccinology approach, which employs computational analysis of the entire genome of a bacterium for the prediction and identification of genes. These genes are likely to lead to the expression of proteins that might be expected to elicit to an immune response; for example, those likely to be expressed on the surface of a micro-organism. Candidate genes are then introduced into an expression system and the recombinant proteins produced are tested for their protective effects in animal models. This method has been used successfully in the development of vaccines against other diseases.[341]

Careful consideration needs to be given to the appropriate vaccination strategies in terms of timing and age of administration, given that the burden of disease is highest in newborns. Maternal immunisation is a promising strategy because it can protect both the mother and the newborn. Alternatively, the vaccine could also be given directly to newborns, health

workers and vulnerable populations. Such an intervention would not only directly protect the newborns, pregnant mothers, and health care workers but would increase the herd immunity, thereby further reducing the carriage and burden of disease.

5.3 Overall conclusion

S. aureus disease is a significant contributor to neonatal and childhood morbidity and mortality in Africa yet is under-recognised. *S. aureus* carriage acquisition by one week of age is high; the mother is the primary source of this acquisition. Interventions are required both in the community and health facilities to reduce the burden of *S. aureus* carriage and disease among children, especially newborns. There is, also, a need for investment in research and continued coordinated population-based surveillance of this disease, strengthening of laboratory infrastructures and the promotion of harmonisation of methods. Finally, a vaccine against *S. aureus* remains an attractive, cost-effective option that merits renewed attention, particularly with additional evidence on the high burden of disease.

References

1. UNICEF, WHO, The World Bank and United Nations. 'Levels & Trends in Child Mortality. Report 2019, Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation'. New York, USA,, **2019**.

2. World Health Organization. Global Health Observatory (GHO) data. **2016**. http://www.who.int/gho/child_health/mortality/causes/en/ (accessed 25 April 2016).

3. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* **2016**; 388: 3027-35.

4. UNICEF, WHO, The World Bank and United Nations. 'Levels & Trends in Child Mortality. Report 2017, Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation'. New York, USA, **2017**.

5. World Health Organization. WHO and Maternal and Child Epidemiology Estimation Group (MCEE) estimates 2018. February 14, 2018 **2018**. http://apps.who.int/gho /data/view.main.CM1002015REG6-CH7?lang=en (accessed June 5 2018).

6. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* **2005**; 352: 39-47.

7. O'Dempsey TJ, McArdle TF, Lloyd-Evans N, et al. Importance of enteric bacteria as a cause of pneumonia, meningitis and septicemia among children in a rural community in The Gambia, West Africa. *Pediatr Infect Dis J* **1994**; 13: 122-8.

8. Enwere G, Biney E, Cheung YB, et al. Epidemiologic and clinical characteristics of community-acquired invasive bacterial infections in children aged 2-29 months in The Gambia. *Pediatr Infect Dis J* **2006**; 25: 700-5.

9. Oluwalana C, Howie SR, Secka O, et al. Incidence of *Haemophilus influenzae* type b disease in The Gambia 14 years after introduction of routine *Haemophilus influenzae* type b conjugate vaccine immunization. *J Pediatr* **2013**; 163: S4-7.

10. Adegbola RA, Secka O, Lahai G, et al. Elimination of *Haemophilus influenzae* type b (Hib) disease from The Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. *Lancet* **2005**; 366: 144-50.

11. Roca A, Hill PC, Townend J, et al. Effects of community-wide vaccination with PCV-7 on pneumococcal nasopharyngeal carriage in the Gambia: a cluster-randomized trial. *PLoS Med* **2011**; 8: e1001107.

12. Mackenzie GA, Hill PC, Jeffries DJ, et al. Effect of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease in The Gambia: a population-based surveillance study. *Lancet Infect Dis* **2016**; 16: 703-11.

13. Wahl B, O'Brien KL, Greenbaum A, et al. Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000-15. *Lancet Glob Health* **2018**; 6: e744-e57.

14. Waters D, Jawad I, Ahmad A, et al. Aetiology of community-acquired neonatal sepsis in low and middle income countries. *J Glob Health* **2011**; 1: 154-70.

15. Hamer DH, Darmstadt GL, Carlin JB, et al. Etiology of bacteremia in young infants in six countries. *Pediatr Infect Dis J* **2015**; 34: e1-8.

16. Simonsen KA, Anderson-Berry AL, Delair SF, et al. Early-onset neonatal sepsis. *Clin Microbiol Rev* **2014**; 27: 21-47.

17. Cortese F, Scicchitano P, Gesualdo M, et al. Early and Late Infections in Newborns: Where Do We Stand? A Review. *Pediatr & Neonatol* **2016**; 57: 265-73.

18. Tiskumara R, Fakharee SH, Liu CQ, et al. Neonatal infections in Asia. *Arch Dis Child Fetal Neonatal Ed* **2009**; 94: F144-8.

19. Okomo U, Akpalu ENK, Le Doare K, et al. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. *Lancet Infect Dis* **2019**; 19: 1219-34.

20. Lee JH, Cho HK, Kim KH, et al. Etiology of invasive bacterial infections in immunocompetent children in Korea (1996-2005): a retrospective multicenter study. *J Korean Med Sci* **2011**; 26: 174-83.

21. Larru B, Gong W, Vendetti N, et al. Bloodstream Infections in Hospitalized Children: Epidemiology and Antimicrobial Susceptibilities. *Pediatr Infect Dis J* **2016**; 35: 507-10.

22. Greenhow TL, Hung YY, Herz AM. Changing epidemiology of bacteremia in infants aged 1 week to 3 months. *Pediatrics* **2012**; 129: e590-6.

23. Onipede AO, Onayade AA, Elusiyan JB, et al. Invasive bacteria isolates from children with severe infections in a Nigerian hospital. *J Infect Dev Ctries* **2009**; 3: 429-36.

24. von Eiff C, Becker K, Machka K, et al. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. *N Engl J Med* **2001**; 344: 11-6.

25. Usuf E, Bojang A, Hill PC, et al. Nasopharyngeal colonization of Gambian infants by *Staphylococcus aureus* and *Streptococcus pneumoniae* before the introduction of pneumococcal conjugate vaccines. *New Microbes New Infect* **2016**; 10: 13-8.

26. Frederiksen MS, Espersen F, Frimodt-Moller N, et al. Changing epidemiology of pediatric *Staphylococcus aureus* bacteremia in Denmark from 1971 through 2000. *Pediatr Infect Dis J* **2007**; 26: 398-405.

27. Cobos-Carrascosa E, Soler-Palacin P, Nieves Larrosa M, et al. *Staphylococcus aureus* Bacteremia in Children: Changes During Eighteen Years. *Pediatr Infect Dis J* **2015**; 34: 1329-34.

28. Gerber JS, Coffin SE, Smathers SA, et al. Trends in the incidence of methicillin-resistant *Staphylococcus aureus* infection in children's hospitals in the United States. *Clin Infect Dis* **2009**; 49: 65-71.

29. Grijalva CG, Nuorti JP, Zhu Y, et al. Increasing incidence of empyema complicating childhood community-acquired pneumonia in the United States. *Clin Infect Dis* **2010**; 50: 805-13.

30. Thorlacius-Ussing L, Sandholdt H, Larsen AR, et al. Age-Dependent increase in incidence of *Staphylococcus aureus* bacteremia, Denmark, 2008-2015. *Emerg Infect Dis* **2019**; 25: 875-82.

31. Ladhani SN, Henderson KL, Muller-Pebody B, et al. Risk of invasive bacterial infections by week of age in infants: prospective national surveillance, England, 2010-2017. *Arch Dis Child* **2019**; 104: 874-78.

32. Pasquali SK, He X, Mohamad Z, et al. Trends in endocarditis hospitalizations at US children's hospitals: impact of the 2007 American Heart Association Antibiotic Prophylaxis Guidelines. *Am Heart J* **2012**; 163: 894-9.

33. Rosenthal LB, Feja KN, Levasseur SM, et al. The changing epidemiology of pediatric endocarditis at a children's hospital over seven decades. *Pediatr Cardiol* **2010**; 31: 813-20.

34. Rushani D, Kaufman JS, Ionescu-Ittu R, et al. Infective endocarditis in children with congenital heart disease: cumulative incidence and predictors. *Circulation* **2013**; 128: 1412-9.

35. Baltimore RS, Gewitz M, Baddour LM, et al. Infective Endocarditis in Childhood: 2015 Update: A Scientific Statement From the American Heart Association. *Circulation* **2015**; 132: 1487-515.

36. Riise OR, Kirkhus E, Handeland KS, et al. Childhood osteomyelitis-incidence and differentiation from other acute onset musculoskeletal features in a population-based study. *BMC Pediatr* **2008**; 8: 45-54.

37. Gafur OA, Copley LA, Hollmig ST, et al. The impact of the current epidemiology of pediatric musculoskeletal infection on evaluation and treatment guidelines. *J Pediatr Orthop* **2008**; 28: 777-85.

38. Riise OR, Kirkhus E, Handeland KS, et al. Childhood osteomyelitis-incidence and differentiation from other acute onset musculoskeletal features in a population-based study. *BMC Pediatr* **2008**; 8: 45.

39. Dartnell J, Ramachandran M, Katchburian M. Haematogenous acute and subacute paediatric osteomyelitis: a systematic review of the literature. *J Bone Joint Surg Br* **2012**; 94: 584-95.

40. Suaya JA, Mera RM, Cassidy A, et al. Incidence and cost of hospitalizations associated with *Staphylococcus aureus* skin and soft tissue infections in the United States from 2001 through 2009. *BMC Infect Dis* **2014**; 14: 296-303.

41. Morgan E, Hohmann S, Ridgeway J, et al. Decreasing incidence of skin and soft tissue infections at 86 U.S. emergency departments, 2009-2014. *Clin Infect Dis* **2018**: 453-59.

42. Darmstadt GL, Saha SK, Choi Y, et al. Population-based incidence and etiology of community-acquired neonatal bacteremia in Mirzapur, Bangladesh: an observational study. *J Infect Dis* **2009**; 200: 906-15.

43. Pavan Kumar DV, Mohan J, Rakesh PS, et al. Bacteriological profile of neonatal sepsis in a secondary care hospital in rural Tamil Nadu, Southern India. *J Family Med Prim* Care **2017**; 6: 735-38.

44. Kanoksil M, Jatapai A, Peacock SJ, et al. Epidemiology, microbiology and mortality associated with community-acquired bacteremia in northeast Thailand: a multicenter surveillance study. *PLoS One* **2013**; 8: e54714.

45. Sigauque B, Roca A, Mandomando I, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J* **2009**; 28: 108-13.

46. Brent AJ, Ahmed I, Ndiritu M, et al. Incidence of clinically significant bacteraemia in children who present to hospital in Kenya: community-based observational study. *Lancet* **2006**; 367: 482-8.

47. Mulholland EK, Ogunlesi OO, Adegbola RA, et al. Etiology of serious infections in young Gambian infants. *Pediatr Infect Dis J* **1999**; 18: S35-41.

48. Mhada TV, Fredrick F, Matee MI, et al. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome. *BMC Public Health* **2012**; 12: 904-09.

49. Ogundare EO, Akintayo AA, Dedeke IOF, et al. Neonatal septicaemia in a rural Nigerian hospital: aetiology, presentation and antibiotic sensitivity pattern. *Br J Med Med Res* **2016**; 12: 1-11.

50. Obaro S, Lawson L, Essen U, et al. Community acquired bacteremia in young children from central Nigeria--a pilot study. *BMC Infect Dis* **2011**; 11: 137-46.

51. Nielsen MV, Sarpong N, Krumkamp R, et al. Incidence and characteristics of bacteremia among children in rural Ghana. *PLoS One* **2012**; 7: e44063.

52. Johnson AW, Osinusi K, Aderele WI, et al. Bacterial aetiology of acute lower respiratory infections in pre-school Nigerian children and comparative predictive features of bacteraemic and non-bacteraemic illnesses. *J Trop Pediatr* **1993**; 39: 97-106.

53. Adegbola RA, Falade AG, Sam BE, et al. The etiology of pneumonia in malnourished and well-nourished Gambian children. *Pediatr Infect Dis J* **1994**; 13: 975-82.

54. Fagbule D, Parakoyi DB, Spiegel R. Acute respiratory infections in Nigerian children: prospective cohort study of incidence and case management. *J Trop Pediatr* **1994**; 40: 279-84.

55. Wall RA, Corrah PT, Mabey DC, et al. The etiology of lobar pneumonia in the Gambia. *Bull World Health Organ* **1986**; 64: 553-8.

56. Adedoyin MA, Fagbule D. Bacterial aetiology of childhood pneumonia. *Nig J Paed* **1987**; 14: 37-40.

57. Pneumonia Etiology Research for Child Health Study G. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet* **2019**; 394: 757-79.

58. Rudan I, Boschi-Pinto C, Biloglav Z, et al. Epidemiology and etiology of childhood pneumonia. *Bull World Health Organ* **2008**; 86: 408-16.

59. Luksic I, Mulic R, Falconer R, et al. Estimating global and regional morbidity from acute bacterial meningitis in children: assessment of the evidence. *Croat Med J* **2013**; 54: 510-8.

60. Roca A, Bassat Q, Morais L, et al. Surveillance of acute bacterial meningitis among children admitted to a district hospital in rural Mozambique. *Clin Infect Dis* **2009**; 48: S172-80.

61. Mwangi I, Berkley J, Lowe B, et al. Acute bacterial meningitis in children admitted to a rural Kenyan hospital: increasing antibiotic resistance and outcome. *Pediatr Infect Dis* J **2002**; 21: 1042-8.

62. Hugo-Hamman CT, de Moor MM, Human DG. Infective endocarditis in South African children. *J Trop Pediatr* **1989**; 35: 154-8.

63. Yameogo NV, Kologo KJ, Yameogo AA, et al. [Infective endocarditis in sub-Saharan african children, cross-sectional study about 19 cases in Ouagadougou at Burkina Faso]. *Ann Cardiol Angeiol* (Paris) **2014**; 63: 7-10.

64. Hodes RM. Endocarditis in Ethiopia. Analysis of 51 cases from Addis Ababa. *Trop Geogr Med* **1993**; 45: 70-2.

65. Omoke NI, Obasi AA. Childhood pyogenic septic arthritis as seen in a teaching hospital South East Nigeria. *Niger J Surg* **2017**; 23: 26-32.

66. Ogunlusi JD, Ogunlusi OO, Oginni LM, et al. Septic arthritis in a Nigerian tertiary hospital. *Iowa Orthop J* **2006**; 26: 45-7.

67. Akinyoola AL, Obiajunwa PO, Oginni LM. Septic arthritis in children. *West Afr J Med* **2006**; 25: 119-23.

68. Allard C, Carignan A, Bergevin M, et al. Secular changes in incidence and mortality associated with *Staphylococcus aureus* bacteraemia in Quebec, Canada, 1991-2005. *Clin Microbiol Infect* **2008**; 14: 421-8.

69. El Atrouni WI, Knoll BM, Lahr BD, et al. Temporal trends in the incidence of *Staphylococcus aureus* bacteremia in Olmsted County, Minnesota, 1998 to 2005: a population-based study. *Clin Infect Dis* **2009**; 49: e130-8.

70. Asgeirsson H, Gudlaugsson O, Kristinsson KG, et al. Low mortality of *Staphylococcus aureus* bacteremia in Icelandic children: nationwide study on incidence and outcome. *Pediatr Infect Dis J* **2015**; 34: 140-4.

71. Groome MJ, Albrich WC, Wadula J, et al. Community-onset *Staphylococcus aureus* bacteraemia in hospitalised African children: high incidence in HIV-infected children and high prevalence of multidrug resistance. *Paediatr Int Child Health* **2012**; 32: 140-6.

72. Naidoo R, Nuttall J, Whitelaw A, et al. Epidemiology of *Staphylococcus aureus* bacteraemia at a tertiary children's hospital in Cape Town, South Africa. *PLoS One* **2013**; 8: e78396.

73. Ladhani S, Konana OS, Mwarumba S, et al. Bacteraemia due to *Staphylococcus aureus*. *Arch Dis Child* **2004**; 89: 568-71.

74. Laupland KB, Ross T, Gregson DB. *Staphylococcus aureus* bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000-2006. *J Infect Dis* **2008**; 198: 336-43.

75. Kallen AJ, Mu Y, Bulens S. Active Bacterial Core surveillance (ABCs) MRSA Investigators of the Emerging Infections Program. Health care–associated invasive MRSA infections, 2005-2008. *JAMA* **2010**; 304: 641-48.

76. Laupland KB, Church DL, Mucenski M, et al. Population-based study of the epidemiology of and the risk factors for invasive *Staphylococcus aureus* infections. *J Infect Dis* **2003**; 187: 1452-9.

77. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* **2007**; 298: 1763-71.

78. Tong SY, van Hal SJ, Einsiedel L, et al. Impact of ethnicity and socio-economic status on *Staphylococcus aureus* bacteremia incidence and mortality: a heavy burden in Indigenous Australians. *BMC Infect Dis* **2012**; 12: 249-57.

79. McMullan BJ, Bowen A, Blyth CC, et al. Epidemiology and mortality of *Staphylococcus aureus* bacteremia in australian and New Zealand children. *JAMA Pediatr* **2016**; 170: 979-86.

80. Williamson DA, Lim A, Thomas MG, et al. Incidence, trends and demographics of *Staphylococcus aureus* infections in Auckland, New Zealand, 2001-2011. *BMC Infect Dis* **2013**; 13: 569-77.

81. Fortuin-de Smidt MC, Singh-Moodley A, Badat R, et al. *Staphylococcus aureus* bacteraemia in Gauteng academic hospitals, South Africa. *Int J Infect Dis* **2015**; 30: 41-8.

82. Madhi SA, Petersen K, Madhi A, et al. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. *Clin Infect Dis* **2000**; 31: 170-6.

83. Cannas G, Merazga S, Virot E. Sickle Cell Disease and Infections in High- and Low-Income Countries. *Mediterr J Hematol Infect Dis* **2019**; 11: e2019042.

84. Fitzgerald SF, O'Gorman J, Morris-Downes MM, et al. A 12-year review of *Staphylococcus aureus* bloodstream infections in haemodialysis patients: more work to be done. *J Hosp Infect* **2011**; 79: 218-21.

85. Roodyn L. Staphylococcal infections in general practice. Br Med J **1954**; 2: 1322-5.

86. Williams RE. Healthy carriage of *Staphylococcus aureus*: its prevalence and importance. *Bacteriol Rev* **1963**; 27: 56-71.

87. Aktas E, Pazarli O, Kulah C, et al. Determination of *Staphylococcus aureus* carriage in hemodialysis and peritoneal dialysis patients and evaluation of the clonal relationship between carriage and clinical isolates. *Am J Infect Control* **2011**; 39: 421-5.

88. Patel G, Jenkins SG, Mediavilla JR, et al. Clinical and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among patients in an ambulatory hemodialysis center. *Infect Control Hosp Epidemiol* **2011**; 32: 881-8.

89. Sai N, Laurent C, Strale H, et al. Efficacy of the decolonization of methicillin-resistant *Staphylococcus aureus* carriers in clinical practice. *Antimicrob Resist Infect Control* **2015**; 4: 56-73.

90. Zacharioudakis IM, Zervou FN, Ziakas PD, et al. Meta-analysis of methicillin-resistant *Staphylococcus aureus* colonisation and risk of infection in dialysis patients. *J Am Soc Nephrol* **2014**; 25: 2131-41.

91. Kim MW, Greenfield BK, Snyder RE, et al. The association between community-associated *Staphylococcus aureus* colonization and disease: a meta-analysis. *BMC Infect Dis* **2018**; 18: 86-97.

92. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* **2005**; 5: 751-62.

93. Senn L, Basset P, Nahimana I, et al. Which anatomical sites should be sampled for screening of methicillin-resistant *Staphylococcus aureus* carriage by culture or by rapid PCR test? *Clin Microbiol Infect* **2012**; 18: E31-3.

94. Huang YC, Chao AS, Chang SD, et al. Association of *Staphylococcus aureus* colonization in parturient mothers and their babies. *Pediatr Infect Dis J* **2009**; 28: 742-4.

95. Miller LG, Eells SJ, Taylor AR, et al. *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. *Clin Infect Dis* **2012**; 54: 1523-35.

96. Regev-Yochay G, Raz M, Carmeli Y, et al. Parental *Staphylococcus aureus* carriage is associated with staphylococcal carriage in young children. *Pediatr Infect Dis J* **2009**; 28: 960-5.

97. Lebon A, Moll HA, Tavakol M, et al. Correlation of bacterial colonization status between mother and child: the Generation R Study. *J Clin Microbiol* **2010**; 48: 960-2.

98. Ebruke C, Dione MM, Walter B, et al. High genetic diversity of *Staphylococcus aureus* strains colonising the nasopharynx of Gambian villagers before widespread use of pneumococcal conjugate vaccines. *BMC Microbiol* **2016**; 16: 38-46.

99. Dey S, Rosales-Klintz S, Shouche S, et al. Prevalence and risk factors for nasal carriage of *Staphylococcus aureus* in children attending anganwaries (preschools) in Ujjain, India. *BMC Res Notes* **2013**; 6: 265-72.

100. Peacock SJ, Justice A, Griffiths D, et al. Determinants of acquisition and carriage of *Staphylococcus aureus* in infancy. *J Clin Microbiol* **2003**; 41: 5718-25.

101. Kwambana BA, Barer MR, Bottomley C, et al. Early acquisition and high nasopharyngeal co-colonisation by *Streptococcus pneumoniae* and three respiratory pathogens amongst Gambian new-borns and infants. *BMC Infect Dis* **2011**; 11: 175-82.

102. Jimenez-Truque N, Tedeschi S, Saye EJ, et al. Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Pediatrics* **2012**; 129: e1252-9.

103. Roca A, Oluwalana C, Bojang AD, et al. Oral azithromycin given during labour decreases bacterial carriage in the mothers and their offspring: a double-blind randomized trial. *Clin Microbiol Infect* **2016**; 22: e1-e9.

104. Schaumburg F, Alabi AS, Mombo-Ngoma G, et al. Transmission of *Staphylococcus aureus* between mothers and infants in an African setting. *Clin Microbiol Infect* **2014**; 20: O390-6.

105. Leshem E, Maayan-Metzger A, Rahav G, et al. Transmission of *Staphylococcus aureus* from mothers to newborns. *Pediatr Infect Dis J* **2012**; 31: 360-3.

106. Bourgeois-Nicolaos N, Lucet JC, Daubie C, et al. Maternal vaginal colonisation by *Staphylococcus aureus* and newborn acquisition at delivery. *Paediatr Perinat Epidemiol* **2010**; 24: 488-91.

107. Roca A, Bojang A, Camara B, et al. Maternal colonization with *Staphylococcus aureus* and Group B *streptococcus* is associated with colonization in newborns. *Clin Microbiol Infect* **2017**; 23: 974-79.

108. Pinter DM, Mandel J, Hulten KG, et al. Maternal-infant perinatal transmission of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *Am J Perinatol* **2009**; 26: 145-51.

109. Andersen BM, Lindemann R, Bergh K, et al. Spread of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive unit associated with understaffing, overcrowding and mixing of patients. *J Hosp Infect* **2002**; 50: 18-24.

110. Blok HE, Troelstra A, Kamp-Hopmans TE, et al. Role of healthcare workers in outbreaks of methicillin-resistant *Staphylococcus aureus*: a 10-year evaluation from a Dutch university hospital. *Infect Control Hosp Epidemiol* **2003**; 24: 679-85.

111. Price JR, Cole K, Bexley A, et al. Transmission of *Staphylococcus aureus* between health-care workers, the environment, and patients in an intensive care unit: a longitudinal cohort study based on whole-genome sequencing. *Lancet Infect Dis* **2017**; 17: 207-14.

112. Pittet D, Allegranzi B, Sax H, et al. Evidence-based model for hand transmission during patient care and the role of improved practices. *Lancet Infect Dis* **2006**; 6: 641-52.

113. Longtin Y, Schneider A, Tschopp C, et al. Contamination of stethoscopes and physicians' hands after a physical examination. *Mayo Clin Proc* **2014**; 89: 291-9.

114. Haydon DT, Cleaveland S, Taylor LH, et al. Identifying reservoirs of infection: a conceptual and practical challenge. *Emerg Infect Dis* **2002**; 8: 1468-73.

115. Wagenvoort JH, Toenbreker HM, Nurmohamed A, et al. Transmission of methicillin-resistant *Staphylococcus aureus* within a household. *Eur J Clin Microbiol Infect Dis* **1997**; 16: 399-400.

116. Cook HA, Furuya EY, Larson E, et al. Heterosexual transmission of community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* **2007**; 44: 410-3.

117. Knox J, Uhlemann AC, Miller M, et al. Environmental contamination as a risk factor for intra-household *Staphylococcus aureus* transmission. *PLoS One* **2012**; 7: e49900.

118. Uhlemann AC, Knox J, Miller M, et al. The environment as an unrecognized reservoir for community-associated methicillin resistant *Staphylococcus aureus* USA300: a case-control study. *PLoS One* **2011**; 6: e22407.

119. Fritz SA, Hogan PG, Singh LN, et al. Contamination of environmental surfaces with *Staphylococcus aureus* in households with children infected with methicillin-resistant *S. aureus. JAMA Pediatr* **2014**; 168: 1030-8.

120. Brennan L, Lilliebridge RA, Cheng AC, et al. Community-associated meticillin-resistant *Staphylococcus aureus* carriage in hospitalized patients in tropical northern Australia. *J Hosp Infect* **2013**; 83: 205-11.

121. Embil JM, Dyck B, Plourde P. Prevention and control of infections in the home. *Can Med Assoc J* **2009**; 180: E82-6.

122. Kassem, II. Chinks in the armor: the role of the nonclinical environment in the transmission of *Staphylococcus* bacteria. *Am J Infect Control* **2011**; 39: 539-41.

123. Schaumburg F, Pauly M, Anoh E, et al. *Staphylococcus aureus* complex from animals and humans in three remote African regions. *Clin Microbiol Infect* **2015**; 21: 345 e1-8.

124. Creech CB, Al-Zubeidi DN, Fritz SA. Prevention of Recurrent Staphylococcal Skin Infections. *Infect Dis Clin North Am* **2015**; 29: 429-64.

125. Bogaert D, van Belkum A, Sluiter M*ea*. Colonization by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet* **2004**; 363: 1871- 72.

126. Ogston A. Report upon Micro-Organisms in Surgical Diseases. Br Med J **1881**; 1: 369 b2-75.

127. Elek SD. *Staphylococcus pyogenes* and its relation to disease. Edinburgh: E. & S. Livingstone; 1959.

128. Rosenbach FJ. Mikro-organismen bei den Wund-Infections-Krankheiten des Menschen; 1884.

129. Todar K. *Staphylococcus aureus* and Staphylococcal Disease. In: Todar K, editor. Todar's Onine Textbook of Bacteriology. Madison, Wisconsin; 2012.

130. Longe JL. The Gale Encyclopedia of Medicine. 5th ed. Michigan, USA: Farmington Hills; 2015.

131. van Smeden J, Bouwstra JA. Stratum Corneum Lipids: Their role for the skin barrier function in healthy subjects and atopic dermatitis patients. *Curr Probl Dermatol* **2016**; 49: 8-26.

132. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell, Sixth Edition: Taylor & Francis Group; 2014.

133. Verkaik NJ, Lebon A, de Vogel CP, et al. Induction of antibodies by *Staphylococcus aureus* nasal colonization in young children. *Clin Microbiol Infect* **2010**; 16: 1312-7.

134. Verkaik NJ, de Vogel CP, Boelens HA, et al. Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of *Staphylococcus aureus*. *J Infect Dis* **2009**; 199: 625-32.

135. Nouwen JL, Fieren MW, Snijders S, et al. Persistent (not intermittent) nasal carriage of *Staphylococcus aureus* is the determinant of CPD-related infections. *Kidney Int* **2005**; 67: 1084-92.

136. Luzar MA, Coles GA, Faller B, et al. *Staphylococcus aureus* nasal carriage and infection in patients on continuous ambulatory peritoneal dialysis. *N Engl J Med* **1990**; 322: 505-9.

137. Wertheim HF, Vos MC, Ott A, et al. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *Lancet* **2004**; 364: 703-5.

138. Dryla A, Prustomersky S, Gelbmann D, et al. Comparison of antibody repertoires against *Staphylococcus aureus* in healthy individuals and in acutely infected patients. *Clin Diagn Lab Immunol* **2005**; 12: 387-98.

139. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol Microbiol* **2003**; 48: 1429-49.

140. Krakauer T. Interleukin-8 production by human monocytic cells in response to staphylococcal exotoxins is direct and independent of interleukin-1 and tumor necrosis factor-alpha. *J Infect Dis* **1998**; 178: 573-7.

141. Kobayashi SD, Voyich JM, Burlak C, et al. Neutrophils in the innate immune response. *Arch Immunol Ther Exp* (Warsz) **2005**; 53: 505-17.

142. Spaan AN, Surewaard BG, Nijland R, et al. Neutrophils versus *Staphylococcus aureus*: a biological tug of war. *Annu Rev Microbiol* **2013**; 67: 629-50.

143. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* **2008**; 46 Suppl 5: S350-9.

144. Thomer L, Schneewind O, Missiakas D. Pathogenesis of *Staphylococcus aureus* bloodstream infections. *Annu Rev Pathol* **2016**; 11: 343-64.

145. Liu GY, Essex A, Buchanan JT, et al. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med* **2005**; 202: 209-15.

146. Spaan AN, Henry T, van Rooijen WJM, et al. The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. *Cell Host Microbe* **2013**; 13: 584-94.

147. Scherr TD, Heim CE, Morrison JM, et al. Hiding in plain sight: interplay between staphylococcal biofilms and host immunity. *Front Immunol* **2014**; 5: 37-44.

148. Prabhakara R, Harro JM, Leid JG, et al. Murine immune response to a chronic *Staphylococcus aureus* biofilm infection. *Infect Immun* **2011**; 79: 1789-96.

149. Hanke ML, Kielian T. Deciphering mechanisms of staphylococcal biofilm evasion of host immunity. *Front Cell Infect Microbiol* **2012**; 2: 62-74.

150. Patel R. Biofilms and antimicrobial resistance. *Clin Orthop Relat Res* **2005**: 41-7.

151. Valour F, Trouillet-Assant S, Riffard N, et al. Antimicrobial activity against intraosteoblastic *Staphylococcus aureus*. *Antimicrob Agents Chemother* **2015**; 59: 2029-36.

152. Hanssen AM, Kindlund B, Stenklev NC, et al. Localization of *Staphylococcus aureus* in tissue from the nasal vestibule in healthy carriers. *BMC Microbiol* **2017**; 17: 89-100.

153. Dilnessa T, Demeke G, Mengistu G, et al. Emerging blood culture technologies for isolation of blood pathogens at clinical microbiology laboratories. *J Med Microbiol Diagn* **2016**; 5: 227.

154. Magadia RR, Weinstein MP. Laboratory diagnosis of bacteremia and fungemia. *Infect Dis Clin North Am* **2001**; 15: 1009-24.

155. Iroh Tam PY, Bernstein E, Ma X, et al. Blood culture in evaluation of pediatric community-acquired pneumonia: A systematic review and meta-analysis. *Hosp Pediat*r **2015**; 5: 324-36.

156. Weinstein MP. Blood culture contamination: persisting problems and partial progress. *J Clin Microbiol* **2003**; 41: 2275-8.

157. Buttery JP. Blood cultures in newborns and children: optimising an everyday test. *Arch Dis Child Fetal Neonatal Ed* **2002**; 87: F25-8.

158. McNally LM, Jeena PM, Gajee K, et al. Effect of age, polymicrobial disease, and maternal HIV status on treatment response and cause of severe pneumonia in South African children: a prospective descriptive study. *Lancet* **2007**; 369: 1440-51.

159. Chokephaibulkit K, Sitthitrai P, Wanprapa N, et al. Comparison of BACTEC automated blood culture system and conventional system in hospitalized pediatric patients. *J Med Assoc Thai* **1999**; 82: 1011-6.

160. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clin Microbiol Rev* **2010**; 23: 467-92.

161. Lagunju IA, Falade AG, Akinbami FO, et al. Childhood bacterial meningitis in Ibadan, Nigeria--antibiotic sensitivity pattern of pathogens, prognostic indices and outcome. *Afr J Med Med Sci* **2008**; 37: 185-91.

162. Adhikari S, Gauchan E, BK G, et al. Effect of Antibiotic Pretreatment on Cerebrospinal Fluid Profiles of Children with Acute Bacterial Meningitis. *Nepal J Med Sci* **2013**; 2 135- 39.

163. Liesenfeld O, Lehman L, Hunfeld KP, et al. Molecular diagnosis of sepsis: New aspects and recent developments. *Eur J Microbiol Immunol* **2014**; 4: 1-25.

164. Singhal N, Kumar M, Kanaujia PK, et al. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front Microbiol* **2015**; 6: 791-807.

165. Szabados F, Woloszyn J, Richter C, et al. Identification of molecularly defined *Staphylococcus aureus* strains using matrix-assisted laser desorption/ionization time of flight mass spectrometry and the Biotyper 2.0 database. *J Med Microbiol* **2010**; 59: 787-90.

166. The Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Tests. 29th ed. Wayne, PA; 2017.

167. Koreen L, Ramaswamy SV, Graviss EA, et al. Spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol* **2004**; 42: 792-9.

168. Naylor S. Biomarkers: current perspectives and future prospects. *Expert Rev Mol Diagn* **2003**; 3: 525-9.

169. Pichereau S, Moran JJ, Hayney MS, et al. Concentration-dependent effects of antimicrobials on *Staphylococcus aureus* toxin-mediated cytokine production from peripheral blood mononuclear cells. *J Antimicrob Chemother* **2012**; 67: 123-9.

170. Rose WE, Eickhoff JC, Shukla SK, et al. Elevated serum interleukin-10 at time of hospital admission is predictive of mortality in patients with *Staphylococcus aureus* bacteremia. *J Infect Dis* **2012**; 206: 1604-11.

171. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* **2011**; 52: e18-55.

172. Schweizer ML, Furuno JP, Harris AD, et al. Comparative effectiveness of nafcillin or cefazolin versus vancomycin in methicillin-susceptible *Staphylococcus aureus* bacteremia. *BMC Infect Dis* **2011**; 11: 279-85.

173. Agarwal R, Bartsch SM, Kelly BJ, et al. Newer glycopeptide antibiotics for treatment of complicated skin and soft tissue infections: systematic review, network meta-analysis and cost analysis. *Clin Microbiol Infect* **2018**; 24: 361-68.

174. World Health Organization. Pocket book of hospital care for children. Guidelines for the management of common illnesses with limited resources. Geneva: World Health Organization; 2005.

175. Ministry of Health and Social Welfare. Standard Treatment Guidelines. Banjul, The Gambia: Ministry of Health and Social Welfare, The Gambia, **2017**.

176. World Health Organization. Antimicrobial reisstance. November 2017 **2017**. http://www.who.int/mediacentre/factsheets/fs194/en/ (accessed November 14, 2017 2017).

177. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations United Kingdom, **2016**.

178. Williams PCM, Isaacs D, Berkley JA. Antimicrobial resistance among children in sub-Saharan Africa. *Lancet Infect Dis* **2018**; 18: e33-e44.

179. World Health Organization. Antimicrobial resistance: global report on surveillance. 2014. Geneva, Switzerland: World Health Organization; 2014.

180. Fleming A. On the antibacterial action of cultures of a Penicillium, with special reference to their use in isolation of *B. influenzae*. *Br J Exp Pathol* ***19*29**; 10: 226-36.

181. Zou MX, Zhou RR, Wu WJ, et al. Antimicrobial resistance and molecular epidemiological characteristics of clinical isolates of *Staphylococcus aureus* in Changsha area. *Chin Med J* (Engl) **2012**; 125: 2289-94.

182. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* **2004**; 32: 470-85.

183. Holland TL, Arnold C, Fowler VG, Jr. Clinical management of *Staphylococcus aureus* bacteremia: a review. *JAMA* **2014**; 312: 1330-41.

184. Walters M, Lonsway D, Rasheed K, et al. Investigation and Control of Vancomycin-resistant *Staphylococcus aureus*: A Guide for Health Departments and Infection Control Personnel. Atlanta, GA **2015**.

185. Lindsay JA. Genomic variation and evolution of *Staphylococcus aureus*. *Int J Med Microbiol* **2010**; 300: 98-103.

186. Uhlemann AC, Otto M, Lowy FD, et al. Evolution of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus*. *Infect Genet Evol* **2014**; 21: 563-74.

187. Abdulgader SM, Shittu AO, Nicol MP, et al. Molecular epidemiology of Methicillin-resistant *Staphylococcus aureus* in Africa: a systematic review. Front Microbiol **2015**; 6: 348-69.

188. Dantes R, Mu Y, Belflower R, et al. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA Intern Med* **2013**; 173: 1970-8.

189. Centers for Disease Control and Prevention. ABCs report: methicillin-resistant *Staphylococcus aureus*, 2014. **2014**. https://www.cdc.gov/abcs/reports-findings/survreports/mrsa14.html.

190. Klein EY, Mojica N, Jiang W, et al. Trends in methicillin-resistant *Staphylococcus aureus* hospitalizations in the United States, 2010-2014. *Clin Infect Dis* **2017**; 65: 1921-23.

191. Laupland KB, Lyytikainen O, Sogaard M, et al. The changing epidemiology of *Staphylococcus aureus* bloodstream infection: a multinational population-based surveillance study. *Clin Microbiol Infect* **2013**; 19: 465-71.

192. Song JH, Hsueh PR, Chung DR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. *J Antimicrob Chemother* **2011**; 66: 1061-9.

193. Falagas ME, Karageorgopoulos DE, Leptidis J, et al. MRSA in Africa: filling the global map of antimicrobial resistance. *PLoS One* **2013**; 8: e68024.

194. Ashley EA, Lubell Y, White NJ, et al. Antimicrobial susceptibility of bacterial isolates from community acquired infections in Sub-Saharan Africa and Asian low and middle income countries. *Trop Med Int Health* **2011**; 16: 1167-79.

195. Risk R, Naismith H, Burnett A, et al. Rational prescribing in paediatrics in a resource-limited setting. *Arch Dis Child* **2013**; 98: 503-9.

196. National Institute for Health Care Excellence. Meningitis (bacterial) and meningococcal septicaemia in under 16s: recognition, diagnosis and management guideline (NICE guideline CG 102). **2015**. https://www.nice.org.uk/guidance/CG102/chapter/1-Guidance#management-in-secondary-care (accessed October 17 2018).

197. de Kraker ME, Davey PG, Grundmann H, et al. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med* **2011**; 8: e1001104.

198. Cohen-Wolkowiez M, Benjamin DK, Jr., Fowler VG, Jr., et al. Mortality and neurodevelopmental outcome after *Staphylococcus aureus* bacteremia in infants. *Pediatr Infect Dis J* **2007**; 26: 1159-61.

199. Cosgrove SE, Qi Y, Kaye KS, et al. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect Control Hosp Epidemiol* **2005**; 26: 166-74.

200. Burke RE, Halpern MS, Baron EJ, et al. Pediatric and neonatal *Staphylococcus aureus* bacteremia: epidemiology, risk factors, and outcome. *Infect Control Hosp Epidemiol* **2009**; 30: 636-44.

201. Goetghebeur M, Landry PA, Han D, et al. Methicillin-resistant *Staphylococcus aureus*: A public health issue with economic consequences. *Can J Infect Dis Med Microbiol* **2007**; 18: 27-34.

202. Filice GA, Nyman JA, Lexau C, et al. Excess costs and utilization associated with methicillin resistance for patients with *Staphylococcus aureus* infection. *Infect Control Hosp Epidemiol* **2010**; 31: 365-73.

203. MacNeal WJ, Frisbee FC. One hundred patients with *Staphyloccocus* septicemia receiving bacteriophage service. *Am J Med Sci* **1936**; 191: 179–89.

204. Hill PC, Birch M, Chambers S, et al. Prospective study of 424 cases of *Staphylococcus aureus* bacteraemia: determination of factors affecting incidence and mortality. *Intern Med J* **2001**; 31: 97-103.

205. Cosgrove SE, Sakoulas G, Perencevich EN, et al. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis* **2003**; 36: 53-9.

206. Nickerson EK, West TE, Day NP, et al. *Staphylococcus aureus* disease and drug resistance in resource-limited countries in south and east Asia. *Lancet Infect Dis* **2009**; 9: 130-5.

207. Lemmen SW, Hafner H, Zolldann D, et al. Distribution of multi-resistant Gram-negative versus Gram-positive bacteria in the hospital inanimate environment. *J Hosp Infect* **2004**; 56: 191-7.

208. Bhalla A, Pultz NJ, Gries DM, et al. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. *Infect Control Hosp Epidemiol* **2004**; 25: 164-7.

209. Allegranzi B, Bagheri Nejad S, Combescure C, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *Lancet* **2011**; 377: 228-41.

210. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am *J Infect Control* **2008**; 36: 309-32.

211. Simmons S, Morgan M, Hopkins T, et al. Impact of a multi-hospital intervention utilising screening, hand hygiene education and pulsed xenon ultraviolet (PXUV) on the rate of hospital associated meticillin resistant *Staphylococcus aureus* infection. *J Infect.* Prevent. **2013**; 14: 172–74.

212. Lopez-Alcalde J, Mateos-Mazon M, Guevara M, et al. Gloves, gowns and masks for reducing the transmission of meticillin-resistant *Staphylococcus aureus* (MRSA) in the hospital setting. *Cochrane Database Syst Rev* **2015**: CD007087.

213. Erasmus V, Huis A, Oenema A, et al. The ACCOMPLISH study. A cluster randomised trial on the cost-effectiveness of a multicomponent intervention to improve hand hygiene compliance and reduce healthcare associated infections. *BMC Public Health* **2011**; 11: 721-27.

214. Erasmus V, Daha TJ, Brug H, et al. Systematic review of studies on compliance with hand hygiene guidelines in hospital care. *Infect Control Hosp Epidemiol* **2010**; 31: 283-94.

215. Bedoya G, Dolinger A, Rogo K, et al. Observations of infection prevention and control practices in primary health care, Kenya. *Bull World Health Organ* **2017**; 95: 503-16.

216. Abdella NM, Tefera MA, Eredie AE, et al. Hand hygiene compliance and associated factors among health care providers in Gondar University Hospital, Gondar, North West Ethiopia. *BMC Public Health* **2014**; 14: 96-102.

217. Yawson AE, Hesse AA. Hand hygiene practices and resources in a teaching hospital in Ghana. *J Infect Dev Ctries* **2013**; 7: 338-47.

218. World Health Organization. WHO guidelines on hand hygiene in health care. Geneva, Switzerland; 2009.

219. Wasserman S, Messina AP. Bundles of Infection and Safety. In: Bearman G, ed. Guide to Infection Control in the Hospital. 6th ed: International Society for Infectious Diseases; 2018.

220. Lemmen SW, Lewalter K. Antibiotic stewardship and horizontal infection control are more effective than screening, isolation and eradication. *Infection* **2018**; 46: 581-90.

221. van Rijen M, Bonten M, Wenzel R, et al. Mupirocin ointment for preventing *Staphylococcus aureus* infections in nasal carriers. *Cochrane Database Syst Rev* **2008**: CD006216.

222. Liu Z, Norman G, Iheozor-Ejiofor Z, et al. Nasal decontamination for the prevention of surgical site infection in *Staphylococcus aureus* carriers. *Cochrane Database Syst Rev* **2017**; 5: CD012462.

223. Fattom A, Matalon A, Buerkert J, et al. Efficacy profile of a bivalent *Staphylococcus aureus* glycoconjugated vaccine in adults on hemodialysis: phase III randomized study. *Hum Vacc Immunother* **2015**; 11: 632–41.

224. Fowler VG, Allen KB, Moreira ED, et al. Effect of an investigational vaccine for preventing *Staphylococcus aureus* infections after cardiothoracic surgery: a randomized trial. *JAMA* **2013**; 309: 1368-78.

225. Rupp ME, Holley HP, Jr., Lutz J, et al. Phase II, randomized, multicenter, double-blind, placebo-controlled trial of a polyclonal anti-*Staphylococcus aureus* capsular polysaccharide immune globulin in treatment of *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother* **2007**; 51: 4249-54.

226. Giersing BK, Dastgheyb SS, Modjarrad K, et al. Status of vaccine research and development of vaccines for *Staphylococcus aureus*. *Vaccine* **2016**; 34: 2962-66.

227. Weisman LE, Thackray HM, Steinhorn RH, et al. A randomized study of a monoclonal antibody (pagibaximab) to prevent staphylococcal sepsis. *Pediatrics* **2011**; 128: 271-9.

228. Fowler Jr VG, Proctor RA. Where does a *Staphylococcus aureus* vaccine stand? *Clin Microbiol Infect* **2014**; 20: 66–75.

229. O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* **2009**; 374: 893-902.

230. Stevens GA, Alkema L, Black RE, et al. Guidelines for Accurate and Transparent Health Estimates Reporting: the GATHER statement. *Lancet* **2016**; 388: e19-e23.

231. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* **2009**; 6: e1000100.

232. The African Union Commission. Regional Economic Communities (RECs). **2017**. https//:www.au.int/en/organs/recs (accessed 14 July 2018).

233. Reeves BC, Deeks JJ, Higgins JPT, et al. Including non-randomized studies. In: Higgins JPT, Green S, eds. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 ed: The Cochrane Collaboration; 2011.

234. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* **1986**; 7: 177–88.

235. United Nations Population Fund. World Population Prospects 2017 File POP/7-1: Total population (both sexes combined) by five-year age group, region, subregion and country, 1950-2100 (thousands) **2017**. https://esa.un.org/unpd/wpp/Download/ Standard/Population/ (accessed February 02 2018).

236. The Joint United Nations Programme on HIV and AIDS (UNAIDS). HIV Estimates among children 0-4 years. 2017.

237. United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, **2017**.

238. United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, **2016**.

239. African Union Commission. African Union Handbook: A guide for those working with and within the African Union. 6th ed. Addis Ababa: African Union Commission and New Zealand Ministry of Foreign Affairs and Trade/Manatū Aorere; 2019.

240. McAllister DA, Liu L, Shi T, et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. *Lancet Glob Health* **2019**; 7: e47-e57.

241. Thriemer K, Ley B, Ame S, et al. The burden of invasive bacterial infections in Pemba, Zanzibar. *PLoS One* **2012**; 7: e30350.

242. Odutola A, Bottomley C, Zaman SA, et al. *Staphylococcus aureus* bacteremia in children of rural areas of The Gambia, 2008 - 2015. *Emerg Infect Dis* **2019**; 25: 701-09.

243. Mackenzie GA, Plumb ID, Sambou S, et al. Monitoring the introduction of pneumococcal conjugate vaccines into West Africa: design and implementation of a population-based surveillance system. *PLoS Med* **2012**; 9: e1001161.

244. Bahwere P, Levy J, Hennart P, et al. Community-acquired bacteremia among hospitalized children in rural central Africa. *Int J Infect Dis* **2001**; 5: 180-8.

245. Traore Y, Tameklo TA, Njanpop-Lafourcade BM, et al. Incidence, seasonality, age distribution, and mortality of pneumococcal meningitis in Burkina Faso and Togo. *Clin Infect Dis* **2009**; 48: S181-9.

246. Breiman RF, Cosmas L, Njenga M, et al. Severe acute respiratory infection in children in a densely populated urban slum in Kenya, 2007-2011. *BMC Infect Dis* **2015**; 15: 95-104.

247. Sigauque B, Roca A, Bassat Q, et al. Severe pneumonia in Mozambican young children: clinical and radiological characteristics and risk factors. *J Trop Pediatr* **2009**; 55: 379-87.

248. Antia-Obong OE, Utsalo SJ, Udo JJ, et al. Neonatal septicaemia in Calabar, Nigeria. *Cent Afr J Med* **1992**; 38: 161-5.

249. Klingenberg C, Olomi R, Oneko M, et al. Neonatal morbidity and mortality in a Tanzanian tertiary care referral hospital. *Ann Trop Paediatr* **2003**; 23: 293-9.

250. Ojukwu JU, Abonyi LE, Ugwu J, et al. Neonatal septicemia in high risk babies in South-Eastern Nigeria. *J Perinat Med* **2006**; 34: 166-72.

251. Forgie IM, O'Neill KP, Lloyd-Evans N, et al. Etiology of acute lower respiratory tract infections in Gambian children: II. Acute lower respiratory tract infection in children ages one to nine years presenting at the hospital. *Pediatr Infect Dis J* **1991**; 10: 42-7.

252. Sow SO, Diallo S, Campbell JD, et al. Burden of invasive disease caused by *Haemophilus influenzaeHaemophilus influenzae* type b in Bamako, Mali: impetus for routine infant immunization with conjugate vaccine. *Pediatr Infect Dis J* **2005**; 24: 533-7.

253. Nantanda R, Hildenwall H, Peterson S, et al. Bacterial aetiology and outcome in children with severe pneumonia in Uganda. *Ann Trop Paediatr* **2008**; 28: 253-60.

254. Benet T, Sylla M, Messaoudi M, et al. Etiology and Factors Associated with Pneumonia in Children under 5 Years of Age in Mali: A Prospective Case-Control Study. *PLoS One* **2015**; 10: e0145447.

255. Johnson AW, Osinusi K, Aderele WI, et al. Etiologic agents and outcome determinants of community-acquired pneumonia in urban children: a hospital-based study. *J Natl Med Assoc* **2008**; 100: 370-85.

256. El Mdaghri N, Jilali N, Belabbes H, et al. Epidemiological profile of invasive bacterial diseases in children in Casablanca, Morocco: antimicrobial susceptibilities and serotype distribution. *East Mediterr Health J* **2012**; 18: 1097-101.

257. Ba O, Fleming JA, Dieye Y, et al. Hospital surveillance of childhood bacterial meningitis in Senegal and the introduction of *Haemophilus influenzae* type b conjugate vaccine. *Am J Trop Med Hyg* **2010**; 83: 1330-5.

258. Akpede OG, Skyes RM. Malaria with bacteraemia in acutely febrile school pre-children without localising signs: Coincidence or association/complication. *J Trop Med Hyg* **1993**; 96: 146 - 50.

259. Kibuuka A, Byakika-Kibwika P, Achan J, et al. Bacteremia Among Febrile Ugandan Children Treated with Antimalarials Despite a Negative Malaria Test. *Am J Trop Med Hyg* **2015**; 93: 276-80.

260. Isendahl J, Manjuba C, Rodrigues A, et al. Prevalence of community-acquired bacteraemia in Guinea-Bissau: an observational study. *BMC Infect Dis* **2014**; 14: 3859.

261. Onubogu UC, Anochie IC, Akani NA. Prevalence of bacteraemia in febrile, under-five children in the children's outpatient clinic of University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. *Nig J Paed* **2015**; 42: 93-97.

262. West BA, Tabansi PN. Prevalence of neonatal septicaemia in the University of Port Harcourt Teaching Hospital, Nigeria. *Nig J Paed* **2014**; 41: 33-37.

263. Owa JA, Olusanya O. Neonatal bacteraemia in Wesley Guild Hospital, Ilesha, Nigeria. *Ann Trop Paediatr* **1988**; 8: 80-4.

264. Kayange N, Kamugisha E, Mwizamholya DL, et al. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. *BMC Pediatr* **2010**; 10: 39-47.

265. Mkony MF, Mizinduko MM, Massawe A, et al. Management of neonatal sepsis at Muhimbili National Hospital in Dar es Salaam: diagnostic accuracy of C-reactive protein and newborn scale of sepsis and antimicrobial resistance pattern of etiological bacteria. *BMC Pediatr* **2014**; 14: 293-99.

266. Kiwanuka J, Bazira J, Mwanga J, et al. The microbial spectrum of neonatal sepsis in Uganda: recovery of culturable bacteria in mother-infant pairs. *PLoS One* **2013**; 8: e72775.

267. John B, David M, Mathias L, et al. Risk factors and practices contributing to newborn sepsis in a rural district of Eastern Uganda, August 2013: a cross sectional study. *BMC Res Notes* **2015**; 8: 339-50.

268. Mugalu J, Nakakeeto MK, Kiguli S, et al. Aetiology, risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. *Afr Health Sci* **2006**; 6: 120-6.

269. Akpede O, Abiodun PO, Sykes M, et al. Childhood bacterial meningitis beyond the neonatal period in southern Nigeria: changes in organisms/antibiotic susceptibility. East *Afr Med J* **1994**; 71: 14-20.

270. Adejuyigbe EA, Adeodu OO, Ako-Nai KA, et al. Septicaemia in high risk neonates at a teaching hospital in Ile-Ife, Nigeria. *East Afr Med J* **2001**; 78: 540-3.

271. Lepage P, Bogaerts J, Van Goethem C, et al. Community-acquired bacteraemia in African children. *Lancet* **1987**; 1: 1458-61.

272. The Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Twelfth Edition (M02-A12). Wayne, Pennsylvania, USA, **2015**.

273. United Nations Children's Fund. UNICEF Data: Monitoring the Situation of Children and Women. **2017**. https://data.unicef.org/topic/child-health/pneumonia/# (accessed October 25 2017).

274. Rudan I, O'Brien KL, Nair H, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. *J Glob Health* **2013**; 3: 010401.

275. Morakinyo OM, Fagbamigbe AF. Neonatal, infant and under-five mortalities in Nigeria: An examination of trends and drivers (2003-2013). *PLoS One* **2017**; 12: e0182990.

276. Scott JA, Hall AJ. The value and complications of percutaneous transthoracic lung aspiration for the etiologic diagnosis of community-acquired pneumonia. Chest **1999**; 116: 1716-32.

277. Asghar R, Banajeh S, Egas J, et al. Chloramphenicol versus ampicillin plus gentamicin for community acquired very severe pneumonia among children aged 2-59 months in low resource settings: multicentre randomised controlled trial (SPEAR study). *BMJ* **2008**; 336: 80-4.

278. Bates JH, Campbell GD, Barron AL, et al. Microbial etiology of acute pneumonia in hospitalized patients. *Chest* **1992**; 101: 1005-12.

279. Zar HJ, Tannenbaum E, Hanslo D, et al. Sputum induction as a diagnostic tool for community-acquired pneumonia in infants and young children from a high HIV prevalence area. *Pediatr Pulmonol* **2003**; 36: 58-62.

280. Falade AG, Mulholland EK, Adegbola RA, et al. Bacterial isolates from blood and lung aspirate cultures in Gambian children with lobar pneumonia. *Ann Trop Paediatr* **1997**; 17: 315-9.

281. Pedersen M, Benfield TL, Skinhoej P, et al. Haematogenous *Staphylococcus aureus* meningitis. A 10-year nationwide study of 96 consecutive cases. *BMC Infect Dis* **2006**; 6: 49.

282. Power Coombs MR, Kronforst K, Levy O. Neonatal host defense against Staphylococcal infections. *Clin Dev Immunol* **2013**; 2013: 826303.

283. Feikin DR, Scott JA, Gessner BD. Use of vaccines as probes to define disease burden. *Lancet* **2014**; 383: 1762-70.

284. Mackenzie G. The definition and classification of pneumonia. *Pneumonia (Nathan)* **2016**; 8: 14-15.

285. Hill PC, Onyeama CO, Ikumapayi UN, et al. Bacteraemia in patients admitted to an urban hospital in West Africa. BMC Infect Dis **2007**; 7: 2-9.

286. Enwere G, Van Hensbroek MB, Adegbola R, et al. Bacteraemia in cerebral malaria. *Ann Trop Paediatr* **1998**; 18: 275-8.

287. Darboe S, Okomo U, Muhammad AK, et al. Community-acquired invasive bacterial disease in urban Gambia, 2005-2015: A hospital-based surveillance. *Clin Infect Dis* **2019**; 69: S105-S13.

288. Greenhow TL, Hung YY, Herz A. Bacteremia in children 3 to 36 months old after introduction of conjugated pneumococcal vaccines. *Pediatrics* **2017**; 139: e20162098.

289. Mika M, Maurer J, Korten I, et al. Influence of the pneumococcal conjugate vaccines on the temporal variation of pneumococcal carriage and the nasal microbiota in healthy infants: a longitudinal analysis of a case-control study. *Microbiome* **2017**; 5: 85-99.

290. Biesbroek G, Wang X, Keijser BJ, et al. Seven-valent pneumococcal conjugate vaccine and nasopharyngeal microbiota in healthy children. *Emerg Infect Dis* **2014**; 20: 201-10.

291. Revey-Yochay G, Dagan R, Raz M, et al. Association between carriage of *Streptococcus pneumoniae* and *Staphylococcus aureus* in children. *JAMA* **2004**; 292: 716-20.

292. van Gils EJ, Hak E, Veenhoven RH, et al. Effect of seven-valent pneumococcal conjugate vaccine on *Staphylococcus aureus* colonisation in a randomised controlled trial. *PLoS One* **2011**; 6: e20229.

293. Veenhoven R, Bogaert D, Uiterwaal C, et al. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomised study. *Lancet* **2003**; 361: 2189-95.

294. Scott S, Odutola A, Mackenzie G, et al. Coverage and timing of children's vaccination: an evaluation of the expanded programme on immunisation in The Gambia. *PLoS One* **2014**; 9: e107280.

295. Mwesigwa J, Okebe J, Affara M, et al. On-going malaria transmission in The Gambia despite high coverage of control interventions: a nationwide cross-sectional survey. *Malar J* **2015**; 14: 314-22.

296. The Gambia Bureau of Statistics. The Gambia Multiple Indicator Cluster Survey 2018, Survey Findings Report. Banjul, The Gambia: The Gambia Bureau of Statistics, **2019**.

297. The Joint United Nations Programme on HIV and AIDS (UNAIDS). Country factsheets GAMBIA 2018. HIV and AIDS Estimates. **2019**. https://www.unaids.org/en/ regionscountries/countries/gambia (accessed December 23 2019).

298. Arifeen SE, Saha SK, Rahman S, et al. Invasive pneumococcal disease among children in rural Bangladesh: results from a population-based surveillance. *Clin Infect Dis* **2009**; 48: S103-13.

299. AIDS in Kenya. Nairobi, Republic of Kenya: Ministry of Health, **2001**.

300. Menendez C, Bardaji A, Sigauque B, et al. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. *PLoS One* **2008**; 3: e1934.

301. Zellweger RM, Carrique-Mas J, Limmathurotsakul D, et al. A current perspective on antimicrobial resistance in Southeast Asia. *J Antimicrob Chemother* **2017;** 72: 2963–72.

302. Uzodimma CC, Njokanma F, Ojo O, et al. Bacterial isolates from blood cultures of children with suspected sepsis in an urban hospital in Lagos: A prospective study using BACTEC blood culture system. *Internet J Pediatr Neonatol* **2013**; 16: 1-6.

303. Madrid L, Seale AC, Kohli-Lynch M, et al. Infant Group B Streptococcal disease incidence and serotypes worldwide: systematic review and meta-analyses. *Clin Infect Dis* **2017**; 65: S160-S72.

304. Bojang A, Kendall L, Usuf E, et al. Prevalence and risk factors for *Staphylococcus aureus* nasopharyngeal carriage during a PCV trial. *BMC Infect Dis* **2017**; 17: 588-92.

305. Wang X, Zhang N, Glorieux S, et al. Herpes simplex virus type 1 infection facilitates invasion of *Staphylococcus aureus* into the nasal mucosa and nasal polyp tissue. *PLoS One* **2012**; 7: e39875.

306. Lewis SS, Walker VJ, Lee MS, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* pneumonia in community hospitals. *Infect Control Hosp Epidemiol* **2014**; 35: 1452-7.

307. Rohner P, Pepey B, Auckenthaler R. Advantage of combining resin with lytic BACTEC blood culture media. *J Clin Microbiol* **1997**; 35: 2634-8.

308. Madhi SA, Petersen K, Madhi A, et al. Impact of human immunodeficiency virus type 1 on the disease spectrum of *Streptococcus pneumoniae* in South African children. *Pediatr Infect Dis J* **2000**; 19: 1141-7.

309. Department of Health. High impact intervention: taking blood cultures; a summary of best practice. London: Department of Health, **2010**.

310. Mejer N, Westh H, Schonheyder HC, et al. Stable incidence and continued improvement in short term mortality of *Staphylococcus aureus* bacteraemia between 1995 and 2008. *BMC Infect Dis* **2012**; 12: 260-66.

311. Paule SM, Pasquariello AC, Hacek DM, et al. Direct detection of *Staphylococcus aureus* from adult and neonate nasal swab specimens using real-time polymerase chain reaction. *J Mol Diagn* **2004**; 6: 191-6.

312. Andremont A. Commensal flora may play key role in spreading antibiotic resistance. *Am Soc Microbiol News* **2003**; 69: 601 - 07.

313. Bojang A, Camara B, Jagne Cox I, et al. Long-term impact of oral azithromycin taken by Gambian women during labor on prevalence and antibiotic susceptibility of *Streptococcus pneumoniae* and *Staphylococcus aureus* in their infants: Follow-up of a randomized clinical trial. *Clin Infect Dis* **2018**; 67: 1191-97.

314. Lindsay JA, Moore CE, Day NP, et al. Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. *J Bacteriol* **2006**; 188: 669-76.

315. The Gambia Bureau of Statistics (GBOS) and ICF International. The Gambia Demographic and Health Survey 2013. Banjul, The Gambia, and Rockville, Maryland, USA: GBOS and ICF International., **2014**.

316. McAllister SK, Albrecht VS, Fosheim GE, et al. Evaluation of the impact of direct plating, broth enrichment, and specimen source on recovery and diversity of methicillin-resistant *Staphylococcus aureus* isolates among HIV-infected outpatients. *J Clin Microbiol* **2011**; 49: 4126-30.

317. Maayan-Metzger A, Strauss T, Rubin C, et al. Clinical evaluation of early acquisition of *Staphylococcus aureus* carriage by newborns. *Int J Infect Dis* **2017**; 64: 9-14.

318. Ashour MS, Abdelaziz AA, Hefni H, et al. Microbial contamination of cosmetics and personal care items in Egypt--body lotions and talcum powders. *J Clin Pharm Ther* **1989**; 14: 207-12.

319. Pabst O, Cerovic V, Hornef M. Secretory IgA in the coordination of establishment and maintenance of the microbiota. *Trends Immunol* **2016**; 37: 287-96.

320. Lin J, Wu C, Yan C, et al. A prospective cohort study of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* carriage in neonates: the role of maternal carriage and phenotypic and molecular characteristics. *Infect Drug Resist* **2018**; 11: 555-65.

321. Gonzalez R, Maldonado A, Martin V, et al. Breast milk and gut microbiota in African mothers and infants from an area of high HIV prevalence. *PLoS One* **2013**; 8: e80299.

322. Ojo-Okunola A, Nicol M, du Toit E. Human breast milk bacteriome in health and disease. *Nutrients* **2018**; 10: E1643.

323. Rodriguez JM. The Origin of human milk bacteria: Is there a bacterial entero-mammary pathway during late pregnancy and lactation? *Adv Nutr* **2014**; 4: 779-84.

324. Eibach D, Nagel M, Hogan B, et al. Nasal carriage of *Staphylococcus aureus* among children in the Ashanti Region of Ghana. *PLoS One* **2017**; 12: e0170320.

325. De Boeck H, Vandendriessche S, Hallin M, et al. *Staphylococcus aureus* nasal carriage among healthcare workers in Kisangani, the Democratic Republic of the Congo. *Eur J Clin Microbiol Infect Dis* **2015**; 34: 1567-72.

326. Legese H, Kahsay AG, Kahsay A, et al. Nasal carriage, risk factors and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* among healthcare workers in Adigrat and Wukro hospitals, Tigray, Northern Ethiopia. *BMC Res Notes* **2018**; 11: 250-55.

327. Egyir B, Guardabassi L, Esson J, et al. Insights into nasal carriage of *Staphylococcus aureus* in an urban and a rural community in Ghana. *PLoS One* **2014**; 9: e96119.

328. Ngoa UA, Frieder Schaumburg, Adegnika AA, et al. Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa. *Acta Tropica* **2012**; 124: 42-47.

329. Saito H, Inoue K, Ditai J, et al. Alcohol-based hand rub and incidence of healthcare associated infections in a rural regional referral and teaching hospital in Uganda ('WardGel' study). *Antimicrob Resist Infect Control* **2017**; 6: 129-40.

330. van Belkum A, Verkaik NJ, de Vogel CP, et al. Reclassification of *Staphylococcus aureus* nasal carriage types. *J Infect Dis* **2009**; 199: 1820-6.

331. Chan GJ, Lee AC, Baqui AH, et al. Prevalence of early-onset neonatal infection among newborns of mothers with bacterial infection or colonization: a systematic review and meta-analysis. *BMC Infect Dis* **2015**; 15: 118-33.

332. Lloyd-Evans N, O'Dempsey TJ, Baldeh I, et al. Nasopharyngeal carriage of pneumococci in Gambian children and in their families. *Pediatr Infect Dis* J **1996**; 15: 866-71.

333. Stappers MH, Hagen F, Reimnitz P, et al. Direct molecular versus culture-based assessment of Gram-positive cocci in biopsies of patients with major abscesses and diabetic foot infections. *Eur J Clin Microbiol Infect Dis* **2015**; 34: 1885-92.

334. Sakr A, Bregeon F, Mege JL, et al. *Staphylococcus aureus* nasal nolonisation: An update on mechanisms, epidemiology, risk factors, and subsequent infections. *Front Microbiol* **2018**; 9: 2419-33.

335. Reta A, Wubie M, Mekuria G. Nasal colonization and antimicrobial susceptibility pattern of *Staphylococcus aureus* among pre-school children in Ethiopia. *BMC Res Notes* **2017**; 10: 746.

336. Darmstadt GL, Oot DA, Lawn JE. Newborn survival: changing the trajectory over the next decade. *Health Policy Plan* **2012**; 27: 1-5.

337. World Health Organization. WHO Statistical Information System (WHOSIS). 2018. 14 February, 2018 **2018**. http://apps.who.int/gho/data/view.main.CM1002015REG7-CH8?lang=en (accessed 15 August 2018).

338. Blencowe H, Cousens S, Mullany LC, et al. Clean birth and postnatal care practices to reduce neonatal deaths from sepsis and tetanus: a systematic review and Delphi estimation of mortality effect. *BMC Public Health* **2011**; 11: S11-S29.

339. Economic Commission for Africa. MDG Report 2015: Assessing Progress in Africa toward the Millennium Development Goals. Addis Ababa: United Nations Economic Commission for Africa, African Union, African Development Bank and United Nations Development Programme, **2015**.

340. Sinha A, Sazawal S, Pradhan A, et al. Chlorhexidine skin or cord care for prevention of mortality and infections in neonates. *Cochrane Database Syst Rev* **2015**: CD007835.

341. Seib KL, Zhao X, Rappuoli R. Developing vaccines in the era of genomics: a decade of reverse vaccinology. *Clin Microbiol Infect* **2012**; 18: 109-16.

Appendices

Appendix 1: GATHER Checklist of information in new reports of global health estimates

Item #	Checklist item	Reported on page #
Objectives and funding		
1	Define the indicator(s), populations (including age, sex, and geographic entities), and time period(s) for which estimates were made.	61-62
2	List the funding sources for the work.	1
Data Inputs		
For all data inputs from multiple sources that are synthesized as part of the study:		
3	Describe how the data were identified and how the data were accessed.	63-64
4	Specify the inclusion and exclusion criteria. Identify all ad-hoc exclusions.	61 - 62
5	Provide information on all included data sources and their main characteristics. For each data source used, report reference information or contact name/institution, population represented, data collection method, year(s) of data collection, sex and age range, diagnostic criteria or measurement method, and sample size, as relevant.	61-64
6	Identify and describe any categories of input data that have potentially important biases (e.g., based on characteristics listed in item 5).	64-65
For data inputs that contribute to the analysis but were not synthesized as part of the study:		
7	Describe and give sources for any other data inputs.	63, 247-248
For all data inputs:		
8	Provide all data inputs in a file format from which data can be efficiently extracted (e.g., a spreadsheet rather than a PDF), including all relevant meta-data listed in item 5. For any data inputs that cannot be shared because of ethical or legal reasons, such as third-party ownership, provide a contact name or the name of the institution that retains the right to the data.	Appendix 3,
Data analysis		
9	Provide a conceptual overview of the data analysis method. A diagram may be helpful.	57
10	Provide a detailed description of all steps of the analysis, including mathematical formulae. This description should cover, as relevant, data cleaning, data pre-processing, data adjustments and weighting of data sources, and mathematical or statistical model(s).	54 – 60
11	Describe how candidate models were evaluated and how the final model(s) were selected.	ND
12	Provide the results of an evaluation of model performance, if done, as well as the results of any relevant sensitivity analysis.	ND
13	Describe methods for calculating uncertainty of the estimates. State which sources of uncertainty were, and were not, accounted for in the uncertainty analysis.	73
14	State how analytic or statistical source code used to generate estimates can be accessed.	Appendices 5 - 11
Results and Discussion		
15	Provide published estimates in a file format from which data can be efficiently extracted.	Appendices 5 - 11
16	Report a quantitative measure of the uncertainty of the estimates (e.g. uncertainty intervals).	ND
17	Interpret results in light of existing evidence. If updating a previous set of estimates, describe the reasons for changes in estimates.	73-102
18	Discuss limitations of the estimates. Include a discussion of any modelling assumptions or data limitations that affect interpretation of the estimates.	102-113

Appendix 2: Search strategy and numbers of articles in Medline, Embase and Global Health January 1980 - May 2019

Concept	Search Terms	Number of articles
African Countries		
	Africa*	288989
1	Algeria*	4011
2	Angola*	1494
3	Benin*	3233
4	Botswana or Batswana	2090
5	Burkina*	3782
6	Burundi*	861
7	Cameroon*	6585
8	Cape Verde	468
9	Chad*	2776
10	Comoros*	429
11	Congo*	15060
12	Cote?d'ivoire OR Ivory?coast OR Ivorian*	146
13	Djibouti	363
14	Egypt*	20272
15	Eritrea*	554
16	Ethiopia*	13123
17	Gabon*	2094
18	Gambia*	7472
19	Ghana*	8946
20	Guinea*	157989
21	Kenya*	18439
22	Lesotho*	603
23	Liberia*	1659
24	Libya*	1516
25	Madagascar*	4705
26	Malawi*	6087
27	Mali OR Malians	3380
28	Mauritania*	637
29	Mauritius*	871
30	Morocco*	6603
31	Mozambi*	1431
32	Namibia*	1431
33	Niger*	44263
34	Nigeria*	31157
35	Rwanda*	2782
36	Sao?tome OR Principe	900
37	Senegal*	8088
38	Seychelles*	715
39	Sierra?leone*	1937
40	Somali*	2492
41	South?Africa*	47803
42	Sudan*	8800
43	Swazi* OR Eswatini	766
44	Tanzania*	12451
45	Togo*	1649
46	Tunisia OR Tunis*	10170
47	Uganda*	13449

48	Zambia*	5273
49	Zimbabwe*	6714
Disease		
50	(Fever* or bacterem* or bacteraem* or septice* or septicae* or blood stream infection* or sepsis).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	363455
51	(Disease or abscesses or arthritis or brain abscess or burn or cellulitis or dermatitis or empyema or endocarditis or endophthalmitis or food poisoning or Fournier Gangrene or impetigo or laryngitis or lung infection or mastitis or meningitis or osteomyelitis or otitis externa or otitis media or paronychia or pericarditis or peritonitis or pleural effusion or pneumonia or pyelonephritis or pyomyositis or respiratory tract infection or scalded skin syndrome or sepsis or septic arthritis or sinusitis or skin infections or skin adj3 infection or tonsillitis or toxic shock syndrome or pharyngitis or tracheitis or urethritis or urinary tract infection or uveitis or vaginitis or vaginosis or wound infection).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	4872199
52	*Staphylococcus aureus* or S aureus or staphylococcal infection* or staphylococcal disease* or staphylococc*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	186692
53	(Africa* or Benin or Burkina* or Cape Verde or Cote d'ivoire or Ivory coast or Ivorian* or Gambia* or Ghana* or Guinea* or Liberia* or Mali or Malians or Mauritania* or Niger* or Nigeria* or Senegal* or Sierra leone* or Togo* or Angola* or Botswana* or Batswana* or Lesotho* or Malawi* or Mozambi* or Namibia* or South Africa* or Swazi* or Zambia* or Zimbabwe* or Burundi* or Ethiopia* or Eritrea* or Kenya* or Rwanda* or Somali* or Sudan* or Tanzania* or Uganda* or Cameron* or Chad* or Congo* or Gabon* or Algeria* or Egypt* or Libya* or Morocco* or Tunisia or Tunis* or Sao tome or Principe* or Mauritius* or Madagascar* or Comoros* or Seychelles* or Djibouti).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	666618
54 (51 or 50)		5004020
55 (54 or 52)		5121044

56 (55 and 53)	149737
Limit 56 to	
"1980-2019"	136324
Humans	98722
English Language	90520

List of countries by sub-region

Central

Burundi, Cameroon, Central African Republic, Chad, Congo Republic, DR Congo, Equatorial Guinea, Gabon, São Tomé and Príncipe

East

Comoros, Djibouti, Eritrea, Ethiopia, Kenya, Madagascar, Mauritius, Rwanda, Seychelles, Somalia, South Sudan, Sudan, Tanzania, Uganda

North

Algeria, Egypt, Libya, Mauritania, Morocco, Sahrawi, Tunisia

Southern

Angola, Botswana, Eswatini (Swaziland), Lesotho, Malawi, Mozambique, Namibia, South Africa, Zambia, Zimbabwe

West

Benin, Burkina Faso, Cabo Verde, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Togo

List of countries by mortality stratum

Stratum 1

Cape Verde, Libya, Mauritius, Seychelles, Tunisia

Stratum 2

Algeria, Egypt, Eritrea, Ethiopia, Gabon, Madagascar, Morocco, Rwanda, Sao tome or Principe, Tanzania

Stratum 3

Benin, Burkina Faso, Burundi, Cameroon, Comoros, Congo, Cote d'Ivoire, Democratic Republic of Congo, Djibouti, Eswatini (Swaziland), Gambia, Ghana, Guinea, Kenya, Lesotho, Liberia, Malawi, Mali, Mauritania, Mozambique, Niger, Nigeria, Sierra Leone, Somali, Equatorial Guinea, South Sudan, Sudan, Togo, Uganda., Zambia,

Stratum 4

Angola, Central African Republic, Chad, Guinea Bissau

Appendix 3: Supplemental Tables and Figures for Chapter 2

Supplemental Table 2.1: Meta-analysis results by parameter, country, number of studies, mortality stratum and region (hospital-based studies)

Parameter	Country	Region	Mortality Stratum	Number of studies	Effect size (95%CI)
Pneumonia					**%**
Bacteraemic aetiologic fraction	Pooled[a]		3	3	6.0 (0.1 – 11.0)
	Mali	West	3	1	0.9 (0.2 – 4.7)
	Nigeria	West	3	1	10.7 (7.2 – 15.7)
	Uganda	East	3	1	5.7 (3.1 – 10.5)
	Morocco	North	2	1	1.3 (0.0 – 7.3)
Meningitis					**Cases per 100,000 population**
All-cause incidence[b]	Pooled		3	3	85.1 (69.2 – 102.6)
	Mozambique	South	3	1	171.9 (110.4 – 246.3)
	Mali	West	3	1	76.9 (69.0 – 85.4)
	Togo/Burkina Faso	West	3	1	79.8 (73.0 – 87.0)
Aetiologic fraction	Nigeria	West	3	1	7.0 (1.0 – 24.0)
	Senegal	West	2	1	2.3 (0.8 – 5.5)
All-cause CFR[c]	Nigeria	West	3	1	27.0 (18.0 – 37.0)
Septicaemia					**Cases per 100,000 population**
Incidence[b]	Pooled		3	6	919.3 (604.5 – 1299.3)
	Pooled Kenya	East	3	2	764.4 (251.6 – 1547.2)
	Pooled Gambia	West	3	2	634.9 (289.9 – 1112.9)
	Ghana	West	3	1	4686.0 (4109.0–5300.8)
	Mozambique	South	3	1	782.0 (727.7 – 838.3)
	Tanzania	East	2	1	159.6 (138.4 – 182.2)
Aetiologic fraction	Pooled		3	4	47.0 (40.0 – 54.0)
	Nigeria	West	3	3	48.0 (40.0 – 56.0)
	Uganda	East	3	1	42.0 (28.0 – 58.0)
	Guinea-Bissau	West	4	1	57.0 (41.0 – 71.0)
CFR	Pooled		3	2	9.0 (6.0 – 12.0)
	Gambia	West	3	1	14.0 (10.0 – 20.0)
	Mozambique	South	3	1	6.0 (3.0 – 11.0)

[a] Summary estimate of the meta-analysis of the countries in the same stratum

[b] Incidence in cases per 100,000 person-years

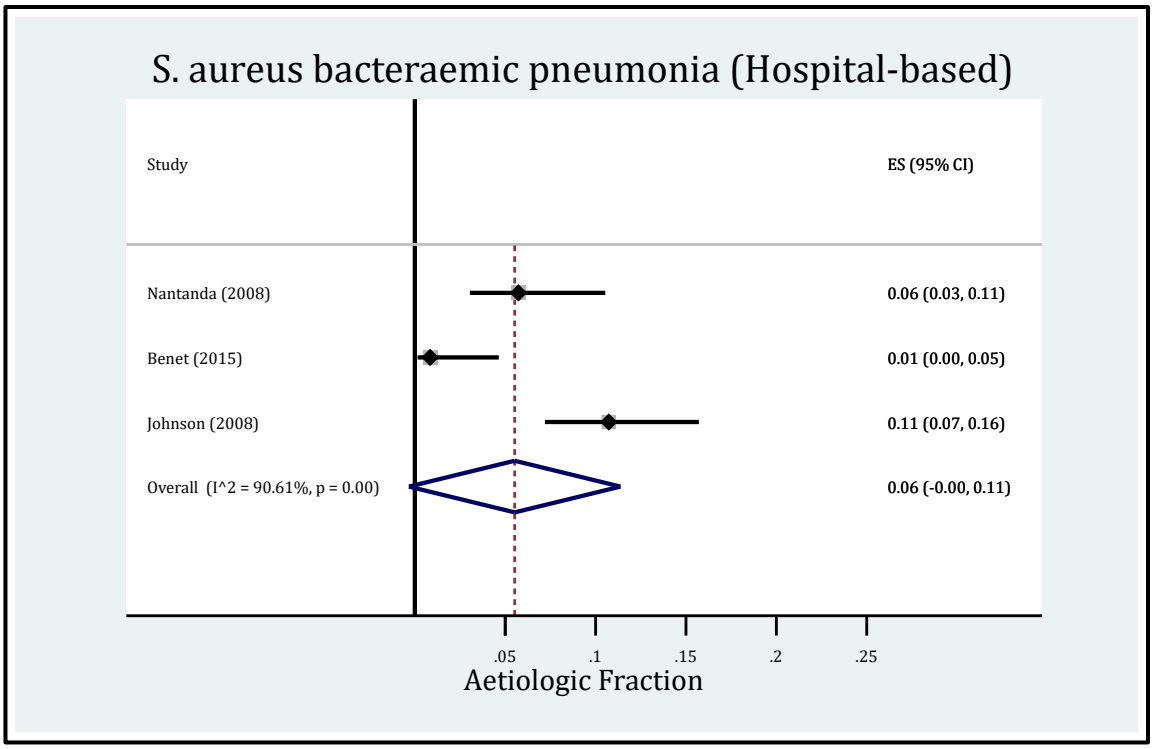
[c] CFR=Case fatality ratio

[d] Incidence in cases per 1,000 live births

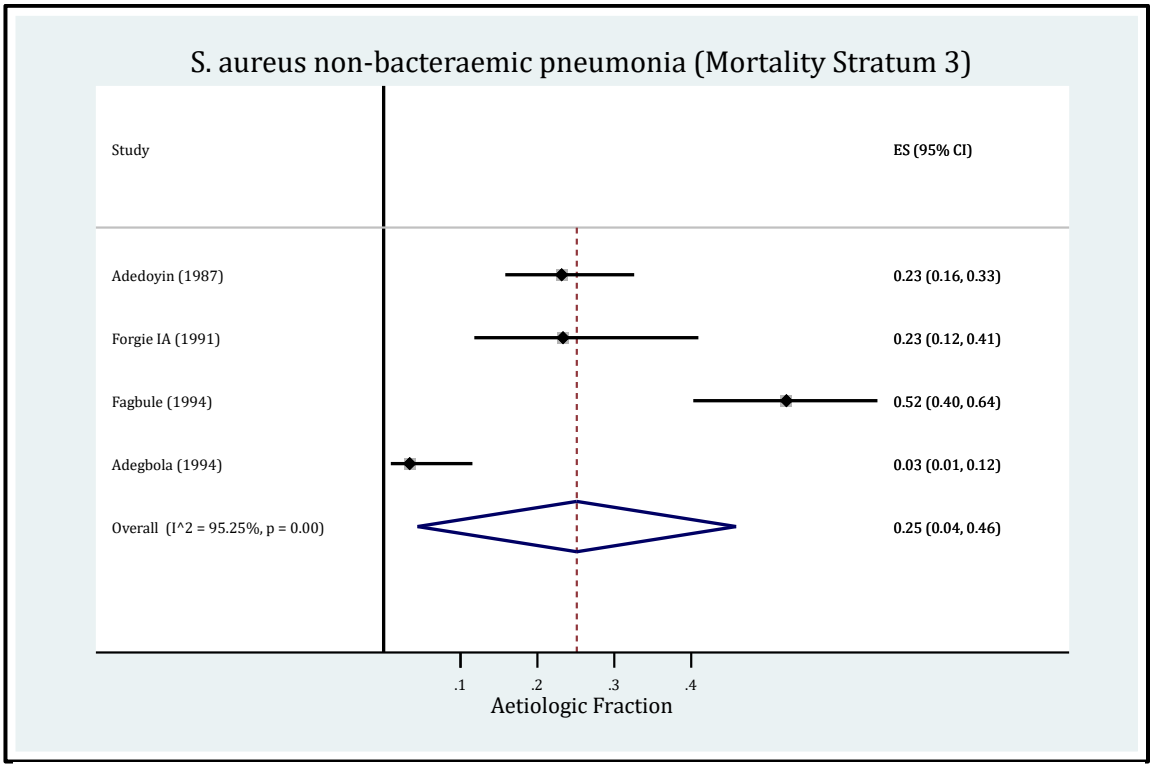
Supplemental Table 2.1: M**eta-analysis results by parameter, country, number of studies, mortality stratum and region (hospital-based studies- continued)**

Parameter	Country	Region	Mortality Stratum	Number of studies	Effect size (95%CI)
Neonatal septicaemia					**Cases per 1,000 live births**
Incidence[d]	Pooled[a]		3	2	7.1 (4.1 – 11.0)
	Gambia	West	3	1	9.0 (7.8 – 10.4)
	Kenya	East	3	1	5.5 (4.6 – 6.4)
Aetiologic fraction	Pooled		3	7	45.0 (27.0 - 63.0)
	Nigeria (Pooled)	West	3	4	40.0 (13.0 – 67.0)
	Tanzania (Pooled)	East	2	3	9.0 (9.0 – 22.0)
	Uganda (Pooled)	East	3	3	52.0 (32.0 – 72.0)
CFR[C]	Pooled		3	5	20.0 (14.0 – 26.0)
	Nigeria	West	3	4	23.0 (14.0 – 32.0)
	Gambia	West	3	1	18.0 (10.0 – 28.0)
	Tanzania	East	2	1	33.3 (4.0 – 78.0)

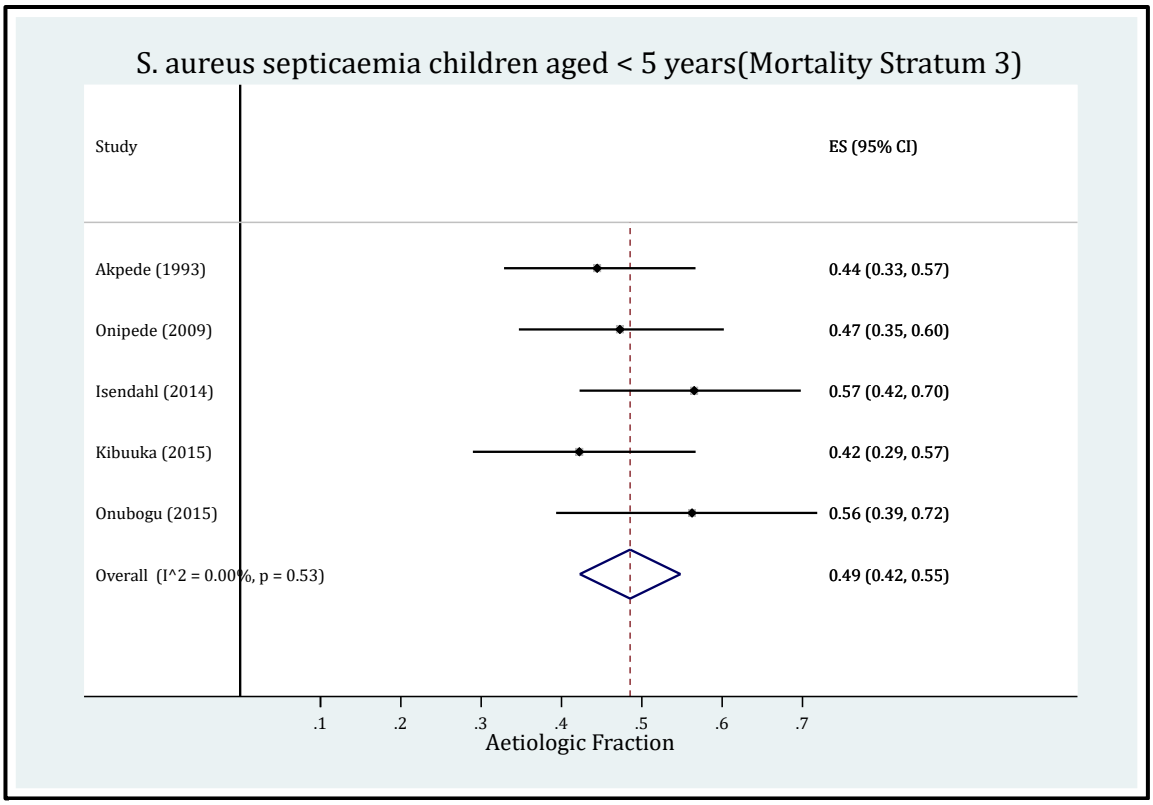
[a] Summary estimate of the meta-analysis of the countries in the same stratum
[b] Incidence in cases per 100,000 person-years
[c] CFR=Case fatality ratio
[d] Incidence in cases per 1,000 live births



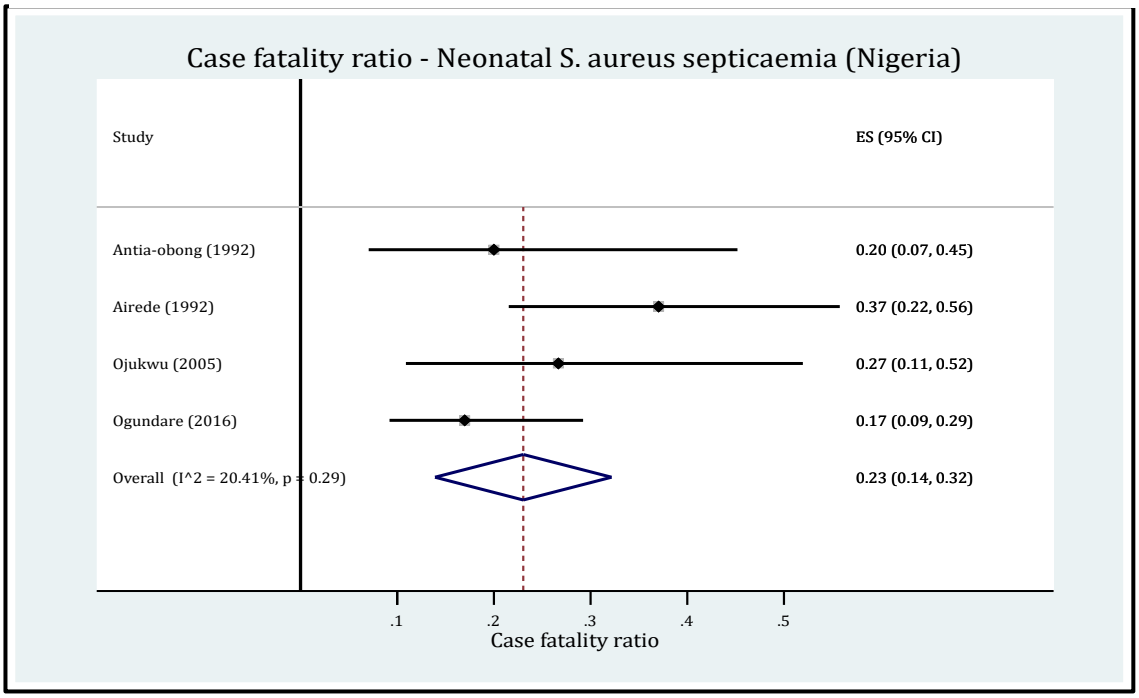
Supplemental Figure 1: Random-effects pooled aetiologic fraction of community-acquired *S. aureus* bacteraemic pneumonia in hospital-based studies in mortality strata 3 in Africa



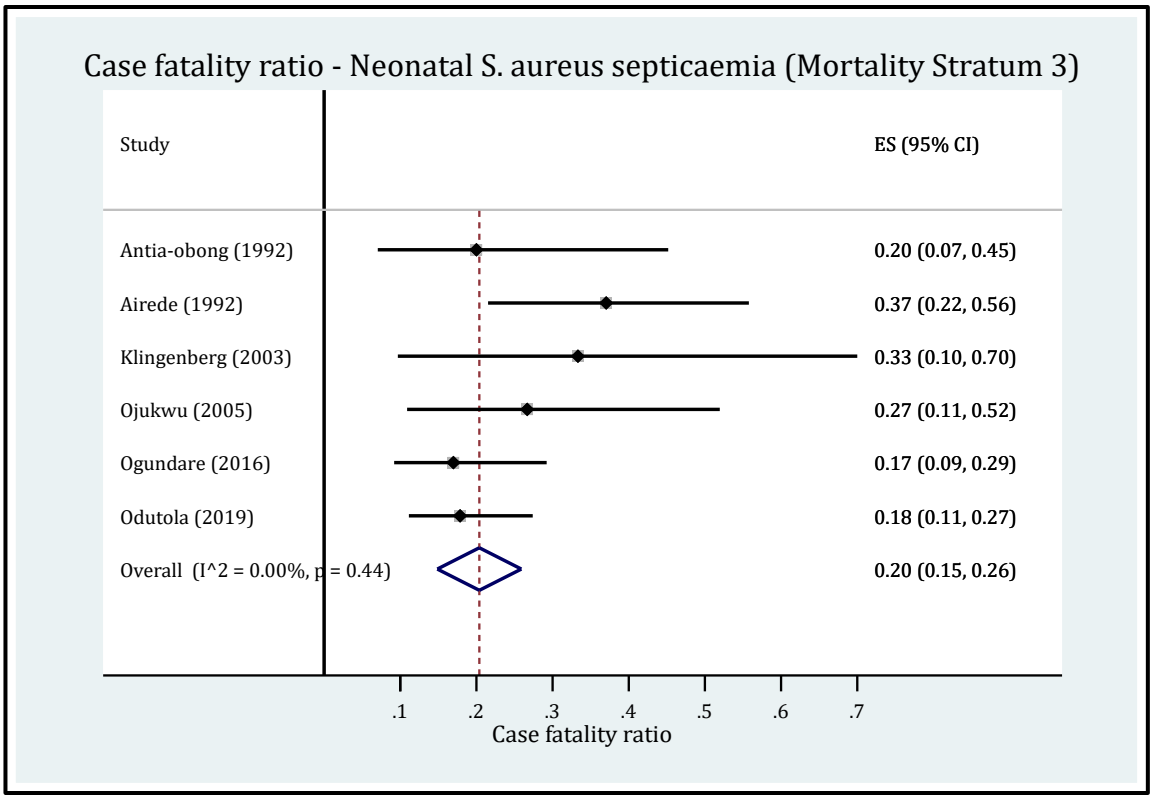
Supplemental Figure 2: Random-effects pooled aetiologic fraction of community-acquired *S. aureus* non-bacteraemic pneumonia in mortality stratum 3



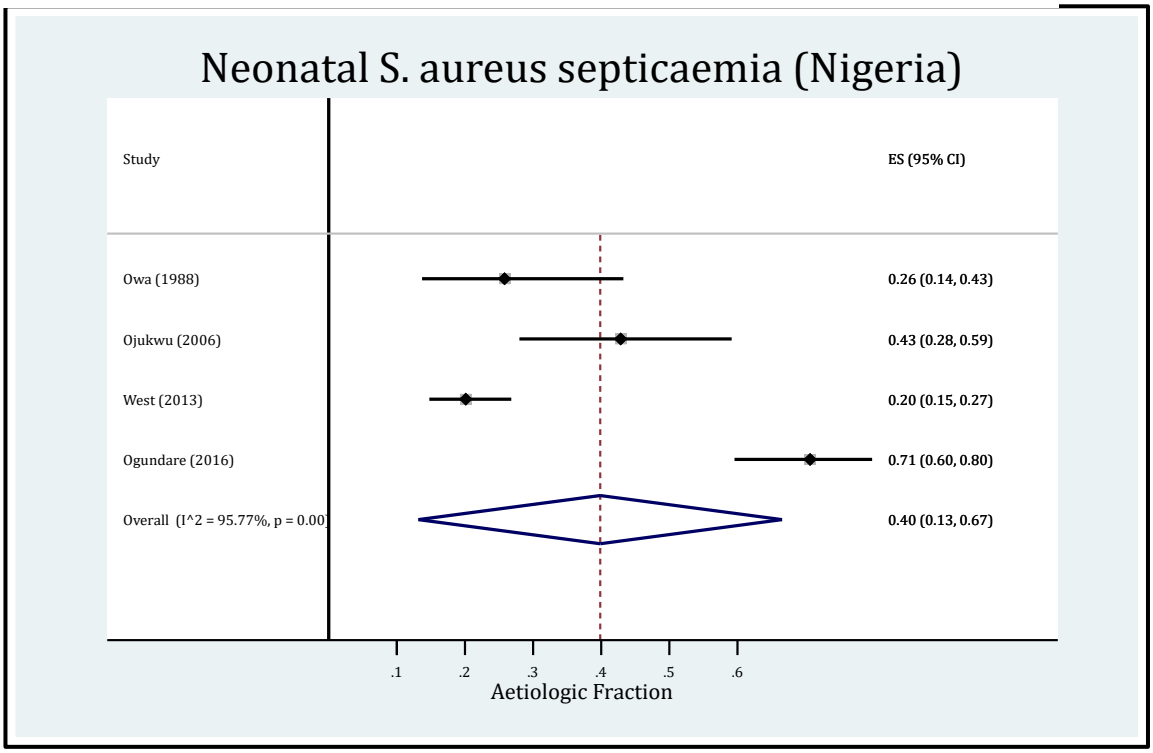
Supplemental Figure 3: Random-effects pooled aetiologic fraction of community-acquired *S. aureus* septicaemia among children aged less than five years in mortality stratum 3



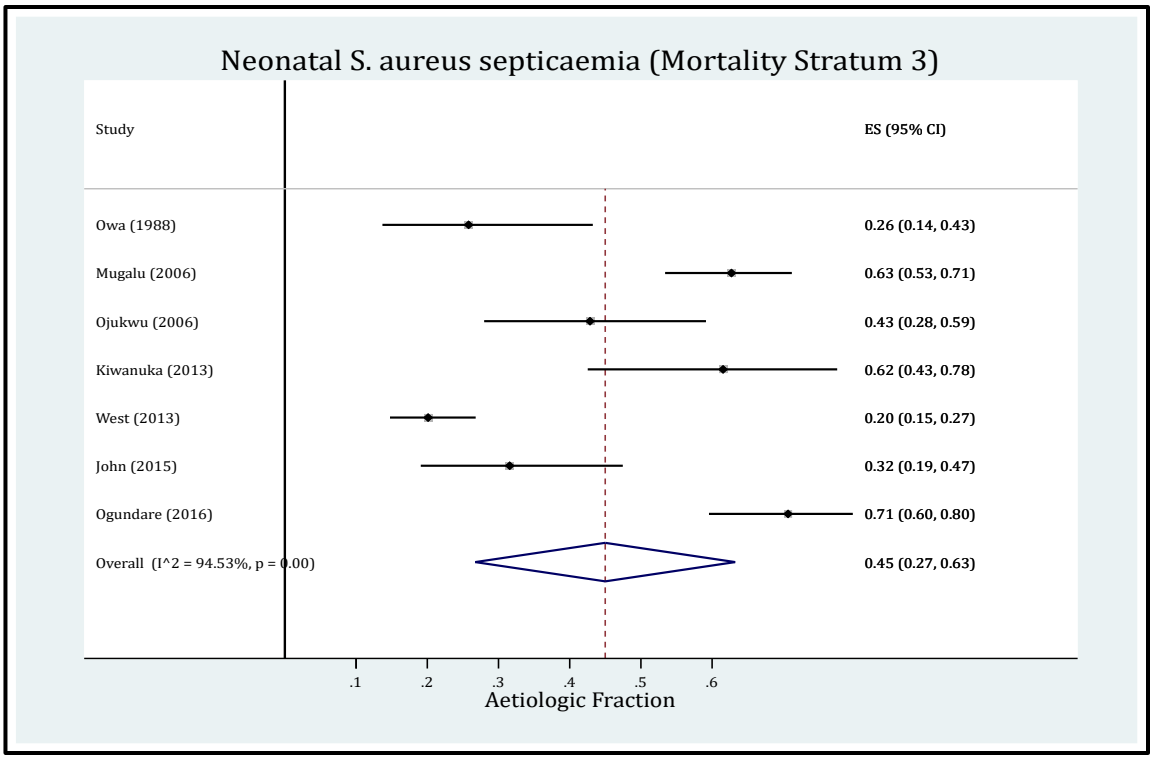
Supplemental Figure 4: Random-effects pooled case fatality ratio of community-acquired neonatal *S. aureus* septicaemia in Nigeria



Supplemental Figure 5: Random-effects pooled case fatality ratio of community-acquired neonatal *S. aureus* septicaemia in mortality stratum 3



Supplemental Figure 6: Random-effects pooled aetiologic fraction of community-acquired neonatal *S. aureus* septicaemia in Nigeria



Supplemental Figure 7: Random-effects pooled aetiologic fraction of community-acquired neonatal *S. aureus* septicaemia in mortality stratum 3

Appendix 4: Population size of children aged less than five years by country in 2015

	Country	Region	Mortality Stratum	Population Size	Percentage
1	Algeria	North	2	4,664,000	2.5
2	Angola	South	4	5,158,000	2.8
3	Benin	West	3	1,739,000	0.9
4	Botswana	South	2	256,000	0.2
5	Burkina Faso	West	3	3,161,000	1.7
6	Burundi	Central	3	1,853,000	1.0
7	Cameroon	Central	3	3,742,000	2.0
8	Cape Verde	West	1	55,000	0.03
9	Central African Republic	Central	4	728,000	0.4
10	Chad	Central	4	2,601,000	1.4
11	Comoros	East	3	117,000	0.1
12	Congo	Central	3	814,000	0.4
13	Cote d'ivoire	West	3	3,765,000	2.0
14	Democratic Republic of Congo	Central	3	14,099,000	7.5
15	Djibouti	East	3	101,000	0.1
16	Egypt	North	2	12,374,000	6.6
17	Equatorial Guinea	Central	3	176,000	0.1
18	Eritrea	East	2	744,000	0.4
19	Ethiopia	East	2	14,901,000	8.0
20	Gabon	Central	2	267,000	0.1
21	Gambia	West	3	352,000	0.2
22	Ghana	West	3	4,023,000	2.1
23	Guinea	West	3	1,949,000	1.0
24	Guinea Bissau	West	4	285,000	0.1
25	Kenya	East	3	6,997,000	3.7
26	Lesotho	South	3	280,000	0.1
27	Liberia	West	3	705,000	0.4
28	Libya	North	1	630,000	0.3
29	Madagascar	East	2	3,700,000	2.0
30	Malawi	South	3	2,888,000	1.6
31	Mali	West	3	3,274,000	1.7
32	Mauritania	North	3	640,000	0.4
33	Mauritius	East	1	69,000	0.1
34	Morocco	North	2	3,457,000	1.9
35	Mozambique	South	3	4,844,000	2.6
36	Namibia	South	2	335,000	0.2
37	Niger	West	3	4,066,000	2.2
38	Nigeria	West	3	31,109,000	16.7
39	Rwanda	East	2	1,735,000	0.9
40	Sao tome and Principe	Central	2	31,000	0.02
41	Senegal	West	2	2,493,000	1.3
42	Seychelles	East	1	8,000	0.01
43	Sierra Leone	West	3	1,135,000	0.6
44	Somali	East	3	2,554,000	1.4
45	South Africa	South	2	5,664,000	3.0
46	South Sudan	East	3	1,882,000	1.0
47	Sudan	East	3	5,859,000	3.1
48	Swaziland/Eswatini	South	3	178,000	0.1

Appendix 4: Population size of children aged less than five years by country in 2015 (continued)

	Country	Region[1]	Mortality Stratum[2]	Population Size[3]	Percentage
49	Tanzania	East	2	9,419,000	5.0
50	Togo	West	3	1,162,000	0.6
51	Tunisia	North	1	1,024,000	0.5
52	Uganda	East	3	7,512,000	4.0
53	Western Sahara	North	2	55,000	0.03
54	Zambia	South	3	2,750,000	1.5
55	Zimbabwe	South	2	2,505,000	1.3
	All countries			**186,884,000**	**100.0**

[1] African Union Commission. African Union Handbook: A guide for those working with and within the African Union. 6th ed. Addis Ababa: African Union Commission and New Zealand Ministry of Foreign Affairs and Trade/Manatū Aorere; 2019.
[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2016.
[3] United Nations Population Fund. World Population Prospects 2017 File POP/7-1: Total population (both sexes combined) by five-year age group, region, subregion and country, 1950-2100 (thousands) 2017. https://esa.un.org/unpd/wpp/Download/Standard/Population/ (accessed February 02 2018).

Appendix 5: Number of cases by clinical syndrome and incidence of *S. aureus* disease by country in 2015 (population-based studies)

Country	Region	MS[1]	Population[1]	Meningitis	Pneumonia	Septicaemia	All cases	Incidence[2]
Burundi	Central	3	1,853,000	252	1,220	2,348	3,821	206
Cameroon	Central	3	3,742,000	510	2,291	4,741	7,541	202
Central African Republic	Central	4	728,000	99	412	922	1,433	197
Chad	Central	4	2,601,000	354	1,611	3,295	5,260	202
Congo	Central	3	814,000	111	437	1,031	1,580	194
Democratic Republic of Congo	Central	3	14,099,000	1,920	9,851	17,863	29,634	210
Equatorial Guinea	Central	3	176,000	24	77	223	324	184
Gabon	Central	2	267,000	36	153	338	527	197
Sao tome or Principe	Central	2	31,000	4	16	39	59	191
Comoros	East	3	117,000	16	92	148	257	219
Djibouti	East	3	101,000	14	51	128	193	191
Eritrea	East	2	744,000	101	502	943	1,546	208
Ethiopia	East	2	14,901,000	2,029	9,073	18,880	29,981	201
Kenya	East	3	6,997,000	953	4,577	3,135	8,664	124
Madagascar	East	2	3,700,000	504	1,865	4,688	7,057	191
Mauritius	East	1	69,000	9	41	87	138	200
Rwanda	East	2	1,735,000	236	667	2,198	3,101	179
Seychelles	East	1	8,000	1	5	10	16	201
Somali	East	3	2,554,000	348	2,347	3,236	5,931	232
South Sudan	East	3	1,882,000	256	0	2,384	2,641	140
Sudan	East	3	5,859,000	798	2,946	7,423	11,167	191
Tanzania	East	2	9,419,000	1,282	6,135	11,934	19,351	205
Uganda	East	3	7,512,000	1,023	4,328	9,518	14,868	198

[1] MS=Mortality Stratum; Population=Population size

[2] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 5: Number of cases by clinical syndrome and incidence of *S. aureus* disease by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population[1]	Meningitis	Pneumonia	Septicaemia	All cases	Incidence[2]
Algeria	North	2	4,664,000	635	2,668	5,909	9,212	198
Egypt	North	2	12,374,000	1,685	3,624	15,678	20,987	170
Libya	North	1	630,000	86	244	798	1,128	179
Mauritania	North	3	640,000	87	341	811	1,239	194
Morocco	North	2	3,457,000	471	1,693	4,380	6,544	189
Tunisia	North	1	1,024,000	139	370	1,297	1,807	176
Western Sahara	North	2	55,000	7	0	70	77	140
Angola	South	4	5,158,000	1,419	1,897	6,535	9,851	191
Botswana	South	2	256,000	70	170	324	565	221
Lesotho	South	3	280,000	38	187	355	580	207
Malawi	South	3	2,888,000	393	1,676	3,659	5,728	198
Mozambique	South	3	4,844,000	1,332	3,410	5,730	10,473	216
Namibia	South	2	335,000	92	213	424	729	218
South Africa	South	2	5,664,000	1,558	3,210	7,176	11,944	211
Swaziland	South	3	178,000	24	78	226	328	184
Zambia	South	3	2,750,000	374	2,126	3,484	5,984	218
Zimbabwe	South	2	2,505,000	689	1,267	3,174	5,129	205
Benin	West	3	1,739,000	237	1,342	2,203	3,782	217
Burkina Faso	West	3	3,161,000	405	2,219	4,005	6,629	210
Cape Verde	West	1	55,000	7	30	70	108	196
Cote d'Ivoire	West	3	3,765,000	513	1,214	4,770	6,497	173
Ghana	West	3	4,023,000	548	1,974	25,349	27,870	693
Guinea	West	3	1,949,000	265	1,279	2,469	4,014	206

[1] MS=Mortality Stratum; Population=Population size

[2] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 5: Number of cases by clinical syndrome and incidence of *S. aureus* disease by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All cases	Incidence[3]
Guinea Bissau	West	4	285,000	39	183	361	583	205
Liberia	West	3	705,000	96	370	893	1,359	193
Mali	West	3	3,274,000	402	2,459	4,148	7,009	214
Niger	West	3	4,066,000	554	3,181	5,152	8,886	219
Nigeria	West	3	31,109,000	4,236	24,009	39,415	67,660	217
Senegal	West	2	2,493,000	339	1,670	3,159	5,168	207
Sierra Leone	West	3	1,135,000	155	614	1,438	2,206	194
Togo	West	3	1,162,000	149	727	1,472	2,348	202
All countries			**186,884,000**	**27,973**	**113,368**	**250,725**	**392,066**	

[1] MS=Mortality Stratum; United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2016.

[2] United Nations Population Fund. World Population Prospects 2017 File POP/7-1: Total population (both sexes combined) by five-year age group, region, subregion and country, 1950-2100 (thousands) 2017. https://esa.un.org/unpd/wpp/Download/Standard/Population/ (accessed February 02 2018).

[3] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 5a: Number of cases by clinical syndrome and incidence of *S. aureus* disease by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All cases	Incidence[3]
Burundi	Central	3	1,853,000	117	4,195	8,006	12,318	665
Cameroon	Central	3	3,742,000	236	7,874	16,168	24,278	649
Central African Republic	Central	4	728,000	46	1,415	3,815	5,276	725
Chad	Central	4	2,601,000	164	5,536	13,629	19,329	743
Congo	Central	3	814,000	51	1,503	3,517	5,072	623
Democratic Republic of Congo	Central	3	14,099,000	888	33,861	60,918	95,667	679
Equatorial Guinea	Central	3	176,000	11	264	760	1,036	589
Gabon	Central	2	267,000	9	124	183	316	118
Sao tome or Principe	Central	2	31,000	1	13	21	35	113
Comoros	East	3	117,000	7	318	506	831	710
Djibouti	East	3	101,000	6	175	436	618	612
Eritrea	East	2	744,000	25	408	511	944	127
Ethiopia	East	2	14,901,000	507	7,371	10,225	18,104	121
Kenya	East	3	6,997,000	441	14,808	25,138	40,387	577
Madagascar	East	2	3,700,000	126	1,516	2,539	4,180	113
Mauritius	East	1	69,000	2	33	47	83	120
Rwanda	East	2	1,735,000	59	542	1,191	1,791	103
Seychelles	East	1	8,000	0	4	5	10	121
Somali	East	3	2,554,000	161	8,068	11,035	19,264	754
South Sudan	East	3	1,882,000	119	0	8,132	8,250	438
Sudan	East	3	5,859,000	369	10,127	25,315	35,811	611
Tanzania	East	2	9,419,000	321	4,985	6,463	11,769	125

[1] MS=Mortality Stratum

[2] Population=Population size

[3] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 5a: Number of cases by clinical syndrome and incidence of *S. aureus* disease by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All cases	Incidence[3]
Uganda	East	3	7,512,000	473	15,417	29,004	44,894	598
Algeria	North	2	4,664,000	159	2,168	3,200	5,527	119
Egypt	North	2	12,374,000	421	2,945	8,491	11,857	96
Libya	North	1	630,000	21	199	432	652	104
Mauritania	North	3	640,000	40	1,171	2,765	3,976	621
Morocco	North	2	3,457,000	118	1,376	2,372	3,866	112
Tunisia	North	1	1,024,000	35	300	703	1,038	101
Western Sahara	North	2	55,000	2	0	38	40	72
Angola	South	4	5,158,000	656	6,522	27,028	34,206	663
Botswana	South	2	256,000	18	138	176	332	130
Lesotho	South	3	280,000	18	644	1,210	1,872	669
Malawi	South	3	2,888,000	182	5,762	12,478	18,422	638
Mozambique	South	3	4,844,000	616	11,722	17,804	30,142	622
Namibia	South	2	335,000	23	173	230	426	127
South Africa	South	2	5,664,000	389	2,608	3,887	6,884	122
Swaziland	South	3	178,000	11	269	769	1,049	589
Zambia	South	3	2,750,000	173	7,307	11,882	19,362	704
Zimbabwe	South	2	2,505,000	172	5,359	1,719	7,250	289
Benin	West	3	1,739,000	110	4,613	7,514	12,236	704
Burkina Faso	West	3	3,161,000	187	7,629	13,658	21,474	679
Cape Verde	West	1	55,000	2	25	38	64	117
Cote d'Ivoire	West	3	3,765,000	237	4,174	16,267	20,679	549
Gambia	West	3	352,000	22	779	1,050	1,852	526
Ghana	West	3	4,023,000	253	6,784	88,603	95,641	2,377
Guinea	West	3	1,949,000	123	4,396	8,421	12,940	664
Guinea Bissau	West	4	285,000	18	631	1,493	2,142	752

[1] MS=Mortality Stratum

[2] Population=Population size

[3] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 5a: Number of cases by clinical syndrome and incidence of *S. aureus* disease by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All cases	Incidence[3]
Liberia	West	3	705,000	44	1,270	3,046	4,361	619
Mali	West	3	3,274,000	186	1,383	18	1,587	48
Niger	West	3	4,066,000	256	10,934	17,568	28,758	707
Nigeria	West	3	31,109,000	1,959	160,562	137,273	299,793	964
Senegal	West	2	2,493,000	85	1,357	1,711	3,153	126
Sierra Leone	West	3	1,135,000	71	2,110	4,904	7,086	624
Togo	West	3	1,162,000	69	2,499	5,021	7,589	653
All countries			**186,884,000**	**10,816**	**376,367**	**629,334**	**1,016,516**	**25,922**

[1] MS=Mortality Stratum; United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2016.

[2] Population=Population size; United Nations Population Fund. World Population Prospects 2017 File POP/7-1: Total population (both sexes combined) by five-year age group, region, subregion and country, 1950-2100 (thousands) 2017. https://esa.un.org/unpd/wpp/Download/Standard/Population/ (accessed February 02 2018).

[3] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 6: Number of deaths by clinical syndrome and mortality rates of *S. aureus* disease by country in 2015 (population-based studies)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All deaths	MR[3]
Burundi	Central	3	1,853,000	122	95	211	428	23
Cameroon	Central	3	3,742,000	364	177	427	969	26
Central African Republic	Central	4	728,000	70	54	83	206	28
Chad	Central	4	2,601,000	258	309	297	863	33
Congo	Central	3	814,000	79	16	93	188	23
Democratic Republic of Congo	Central	3	14,099,000	1,196	742	1,608	3,545	25
Equatorial Guinea	Central	3	176,000	13	7	20	40	23
Gabon	Central	2	267,000	16	6	30	53	20
Sao tome or Principe	Central	2	31,000	2	1	4	6	19
Comoros	East	3	117,000	10	5	13	28	24
Djibouti	East	3	101,000	4	3	12	18	18
Eritrea	East	2	744,000	61	25	85	171	23
Ethiopia	East	2	14,901,000	1,424	502	1,699	3,625	24
Kenya	East	3	6,997,000	442	180	282	904	13
Madagascar	East	2	3,700,000	317	111	422	850	23
Mauritius	East	1	69,000	3	0	8	11	16
Rwanda	East	2	1,735,000	128	31	198	357	21
Seychelles	East	1	8,000	0	0	1	1	15
Somalia	East	3	2,554,000	283	235	291	809	32
South Sudan	East	3	1,882,000	149	129	215	493	26
Sudan	East	3	5,859,000	465	246	668	1,380	24
Tanzania	East	2	9,419,000	689	230	1,074	1,993	21

[1] MS=Mortality Stratum

[2] Population=Population size

[3] MR=Mortality Rate; Mortality rates of *S. aureus* disease are per 100,000 children

Appendix 6: Number of deaths by clinical syndrome and mortality rates of *S. aureus* disease by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All deaths	MR[3]
Uganda	East	3	7,512,000	380	219	857	1,456	19
Algeria	North	2	4,664,000	295	50	532	877	19
Egypt	North	2	12,374,000	760	134	1,411	2,305	19
Libya	North	1	630,000	22	2	72	96	15
Mauritania	North	3	640,000	59	26	73	158	25
Morocco	North	2	3,457,000	355	36	394	786	23
Tunisia	North	1	1,024,000	70	4	117	191	19
Western Sahara	North	2	55,000	6	1	6	13	23
Angola	South	4	5,158,000	818	474	588	1,880	36
Botswana	South	2	256,000	57	5	29	91	36
Lesotho	South	3	280,000	18	15	32	66	23
Malawi	South	3	2,888,000	151	87	329	568	20
Mozambique	South	3	4,844,000	715	188	344	1,248	26
Namibia	South	2	335,000	39	10	38	87	26
South Africa	South	2	5,664,000	497	114	646	1,257	22
Swaziland	South	3	178,000	12	6	20	38	21
Zambia	South	3	2,750,000	164	84	314	561	20
Zimbabwe	South	2	2,505,000	388	90	286	763	30
Benin	West	3	1,739,000	177	91	198	467	27
Burkina Faso	West	3	3,161,000	225	124	360	710	22
Cape Verde	West	1	55,000	2	1	6	9	16
Cote d'Ivoire	West	3	3,765,000	333	175	429	937	25
Gambia	West	3	352,000	22	12	38	72	21

[1] MS=Mortality Stratum

[2] Population=Population size

[3] MR=Mortality Rate; Mortality rates of *S. aureus* disease are per 100,000 children

Appendix 6: Number of deaths by clinical syndrome and mortality rates of *S. aureus* disease by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All deaths	MR[3]
Ghana	West	3	4,023,000	291	104	2,281	2,676	67
Guinea	West	3	1,949,000	174	112	222	509	26
Guinea Bissau	West	4	285,000	26	16	32	75	26
Liberia	West	3	705,000	54	28	80	162	23
Mali	West	3	3,274,000	301	173	373	847	26
Niger	West	3	4,066,000	283	293	464	1,039	26
Nigeria	West	3	31,109,000	2,834	2,137	3,547	8,518	27
Senegal	West	2	2,493,000	198	64	284	546	22
Sierra Leone	West	3	1,135,000	66	60	129	255	22
Togo	West	3	1,162,000	86	48	133	266	23
All countries			**186,884,000**	**15,977**	**8,083**	**22,407**	**46,467**	

[1] MS=Mortality Stratum; United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2016.

[2] Population=Population size; United Nations Population Fund. World Population Prospects 2017 File POP/7-1: Total population (both sexes combined) by five-year age group, region, subregion and country, 1950-2100 (thousands) 2017. https://esa.un.org/unpd/wpp/Download/Standard/Population/ (accessed February 02 2018).

[3] Mortality rates of *S. aureus* disease are per 100,000 children

Appendix 6a: Number of deaths by clinical syndrome and mortality rates of *S. aureus* disease by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All deaths	MR[3]
Burundi	Central	3	1,853,000	112	355	721	1,188	64
Cameroon	Central	3	3,742,000	326	664	1,455	2,445	65
Central African Republic	Central	4	728,000	62	202	343	608	83
Chad	Central	4	2,601,000	231	1,157	1,227	2,614	100
Congo	Central	3	814,000	71	58	317	446	55
Democratic Republic of Congo	Central	3	14,099,000	1,079	2,774	5,483	9,335	66
Equatorial Guinea	Central	3	176,000	12	25	68	105	60
Gabon	Central	2	267,000	15	5	16	36	14
Sao tome or Principe	Central	2	31,000	2	0	2	4	13
Comoros	East	3	117,000	9	18	45	73	62
Djibouti	East	3	101,000	4	11	39	54	54
Eritrea	East	2	744,000	55	20	46	121	16
Ethiopia	East	2	14,901,000	1,276	409	920	2,604	17
Kenya	East	3	6,997,000	408	630	2,262	3,301	47
Madagascar	East	2	3,700,000	286	90	229	605	16
Mauritius	East	1	69,000	2	0	4	7	10
Rwanda	East	2	1,735,000	117	25	107	249	14
Seychelles	East	1	8,000	0	0	0	1	10
Somali	East	3	2,554,000	252	880	993	2,125	83
South Sudan	East	3	1,882,000	135	0	732	867	46
Sudan	East	3	5,859,000	422	933	2,278	3,633	62
Tanzania	East	2	9,419,000	628	186	582	1,396	15

[1] MS=Mortality Stratum

[2] Population=Population size

[3] MR=Mortality Rate; Mortality rates of *S. aureus* disease are per 100,000 children

Appendix 6a: Number of deaths by clinical syndrome and mortality rates of *S. aureus* disease by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All deaths	MR[3]
Uganda	East	3	7,512,000	358	779	2,610	3,747	50
Algeria	North	2	4,664,000	272	41	288	601	13
Egypt	North	2	12,374,000	703	109	764	1,576	13
Libya	North	1	630,000	22	2	39	62	10
Mauritania	North	3	640,000	53	97	249	399	62
Morocco	North	2	3,457,000	317	124	213	654	16
Tunisia	North	1	1,024,000	64	13	63	140	13
Western Sahara	North	2	55,000	5	0	3	8	15
Angola	South	4	5,158,000	367	1,623	2,433	4,422	89
Botswana	South	2	256,000	25	17	16	58	18
Lesotho	South	3	280,000	17	53	109	178	65
Malawi	South	3	2,888,000	142	298	1,123	1,563	55
Mozambique	South	3	4,844,000	323	647	1,068	2,038	43
Namibia	South	2	335,000	18	34	21	73	14
South Africa	South	2	5,664,000	236	391	350	976	12
Swaziland	South	3	178,000	11	19	69	100	57
Zambia	South	3	2,750,000	152	331	1,069	1,552	58
Zimbabwe	South	2	2,505,000	175	307	155	636	27
Benin	West	3	1,739,000	158	314	676	1,148	68
Burkina Faso	West	3	3,161,000	218	427	1,229	1,874	61
Cape Verde	West	1	55,000	2	2	3	7	11
Cote d'Ivoire	West	3	3,765,000	299	601	1,464	2,365	64

[1] MS=Mortality Stratum

[2] Population=Population size

[3] MR=Mortality Rate; Mortality rates of *S. aureus* disease are per 100,000 children

Appendix 6a: Number of deaths by clinical syndrome and mortality rates of *S. aureus* disease by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[2]	Meningitis	Pneumonia	Septicaemia	All deaths	MR[3]
Gambia	West	3	352,000	20	42	147	209	61
Ghana	West	3	4,023,000	265	355	7,974	8,594	214
Guinea	West	3	1,949,000	156	385	758	1,300	68
Guinea Bissau	West	4	285,000	24	57	134	214	77
Liberia	West	3	705,000	49	96	274	419	61
Mali	West	3	3,274,000	298	97	2	396	12
Niger	West	3	4,066,000	259	1,006	1,581	2,846	72
Nigeria	West	3	31,109,000	2,545	14,257	12,355	29,156	94
Senegal	West	2	2,493,000	179	219	154	552	15
Sierra Leone	West	3	1,135,000	61	204	441	707	64
Togo	West	3	1,162,000	83	163	452	697	61
All countries			**186,884,000**	**13,378**	**31,568**	**56,158**	**101,105**	

[1] MS=Mortality Stratum

[2] Population=Population size

[3] MR=Mortality Rate; Mortality rates of *S. aureus* disease are per 100,000 children

Appendix 7: Number of cases and deaths of *S. aureus* bacteraemia pneumonia by country in 2015 (population-based studies)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Burundi	Central	3	1,853,000	76,281	1.6	1,220	5913	1.6	95
Cameroon	Central	3	3,742,000	143,164	1.6	2,291	11071	1.6	177
Central African Republic	Central	4	728,000	25,731	1.6	412	3368	1.6	54
Chad	Central	4	2,601,000	100,662	1.6	1,611	19275	1.6	308
Congo	Central	3	814,000	27,333	1.6	437	971	1.6	16
Democratic Republic of Congo	Central	3	14,099,000	615,657	1.6	9,851	46226	1.6	740
Equatorial Guinea	Central	3	176,000	4,806	1.6	77	418	1.6	7
Gabon	Central	2	267,000	9,533	1.6	153	355	1.6	6
Sao tome or Principe	Central	2	31,000	977	1.6	16	35	1.6	1
Comoros	East	3	117,000	5,780	1.6	92	300	1.6	5
Djibouti	East	3	101,000	3,188	1.6	51	184	1.6	3
Eritrea	East	2	744,000	31,388	1.6	502	1551	1.6	25
Ethiopia	East	2	14,901,000	567,035	1.6	9,073	31427	1.6	503
Kenya	East	3	6,997,000	269,243	1.7	4,577	10507	1.7	179
Madagascar	East	2	3,700,000	116,583	1.6	1,865	6951	1.6	111
Mauritius	East	1	69,000	2,562	1.6	41	21	1.6	0
Rwanda	East	2	1,735,000	41,664	1.6	667	1929	1.6	31
Seychelles	East	1	8,000	304	1.6	5	1	1.6	0
Somali	East	3	2,554,000	146,691	1.6	2,347	14669	1.6	235
South Sudan	East	3	1,882,000		1.6	0		1.6	129
Sudan	East	3	5,859,000	184,135	1.6	2,946	15551	1.6	249
Tanzania	East	2	9,419,000	383,430	1.6	6,135	14322	1.6	229

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 7: Number of cases and deaths of *S. aureus* bacteraemia pneumonia by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Uganda	East	3	7,512,000	270,471	1.6	4,328	13659	1.6	219
Algeria	North	2	4,664,000	166,754	1.6	2,668	3130	1.6	50
Egypt	North	2	12,374,000	226,501	1.6	3,624	8375	1.6	134
Libya	North	1	630,000	15,280	1.6	244	127	1.6	2
Mauritania	North	3	640,000	21,288	1.6	341	1616	1.6	26
Morocco	North	2	3,457,000	105,827	1.6	1,693	2246	1.6	36
Tunisia	North	1	1,024,000	23,112	1.6	370	234	1.6	4
Western Sahara	North	2	55,000		1.6	0		1.6	0
Angola	South	4	5,158,000	118,577	1.6	1,897	29502	1.6	472
Botswana	South	2	256,000	10,647	1.6	170	307	1.6	5
Lesotho	South	3	280,000	11,717	1.6	187	955	1.6	15
Malawi	South	3	2,888,000	104,755	1.6	1,676	5416	1.6	87
Mozambique	South	3	4,844,000	213,124	1.6	3,410	11757	1.6	188
Namibia	South	2	335,000	13,301	1.6	213	618	1.6	10
South Africa	South	2	5,664,000	200,634	1.6	3,210	7105	1.6	114
Swaziland	South	3	178,000	4,888	1.6	78	354	1.6	6
Zambia	South	3	2,750,000	132,848	1.6	2,126	6020	1.6	96
Zimbabwe	South	2	2,505,000	97,432	1.3	1,267	5582	1.3	73
Benin	West	3	1,739,000	83,873	1.6	1,342	5702	1.6	91
Burkina Faso	West	3	3,161,000	138,710	1.6	2,219	7761	1.6	124
Cape Verde	West	1	55,000	1,898	1.6	30	37	1.6	1
Cote d'Ivoire	West	3	3,765,000	75,899	1.6	1,214	10935	1.6	175

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 7: Number of cases and deaths of *S. aureus* bacteraemia pneumonia by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Gambia	West	3	352,000	14,165	1.6	227	766	1.6	12
Ghana	West	3	4,023,000	123,352	1.6	1,974	6450	1.6	103
Guinea	West	3	1,949,000	79,935	1.6	1,279	7008	1.6	112
Guinea Bissau	West	4	285,000	11,468	1.6	183	1028	1.6	16
Liberia	West	3	705,000	23,094	1.6	370	1737	1.6	28
Mali	West	3	3,274,000	153,660	1.6	2,459	10766	1.6	172
Niger	West	3	4,066,000	198,793	1.6	3,181	18290	1.6	293
Nigeria	West	3	31,109,000	1,500,575	1.6	24,009	133239	1.6	2,132
Senegal	West	2	2,493,000	104,392	1.6	1,670	3984	1.6	64
Sierra Leone	West	3	1,135,000	38,365	1.6	614	3714	1.6	59
Togo	West	3	1,162,000	45,439	1.6	727	2962	1.6	47
All countries			**186,884,000**	**7,086,921**		**113,368**	**496,427**		**8,066**

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 7a: Number of cases and deaths of *S. aureus* bacteraemia pneumonia by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Burundi	Central	3	1,853,000	76,281	6.0	4,577	5,913	6.0	355
Cameroon	Central	3	3,742,000	143,164	6.0	8,590	11,071	6.0	664
Central African Republic	Central	4	728,000	25,731	6.0	1,544	3,368	6.0	202
Chad	Central	4	2,601,000	100,662	6.0	6,040	19,275	6.0	1,157
Congo	Central	3	814,000	27,333	6.0	1,640	971	6.0	58
Democratic Republic of Congo	Central	3	14,099,000	615,657	6.0	36,939	46,226	6.0	2,774
Equatorial Guinea	Central	3	176,000	4,806	6.0	288	418	6.0	25
Gabon	Central	2	267,000	9,533	1.3	124	355	1.3	5
Sao tome or Principe	Central	2	31,000	977	1.3	13	35	1.3	0
Comoros	East	3	117,000	5,780	6.0	347	300	6.0	18
Djibouti	East	3	101,000	3,188	6.0	191	184	6.0	11
Eritrea	East	2	744,000	31,388	1.3	408	1,551	1.3	20
Ethiopia	East	2	14,901,000	567,035	1.3	7,371	31,427	1.3	409
Kenya	East	3	6,997,000	269,243	6.0	16,155	10,507	6.0	630
Madagascar	East	2	3,700,000	116,583	1.3	1,516	6,951	1.3	90
Mauritius	East	1	69,000	2,562	1.3	33	21	1.3	0
Rwanda	East	2	1,735,000	41,664	1.3	542	1,929	1.3	25
Seychelles	East	1	8,000	304	1.3	4	1	1.3	0
Somali	East	3	2,554,000	146,691	6.0	8,801	14,669	6.0	880
South Sudan	East	3	1,882,000		6.0	0		6.0	0
Sudan	East	3	5,859,000	184,135	6.0	11,048	15,551	6.0	933
Tanzania	East	2	9,419,000	383,430	1.3	4,985	14,322	1.3	186

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 7a: Number of cases and deaths of *S. aureus* bacteraemia pneumonia by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Uganda	East	3	7,512,000	270,471	5.7	15,417	13,659	5.7	779
Algeria	North	2	4,664,000	166,754	1.3	2,168	3,130	1.3	41
Egypt	North	2	12,374,000	226,501	1.3	2,945	8,375	1.3	109
Libya	North	1	630,000	15,280	1.3	199	127	1.3	2
Mauritania	North	3	640,000	21,288	6.0	1,277	1,616	6.0	97
Morocco	North	2	3,457,000	105,827	1.3	1,376	2,246	1.3	29
Tunisia	North	1	1,024,000	23,112	1.3	300	234	1.3	3
Western Sahara	North	2	55,000	0	1.3	0	0	1.3	0
Angola	South	4	5,158,000	118,577	6.0	7,115	29,502	6.0	1,770
Botswana	South	2	256,000	10,647	1.3	138	307	1.3	4
Lesotho	South	3	280,000	11,717	6.0	703	955	6.0	57
Malawi	South	3	2,888,000	104,755	6.0	6,285	5,416	6.0	325
Mozambique	South	3	4,844,000	213,124	6.0	12,787	11,757	6.0	705
Namibia	South	2	335,000	13,301	1.3	173	618	1.3	8
South Africa	South	2	5,664,000	200,634	1.3	2,608	7,105	1.3	92
Swaziland	South	3	178,000	4,888	6.0	293	354	6.0	21
Zambia	South	3	2,750,000	132,848	6.0	7,971	6,020	6.0	361
Zimbabwe	South	2	2,505,000	97,432	6.0	5,846	5,582	6.0	335
Benin	West	3	1,739,000	83,873	6.0	5,032	5,702	6.0	342
Burkina Faso	West	3	3,161,000	138,710	6.0	8,323	7,761	6.0	466
Cape Verde	West	1	55,000	1,898	1.3	25	37	1.3	0
Cote d'Ivoire	West	3	3,765,000	75,899	6.0	4,554	10,935	6.0	656

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 7a: Number of cases and deaths of *S. aureus* bacteraemia pneumonia by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Gambia	West	3	352,000	14,165	6.0	850	766	6.0	46
Ghana	West	3	4,023,000	123,352	6.0	7,401	6,450	6.0	387
Guinea	West	3	1,949,000	79,935	6.0	4,796	7,008	6.0	420
Guinea Bissau	West	4	285,000	11,468	6.0	688	1,028	6.0	62
Liberia	West	3	705,000	23,094	6.0	1,386	1,737	6.0	104
Mali	West	3	3,274,000	153,660	0.9	1,383	10,766	0.9	97
Niger	West	3	4,066,000	198,793	6.0	11,928	18,290	6.0	1,097
Nigeria	West	3	31,109,000	1,500,575	10.7	160,562	133,239	10.7	14,257
Senegal	West	2	2,493,000	104,392	1.3	1,357	3,984	1.3	52
Sierra Leone	West	3	1,135,000	38,365	6.0	2,302	3,714	6.0	223
Togo	West	3	1,162,000	45,439	6.0	2,726	2,962	6.0	178
All countries			**186,884,000**	**7,086,921**		**392,069**	**496,427**		**31,568**

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 8: Number of cases and deaths of *S. aureus* non-bacteraemia pneumonia by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Burundi	Central	3	1,853,000	76,281	25.0	19,070	5,100	25.0	1,275
Cameroon	Central	3	3,742,000	143,164	25.0	35,791	33	25.0	8
Central African Republic	Central	4	728,000	25,731	25.0	6,433	3,536	25.0	884
Chad	Central	4	2,601,000	100,662	25.0	25,166	18,839	25.0	4,710
Congo	Central	3	814,000	27,333	25.0	6,833	1,359	25.0	340
Democratic Republic of Congo	Central	3	14,099,000	615,657	25.0	153,914	49,244	25.0	12,311
Equatorial Guinea	Central	3	176,000	4,806	25.0	1,202	551	25.0	138
Gabon	Central	2	267,000	9,533	25.0	2,383	387	25.0	97
Sao tome or Principe	Central	2	31,000	977	25.0	244	47	25.0	12
Comoros	East	3	117,000	5,780	25.0	1,445	353	25.0	88
Djibouti	East	3	101,000	3,188	25.0	797	197	25.0	49
Eritrea	East	2	744,000	31,388	25.0	7,847	1,488	25.0	372
Ethiopia	East	2	14,901,000	567,035	25.0	141,759	32,276	25.0	8,069
Kenya	East	3	6,997,000	269,243	25.0	67,311	10,875	25.0	2,719
Madagascar	East	2	3,700,000	116,583	25.0	29,146	6,548	25.0	1,637
Mauritius	East	1	69,000	2,562	25.0	641	19	25.0	5
Rwanda	East	2	1,735,000	41,664	25.0	10,416	1,966	25.0	492
Seychelles	East	1	8,000	304	25.0	76	1	25.0	0
Somali	East	3	2,554,000	146,691	25.0	36,673	18,066	25.0	4,517
South Sudan	East	3	1,882,000		25.0	0	8,179	25.0	2,045

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 8: Number of cases and deaths of *S. aureus* non-bacteraemia pneumonia by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Sudan	East	3	5,859,000	184,135	25.0	46,034	12,610	25.0	3,153
Tanzania	East	2	9,419,000	383,430	25.0	95,858	18,029	25.0	4,507
Uganda	East	3	7,512,000	270,471	25.0	67,618	14,992	25.0	3,748
Algeria	North	2	4,664,000	166,754	25.0	41,689	3,130	25.0	783
Egypt	North	2	12,374,000	226,501	25.0	56,625	6,400	25.0	1,600
Libya	North	1	630,000	15,280	25.0	3,820	153	25.0	38
Mauritania	North	3	640,000	21,288	25.0	5,322	1,849	25.0	462
Morocco	North	2	3,457,000	105,827	25.0	26,457	2,255	25.0	564
Tunisia	North	1	1,024,000	23,112	25.0	5,778	232	25.0	58
Western Sahara	North	2	55,000		25.0	0	47	25.0	12
Angola	South	4	5,158,000	118,577	25.0	29,644	17,385	25.0	4,346
Botswana	South	2	256,000	10,647	25.0	2,662	218	25.0	55
Lesotho	South	3	280,000	11,717	25.0	2,929	864	25.0	216
Malawi	South	3	2,888,000	104,755	25.0	26,189	4,785	25.0	1,196
Mozambique	South	3	4,844,000	213,124	25.0	53,281	10,868	25.0	2,717
Namibia	South	2	335,000	13,301	25.0	3,325	562	25.0	141
South Africa	South	2	5,664,000	200,634	25.0	50,159	8,487	25.0	2,122
Swaziland	South	3	178,000	4,888	25.0	1,222	424	25.0	106
Zambia	South	3	2,750,000	132,848	25.0	33,212	5,702	25.0	1,426
Zimbabwe	South	2	2,505,000	97,432	25.0	24,358	4,572	25.0	1,143
Benin	West	3	1,739,000	83,873	25.0	20,968	5,859	25.0	1,465
Burkina Faso	West	3	3,161,000	138,710	25.0	34,678	7,657	25.0	1,914
Guinea	West	3	1,949,000	79,935	25.0	19,984	6,815	25.0	1,704

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 8: Number of cases and deaths of *S. aureus* non-bacteraemia pneumonia by country (hospital-based studies) (continued)

Country	Region	MS[1]	Population[1]	All-cause pneumonia cases[2]	AF[1]	*S. aureus* pneumonia cases	All-cause pneumonia deaths[3]	AF[1]	*S. aureus* pneumonia deaths
Cape Verde	West	1	55,000	1,898	25.0	475	10,754	25.0	2,689
Cote d'Ivoire	West	3	3,765,000	75,899	25.0	18,975	13,515	25.0	3,379
Gambia	West	3	352,000	14,165	25.0	3,541	748	25.0	187
Ghana	West	3	4,023,000	123,352	25.0	30,838	6,179	25.0	1,545
Guinea Bissau	West	4	285,000	11,468	25.0	2,867	1,038	25.0	260
Liberia	West	3	705,000	23,094	25.0	5,774	1,807	25.0	452
Mali	West	3	3,274,000	153,660	25.0	38,415	11,138	25.0	2,785
Niger	West	3	4,066,000	198,793	25.0	49,698	16,132	25.0	4,033
Nigeria	West	3	31,109,000	1,500,575	25.0	375,144	141,912	25.0	35,478
Senegal	West	2	2,493,000	104,392	25.0	26,098	3,993	25.0	998
Sierra Leone	West	3	1,135,000	38,365	25.0	9,591	4,116	25.0	1,029
Togo	West	3	1,162,000	45,439	25.0	11,360	2,943	25.0	736
All countries			**186,884,000**	**7,086,921**		**1,771,730**	**507,234**		**126,809**

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] McAllister et al. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. Lancet Glob Health 2019; 7: e47-e57

[3] Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet 2016; 388: 3027-35

Appendix 9: Number of cases of *S. aureus* meningitis by country in 2015 (population-based studies)

Country	Region	MS[1]	Population[1]	All-cause Incidence[2]	All-cause meningitis cases	AF[1]	*S. aureus* meningitis cases
Burundi	Central	3	1,853,000	85.1	1,577	0.16	252
Cameroon	Central	3	3,742,000	85.1	3,184	0.16	510
Central African Republic	Central	4	728,000	85.1	620	0.16	99
Chad	Central	4	2,601,000	85.1	2,213	0.16	354
Congo	Central	3	814,000	85.1	693	0.16	111
Democratic Republic of Congo	Central	3	14,099,000	85.1	11,998	0.16	1,920
Equatorial Guinea	Central	3	176,000	85.1	150	0.16	24
Gabon	Central	2	267,000	85.1	227	0.16	36
Sao tome or Principe	Central	2	31,000	85.1	26	0.16	4
Comoros	East	3	117,000	85.1	100	0.16	16
Djibouti	East	3	101,000	85.1	86	0.16	14
Eritrea	East	2	744,000	85.1	633	0.16	101
Ethiopia	East	2	14,901,000	85.1	12,681	0.16	2,029
Kenya	East	3	6,997,000	85.1	5,954	0.16	953
Madagascar	East	2	3,700,000	85.1	3,149	0.16	504
Mauritius	East	1	69,000	85.1	59	0.16	9
Rwanda	East	2	1,735,000	85.1	1,476	0.16	236
Seychelles	East	1	8,000	85.1	7	0.16	1
Somali	East	3	2,554,000	85.1	2,173	0.16	348
South Sudan	East	3	1,882,000	85.1	1,602	0.16	256
Sudan	East	3	5,859,000	85.1	4,986	0.16	798
Tanzania	East	2	9,419,000	85.1	8,016	0.16	1,282
Uganda	East	3	7,512,000	85.1	6,393	0.16	1,023
Algeria	North	2	4,664,000	85.1	3,969	0.16	635
Egypt	North	2	12,374,000	85.1	10,530	0.16	1,685

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 9: Number of cases of *S. aureus* meningitis by country in 2015 (population-based studies)

Country	Region	MS[1]	Population[1]	All-cause Incidence[2]	All-cause meningitis cases	AF[1]	*S. aureus* meningitis cases
Libya	North	1	630,000	85.1	536	0.16	86
Mauritania	North	3	640,000	85.1	545	0.16	87
Morocco	North	2	3,457,000	85.1	2,942	0.16	471
Tunisia	North	1	1,024,000	85.1	871	0.16	139
Western Sahara	North	2	55,000	85.1	47	0.16	7
Angola	South	4	5,158,000	171.9	8,867	0.16	1,419
Botswana	South	2	256,000	171.9	440	0.16	70
Lesotho	South	3	280,000	85.1	238	0.16	38
Malawi	South	3	2,888,000	85.1	2,458	0.16	393
Mozambique	South	3	4,844,000	171.9	8,327	0.16	1,332
Namibia	South	2	335,000	171.9	576	0.16	92
South Africa	South	2	5,664,000	171.9	9,736	0.16	1,558
Swaziland	South	3	178,000	85.1	151	0.16	24
Zambia	South	3	2,750,000	85.1	2,340	0.16	374
Zimbabwe	South	2	2,505,000	171.9	4,306	0.16	689
Benin	West	3	1,739,000	85.1	1,480	0.16	237
Burkina Faso	West	3	3,161,000	80	2,529	0.16	405
Cape Verde	West	1	55,000	85.1	47	0.16	7
Cote d'Ivoire	West	3	3,765,000	85.1	3,204	0.16	513
Gambia	West	3	352,000	85.1	300	0.16	48
Ghana	West	3	4,023,000	85.1	3,424	0.16	548
Guinea	West	3	1,949,000	85.1	1,659	0.16	265
Guinea Bissau	West	4	285,000	85.1	243	0.16	39
Liberia	West	3	705,000	85.1	600	0.16	96
Mali	West	3	3,274,000	76.8	2,514	0.16	402
Niger	West	3	4,066,000	85.1	3,460	0.16	554
Nigeria	West	3	31,109,000	85.1	26,474	0.16	4,236
Senegal	West	2	2,493,000	85.1	2,122	0.16	339
Sierra Leone	West	3	1,135,000	85.1	966	0.16	155
Togo	West	3	1,162,000	80	930	0.16	149
All countries			**186,884,000**		**174,831**		**27,973**

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 9a: Number of deaths due to *S. aureus* meningitis by country in 2015 (population-based studies)

Country	Region	MS[1]	Care seeking[2]	Not care-seeking	*S. aureus* meningitis cases	Care-seeking deaths	Not care-seeking death	Care-seeking CFR[1]	Not care-seeking CFR[1]	Care-seeking *S. aureus* meningitis deaths	Not care-seeking *S. aureus* meningitis deaths	Total *S. aureus* meningitis deaths
Burundi	Central	3	63	37	252	159	93	24	90	38	84	
Cameroon	Central	3	28	72	510	143	367	24	90	34	330	364
Central African Republic	Central	4	30	70	99	30	69	24	90	7	62	70
Chad	Central	4	26	74	354	92	262	24	90	22	236	258
Congo	Central	3	28	72	111	31	80	24	90	7	72	79
Democratic Republic of Congo	Central	3	42	58	1,920	806	1,113	24	90	194	1,002	1,196
Equatorial Guinea	Central	3	54	46	24	13	11	24	90	3	10	13
Gabon	Central	2	68	32	36	25	12	24	90	6	10	16
Sao tome or Principe	Central	2	69	31	4	3	1	24	90	1	1	2
Comoros	East	3	38	62	16	6	10	24	90	1	9	10
Djibouti	East	3	94	6	14	13	1	24	90	3	1	4
Eritrea	East	2	45	55	101	46	56	24	90	11	50	61
Ethiopia	East	2	30	70	2,029	609	1,420	24	90	146	1,278	1,424
Kenya	East	3	66	34	953	629	324	24	90	151	292	442
Madagascar	East	2	41	59	504	207	297	24	90	50	268	317
Mauritius	East	1	96	4	9	9	0	24	90	2	0	3
Rwanda	East	2	54	46	236	128	109	24	90	31	98	128
Seychelles	East	1	96	4	1	1	0	24	90	0	0	0
Somali	East	3	13	87	348	45	303	24	90	11	272	283
South Sudan	East	3	48	52	256	123	133	24	90	30	120	149

[1] MS=Mortality Stratum; CFR=Case Fatality Ratio (obtained from the literature)

[2] The proportion of children under the age of five years with pneumonia in the preceding two weeks and who had access to health used as proxy obtained from United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

Appendix 9a: Number of deaths due to *S. aureus* meningitis by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Care seeking[2]	Not care-seeking	*S. aureus* meningitis cases	Care-seeking deaths	Not care-seeking death	Care-seeking CFR[1]	Not care-seeking CFR[1]	Care-seeking *S. aureus* meningitis deaths	Not care-seeking *S. aureus* meningitis deaths	Total *S. aureus* meningitis deaths
Sudan	East	3	48	52	798	383	415	24	90	92	373	465
Tanzania	East	2	55	45	1,282	705	577	24	90	169	519	689
Uganda	East	3	80	20	1,023	818	205	24	90	196	184	380
Algeria	North	2	66	34	635	419	216	24	90	101	194	295
Egypt	North	2	68	32	1,685	1,146	539	24	90	275	485	760
Libya	North	1	97	3	86	83	3	24	90	20	2	22
Mauritania	North	3	34	66	87	30	58	24	90	7	52	59
Morocco	North	2	22	78	471	104	367	24	90	25	330	355
Tunisia	North	1	60	40	139	84	56	24	90	20	50	70
Western Sahara	North	2	22	78	7	2	6	24	90	0	5	6
Angola	South	4	49	51	1,419	695	724	24	90	167	651	818
Botswana	South	2	14	86	70	10	61	24	90	2	54	57
Lesotho	South	3	63	37	38	24	14	24	90	6	13	18
Malawi	South	3	78	22	393	307	87	24	90	74	78	151
Mozambique	South	3	55	45	1,332	733	600	24	90	176	540	715
Namibia	South	2	72	28	92	66	26	24	90	16	23	39
South Africa	South	2	88	12	1,558	1,371	187	24	90	329	168	497
Swaziland	South	3	60	40	24	15	10	24	90	3	9	12
Zambia	South	3	70	30	374	262	112	24	90	63	101	164
Zimbabwe	South	2	51	49	689	351	338	24	90	84	304	388
Benin	West	3	23	77	237	54	182	24	90	13	164	177

[1] MS=Mortality Stratum; CFR=Case Fatality Ratio (obtained from the literature)

[2] The proportion of children under the age of five years with pneumonia in the preceding two weeks and who had access to health used as proxy obtained from United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

Appendix 9a: Number of deaths due to *S. aureus* meningitis by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Care seeking[2]	Not care-seeking	*S. aureus* meningitis cases	Care-seeking deaths	Not care-seeking death	Care-seeking CFR[1]	Not care-seeking CFR[1]	Care-seeking *S. aureus* meningitis deaths	Not care-seeking *S. aureus* meningitis deaths	Total *S. aureus* meningitis deaths
Burkina Faso	West	3	52	48	405	210	194	24	90	50	175	225
Cape Verde	West	1	96	4	7	7	0	24	90	2	0	2
Gambia	West	3	68	32	48	33	15	24	90	8	14	22
Ghana	West	3	56	44	548	307	241	24	90	74	217	291
Guinea	West	3	37	63	265	98	167	24	90	24	150	174
Guinea Bissau	West	4	34	66	39	13	26	24	90	3	23	26
Liberia	West	3	51	49	96	49	47	24	90	12	42	54
Mali	West	3	23	77	402	93	310	24	90	22	279	301
Niger	West	3	59	41	554	327	227	24	90	78	204	283
Nigeria	West	3	35	65	4,236	1,483	2,753	24	90	356	2,478	2,834
Senegal	West	2	48	52	339	163	177	24	90	39	159	198
Sierra Leone	West	3	72	28	155	111	43	24	90	27	39	66
Togo	West	3	49	51	149	73	76	24	90	17	68	86
All countries					**27,973**	**13,938**	**14,035**			**3,345**	**12,632**	**15,977**

[1] MS=Mortality Stratum; CFR=Case Fatality Ratio (obtained from the literature)

[2] The proportion of children under the age of five years with pneumonia in the preceding two weeks and who had access to health used as proxy obtained from United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

Appendix 9b: Number of cases of *S. aureus* meningitis by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[1]	All-cause meningitis Incidence[2]	All-cause meningitis cases	AF[1]	*S. aureus* meningitis cases
Burundi	Central	3	1,853,000	85.1	1576.903	0.07	117
Cameroon	Central	3	3,742,000	85.1	3184.442	0.07	236
Central African Republic	Central	4	728,000	85.1	619.528	0.07	46
Chad	Central	4	2,601,000	85.1	2213.451	0.07	164
Congo	Central	3	814,000	85.1	692.714	0.07	51
Democratic Republic of Congo	Central	3	14,099,000	85.1	11998.25	0.07	888
Equatorial Guinea	Central	3	176,000	85.1	149.776	0.07	11
Gabon	Central	2	267,000	85.1	227.217	0.04	9
Sao tome or Principe	Central	2	31,000	85.1	26.381	0.04	1
Comoros	East	3	117,000	85.1	99.567	0.07	7
Djibouti	East	3	101,000	85.1	85.951	0.07	6
Eritrea	East	2	744,000	85.1	633.144	0.04	25
Ethiopia	East	2	14,901,000	85.1	12680.75	0.04	507
Kenya	East	3	6,997,000	85.1	5954.447	0.07	441
Madagascar	East	2	3,700,000	85.1	3148.7	0.04	126
Mauritius	East	1	69,000	85.1	58.719	0.04	2
Rwanda	East	2	1,735,000	85.1	1476.485	0.04	59
Seychelles	East	1	8,000	85.1	6.808	0.04	0
Somali	East	3	2,554,000	85.1	2173.454	0.07	161
South Sudan	East	3	1,882,000	85.1	1601.582	0.07	119
Sudan	East	3	5,859,000	85.1	4986.009	0.07	369

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiology Fraction (obtained from the literature)

[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 9b: Number of cases of *S. aureus* meningitis by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[1]	All-cause meningitis Incidence[2]	All-cause meningitis cases	AF[1]	*S. aureus* meningitis cases
Tanzania	East	2	9,419,000	85.1	8015.569	0.04	321
Uganda	East	3	7,512,000	85.1	6392.712	0.07	473
Algeria	North	2	4,664,000	85.1	3969.064	0.04	159
Egypt	North	2	12,374,000	85.1	10530.27	0.04	421
Libya	North	1	630,000	85.1	536.13	0.04	21
Mauritania	North	3	640,000	85.1	544.64	0.07	40
Morocco	North	2	3,457,000	85.1	2941.907	0.04	118
Tunisia	North	1	1,024,000	85.1	871.424	0.04	35
Western Sahara	North	2	55,000	85.1	46.805	0.04	2
Angola	South	4	5,158,000	171.9	8866.602	0.07	656
Botswana	South	2	256,000	171.9	440.064	0.04	18
Lesotho	South	3	280,000	85.1	238.28	0.07	18
Malawi	South	3	2,888,000	85.1	2457.688	0.07	182
Mozambique	South	3	4,844,000	171.9	8326.836	0.07	616
Namibia	South	2	335,000	171.9	575.865	0.04	23
South Africa	South	2	5,664,000	171.9	9736.416	0.04	389
Swaziland	South	3	178,000	85.1	151.478	0.07	11
Zambia	South	3	2,750,000	85.1	2340.25	0.07	173
Zimbabwe	South	2	2,505,000	171.9	4306.095	0.04	172
Benin	West	3	1,739,000	85.1	1479.889	0.07	110
Burkina Faso	West	3	3,161,000	80	2528.8	0.07	187

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic fraction (obtained from the literature)

[2] Incidence rates of *S. aureus* disease are per 100,000 children

Appendix 9b: Number of cases of *S. aureus* meningitis by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population[1]	All-cause meningitis Incidence[2]	All-cause meningitis cases	AF[1]	*S. aureus* meningitis cases
Cape Verde	West	1	55,000	85.1	46.805	0.04	2
Cote d'Ivoire	West	3	3,765,000	85.1	3204.015	0.07	237
Gambia	West	3	352,000	85.1	299.552	0.07	22
Ghana	West	3	4,023,000	85.1	3423.573	0.07	253
Guinea	West	3	1,949,000	85.1	1658.599	0.07	123
Guinea Bissau	West	4	285,000	85.1	242.535	0.07	18
Liberia	West	3	705,000	85.1	599.955	0.07	44
Mali	West	3	3,274,000	76.8	2514.432	0.07	186
Niger	West	3	4,066,000	85.1	3460.166	0.07	256
Nigeria	West	3	31,109,000	85.1	26473.76	0.07	1,959
Senegal	West	2	2,493,000	85.1	2121.543	0.04	85
Sierra Leone	West	3	1,135,000	85.1	965.885	0.07	71
Togo	West	3	1,162,000	80	929.6	0.07	69
All countries			**186,884,000**	**5,183**	**174,831**		**10,816**

[1] MS=Mortality Stratum; Population=Population size; AF=Aetiologic Fraction (obtained from the literature)

[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 9c: Number of deaths due to *S. aureus* meningitis by country (hospital-based studies) (continued)

Country	Region	MS[1]	Care seeking[2]	Not care-seeking	*S. aureus* meningitis cases	Care-seeking deaths	Not care-seeking death	Care-seeking CFR[1]	Not care-seeking CFR[1]	Care-seeking *S. aureus* meningitis deaths	Not care-seeking *S. aureus* meningitis deaths	*S. aureus* meningitis deaths
Burundi	Central	3	63	37	117	74	43	27	90	20	39	59
Cameroon	Central	3	28	72	236	66	170	27	90	18	153	171
Central African Republic	Central	4	30	70	46	14	32	27	90	4	29	33
Chad	Central	4	26	74	164	43	121	27	90	11	109	121
Congo	Central	3	28	72	51	14	37	27	90	4	33	37
Democratic Republic of Congo	Central	3	42	58	888	373	515	27	90	101	463	564
Equatorial Guinea	Central	3	54	46	11	6	5	27	90	2	5	6
Gabon	Central	2	68	32	9	6	3	27	90	2	3	4
Sao tome or Principe	Central	2	69	31	1	1	0	27	90	0	0	0
Comoros	East	3	38	62	7	3	5	27	90	1	4	5
Djibouti	East	3	94	6	6	6	0	27	90	2	0	2
Eritrea	East	2	45	55	25	11	14	27	90	3	13	16
Ethiopia	East	2	30	70	507	152	355	27	90	41	320	361
Kenya	East	3	66	34	441	291	150	27	90	79	135	213
Madagascar	East	2	41	59	126	52	74	27	90	14	67	81
Mauritius	East	1	96	4	2	2	0	27	90	1	0	1
Rwanda	East	2	54	46	59	32	27	27	90	9	24	33
Seychelles	East	1	96	4	0	0	0	27	90	0	0	0
Somali	East	3	13	87	161	21	140	27	90	6	126	132
South Sudan	East	3	48	52	119	57	62	27	90	15	55	71

[1] MS=Mortality Stratum; CFR=Case Fatality Ratio (obtained from the literature)

[2] The proportion of children under the age of five years with pneumonia in the preceding two weeks and who had access to health used as proxy obtained from United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

Appendix 9c: Number of deaths due to *S. aureus* meningitis by country (hospital-based studies) (continued)

Country	Region	MS[1]	Care seeking[2]	Not care-seeking	*S. aureus* meningitis cases	Care-seeking deaths	Not care-seeking death	Care-seeking CFR[1]	Not care-seeking CFR[1]	Care-seeking *S. aureus* meningitis deaths	Not care-seeking *S. aureus* meningitis deaths	*S. aureus* meningitis deaths
Sudan	East	3	48	52	369	177	192	27	90	48	173	220
Tanzania	East	2	55	45	321	176	144	27	90	48	130	177
Uganda	East	3	80	20	473	378	95	27	90	102	85	187
Algeria	North	2	66	34	159	105	54	27	90	28	49	77
Egypt	North	2	68	32	421	286	135	27	90	77	121	199
Libya	North	1	97	3	21	21	1	27	90	6	1	6
Mauritania	North	3	34	66	40	14	27	27	90	4	24	28
Morocco	North	2	22	78	118	26	92	27	90	7	83	90
Tunisia	North	1	60	40	35	21	14	27	90	6	13	18
Western Sahara	North	2	22	78	2	0	1	27	90	0	1	1
Angola	South	4	49	51	656	322	335	27	90	87	301	388
Botswana	South	2	14	86	18	2	15	27	90	1	14	14
Lesotho	South	3	63	37	18	11	7	27	90	3	6	9
Malawi	South	3	78	22	182	142	40	27	90	38	36	74
Mozambique	South	3	55	45	616	339	277	27	90	92	250	341
Namibia	South	2	72	28	23	17	6	27	90	4	6	10
South Africa	South	2	88	12	389	343	47	27	90	93	42	135
Swaziland	South	3	60	40	11	7	4	27	90	2	4	6
Zambia	South	3	70	30	173	121	52	27	90	33	47	79
Zimbabwe	South	2	51	49	172	88	84	27	90	24	76	100
Benin	West	3	23	77	110	25	84	27	90	7	76	83

[1] MS=Mortality Stratum; CFR=Case Fatality Ratio (obtained from the literature)

[2] The proportion of children under the age of five years with pneumonia in the preceding two weeks and who had access to health used as proxy obtained from United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

Appendix 9c: Number of deaths due to *S. aureus* meningitis by country (hospital-based studies) (continued)

Country	Region	MS[1]	Care seeking[2]	Not care-seeking	*S. aureus* meningitis cases	Care-seeking deaths	Not care-seeking death	Care-seeking CFR[1]	Not care-seeking CFR[1]	Care-seeking *S. aureus* meningitis deaths	Not care-seeking *S. aureus* meningitis deaths	*S. aureus* meningitis deaths
Burkina Faso	West	3	52	48	187	97	90	27	90	26	81	107
Cape Verde	West	1	96	4	2	2	0	27	90	0	0	1
Cote d'Ivoire	West	3	38	62	237	90	147	27	90	24	132	157
Gambia	West	3	68	32	22	15	7	27	90	4	6	10
Ghana	West	3	56	44	253	142	111	27	90	38	100	139
Guinea	West	3	37	63	123	45	77	27	90	12	70	82
Guinea Bissau	West	4	34	66	18	6	12	27	90	2	11	12
Liberia	West	3	51	49	44	23	22	27	90	6	20	26
Mali	West	3	23	77	186	43	143	27	90	12	129	140
Niger	West	3	59	41	256	151	105	27	90	41	94	135
Nigeria	West	3	35	65	1,959	686	1,273	27	90	185	1,146	1,331
Senegal	West	2	48	52	85	41	44	27	90	11	40	51
Sierra Leone	West	3	72	28	71	51	20	27	90	14	18	32
Togo	West	3	49	51	69	34	35	27	90	9	32	41
All countries					**10,254**	**5,270**	**5,546**			**1,423**	**4,992**	**6,415**

[1] MS=Mortality Stratum; CFR=Case Fatality Ratio (obtained from the literature)

[2] The proportion of children under the age of five years with pneumonia in the preceding two weeks and who had access to health used as proxy obtained from United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

Appendix 10: Number of cases and deaths of *S. aureus* septicaemia by country in 2015 (population-based studies)

Country	Region	MS[1]	Population	Incidence Septicaemia[2]	SAB[1] cases	CFR[1]	*S. aureus* deaths
Burundi	Central	3	1,853,000	126.7	2,348	9	211
Cameroon	Central	3	3,742,000	126.7	4,741	9	427
Central African Republic	Central	4	728,000	126.7	922	9	83
Chad	Central	4	2,601,000	126.7	3,295	9	297
Congo	Central	3	814,000	126.7	1,031	9	93
Democratic Republic of Congo	Central	3	14,099,000	126.7	17,863	9	1,608
Equatorial Guinea	Central	3	176,000	126.7	223	9	20
Gabon	Central	2	267,000	126.7	338	9	30
Sao tome or Principe	Central	2	31,000	126.7	39	9	4
Comoros	East	3	117,000	126.7	148	9	13
Djibouti	East	3	101,000	126.7	128	9	12
Eritrea	East	2	744,000	126.7	943	9	85
Ethiopia	East	2	14,901,000	126.7	18,880	9	1,699
Kenya	East	3	6,997,000	44.8	3,135	9	282
Madagascar	East	2	3,700,000	126.7	4,688	9	422
Mauritius	East	1	69,000	126.7	87	9	8
Rwanda	East	2	1,735,000	126.7	2,198	9	198
Seychelles	East	1	8,000	126.7	10	9	1
Somali	East	3	2,554,000	126.7	3,236	9	291
South Sudan	East	3	1,882,000	126.7	2,384	9	215
Sudan	East	3	5,859,000	126.7	7,423	9	668
Tanzania	East	2	9,419,000	126.7	11,934	9	1,074
Uganda	East	3	7,512,000	126.7	9,518	9	857
Algeria	North	2	4,664,000	126.7	5,909	9	532
Egypt	North	2	12,374,000	126.7	15,678	9	1,411
Libya	North	1	630,000	126.7	798	9	72

[1] MS=Mortality stratum; CFR=Case fatality ratio (obtained from literature); SAB=*S. aureus* bacteraemia

[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 10: Number of cases and deaths of *S. aureus* septicaemia by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Population	Septicaemia Incidence[2]	SAB[1] cases	CFR[1]	*S. aureus* deaths
Mauritania	North	3	640,000	126.7	811	9	73
Morocco	North	2	3,457,000	126.7	4,380	9	394
Tunisia	North	1	1,024,000	126.7	1,297	9	117
Western Sahara	North	2	55,000	126.7	70	9	6
Angola	South	4	5,158,000	126.7	6,535	9	588
Botswana	South	2	256,000	126.7	324	9	29
Lesotho	South	3	280,000	126.7	355	9	32
Malawi	South	3	2,888,000	126.7	3,659	9	329
Mozambique	South	3	4,844,000	118.3	5,730	6	344
Namibia	South	2	335,000	126.7	424	9	38
South Africa	South	2	5,664,000	126.7	7,176	9	646
Swaziland	South	3	178,000	126.7	226	9	20
Zambia	South	3	2,750,000	126.7	3,484	9	314
Zimbabwe	South	2	2,505,000	126.7	3,174	9	286
Benin	West	3	1,739,000	126.7	2,203	9	198
Burkina Faso	West	3	3,161,000	126.7	4,005	9	360
Cape Verde	West	1	55,000	126.7	70	9	6
Cote d'Ivoire	West	3	3,765,000	126.7	4,770	9	429
Gambia	West	3	352,000	78	275	14	38
Ghana	West	3	4,023,000	630.1	25,349	9	2,281
Guinea	West	3	1,949,000	126.7	2,469	9	222
Guinea Bissau	West	4	285,000	126.7	361	9	32
Liberia	West	3	705,000	126.7	893	9	80
Mali	West	3	3,274,000	126.7	4,148	9	373
Niger	West	3	4,066,000	126.7	5,152	9	464
Nigeria	West	3	31,109,000	126.7	39,415	9	3,547
Senegal	West	2	2,493,000	126.7	3,159	9	284
Sierra Leone	West	3	1,135,000	126.7	1,438	9	129
Togo	West	3	1,162,000	126.7	1,472	9	133
Total			**186,884,000**		**250,725**		**22,407**

[1] MS=Mortality stratum; CFR=Case fatality ratio (obtained from literature); SAB=*S. aureus* bacteraemia
[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 10a: Number of cases and deaths of *S. aureus* septicaemia by country in 2015 (hospital-based studies)

Country	Region	MS[1]	Population	Incidence all-cause Septicaemia	All-cause septicaemia cases[2]	AF[1]	*S. aureus* cases	CFR[1]	*S. aureus* deaths
Burundi	Central	3	1,853,000	919.3	17034.629	47	8,006	9	721
Cameroon	Central	3	3,742,000	919.3	34400.206	47	16,168	9	1,455
Central African Republic	Central	4	728,000	919.3	6692.504	57	3,815	9	343
Chad	Central	4	2,601,000	919.3	23910.993	57	13,629	9	1,227
Congo	Central	3	814,000	919.3	7483.102	47	3,517	9	317
Democratic Republic of Congo	Central	3	14,099,000	919.3	129612.107	47	60,918	9	5,483
Equatorial Guinea	Central	3	176,000	919.3	1617.968	47	760	9	68
Gabon	Central	2	267,000	146	389.82	47	183	9	16
Sao tome or Principe	Central	2	31,000	146	45.26	47	21	9	2
Comoros	East	3	117,000	919.3	1075.581	47	506	9	45
Djibouti	East	3	101,000	919.3	928.493	47	436	9	39
Eritrea	East	2	744,000	146	1086.24	47	511	9	46
Ethiopia	East	2	14,901,000	146	21755.46	47	10,225	9	920
Kenya	East	3	6,997,000	764.4	53485.068	47	25,138	9	2,262
Madagascar	East	2	3,700,000	146	5402	47	2,539	9	229
Mauritius	East	1	69,000	146	100.74	47	47	9	4
Rwanda	East	2	1,735,000	146	2533.1	47	1,191	9	107
Seychelles	East	1	8,000	146	11.68	47	5	9	0
Somali	East	3	2,554,000	919.3	23478.922	47	11,035	9	993
South Sudan	East	3	1,882,000	919.3	17301.226	47	8,132	9	732
Sudan	East	3	5,859,000	919.3	53861.787	47	25,315	9	2,278
Tanzania	East	2	9,419,000	146	13751.74	47	6,463	9	582
Uganda	East	3	7,512,000	919.3	69057.816	42	29,004	9	2,610

[1] MS=Mortality Stratum; AF=Aetiologic fraction (obtained from literature); CFR=Case fatality ratio
[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 10a: Number of cases and deaths of *S. aureus* septicaemia by country in 2015 (population-based studies)

Country	Region	MS[1]	Population	Incidence all-cause Septicaemia[2]	All-cause septicaemia cases	AF[1]	*S. aureus* cases	CFR[1]	*S. aureus* deaths
Algeria	North	2	4,664,000	146	6809	47	3,200	9	288
Egypt	North	2	12,374,000	146	18066	47	8,491	9	764
Libya	North	1	630,000	146	920	47	432	9	39
Mauritania	North	3	640,000	919.3	5884	47	2,765	9	249
Morocco	North	2	3,457,000	146	5047	47	2,372	9	213
Tunisia	North	1	1,024,000	146	1495	47	703	9	63
Western Sahara	North	2	55,000	146	80	47	38	9	3
Angola	South	4	5,158,000	919.3	47418	57	27,028	9	2,433
Botswana	South	2	256,000	146	374	47	176	9	16
Lesotho	South	3	280,000	919.3	2574	47	1,210	9	109
Malawi	South	3	2,888,000	919.3	26549	47	12,478	9	1,123
Mozambique	South	3	4,844,000	782	37880	47	17,804	6	1,068
Namibia	South	2	335,000	146	489	47	230	9	21
South Africa	South	2	5,664,000	146	8269	47	3,887	9	350
Swaziland	South	3	178,000	919.3	1636	47	769	9	69
Zambia	South	3	2,750,000	919.3	25281	47	11,882	9	1,069
Zimbabwe	South	2	2,505,000	146	3657	47	1,719	9	155
Benin	West	3	1,739,000	919.3	15987	47	7,514	9	676
Burkina Faso	West	3	3,161,000	919.3	29059	47	13,658	9	1,229
Cape Verde	West	1	55,000	146	80	47	38	9	3
Cote d'Ivoire	West	3	3,765,000	919.3	34612	47	16,267	9	1,464

[1] MS=Mortality Stratum; AF=Aetiologic fraction (obtained from the literature); CFR=Case fatality ratio (obtained from the literature)
[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 10a: Number of cases and deaths of *S. aureus* septicaemia by country in 2015 (population-based studies)

Country	Region	MS[1]	Population	Incidence all-cause Septicaemia[2]	All-cause septicaemia cases	AF[1]	*S. aureus* cases	CFR[1]	*S. aureus* deaths
Gambia	West	3	352,000	634.9	2,235	47	1,050	14	147
Ghana	West	3	4,023,000	4686	188,518	47	88,603	9	7,974
Guinea	West	3	1,949,000	919.3	17,917	47	8,421	9	758
Guinea Bissau	West	4	285,000	919.3	2,620	57	1,493	9	134
Liberia	West	3	705,000	919.3	6,481	47	3,046	9	274
Mali	West	3	3,274,000	919.3	30,098	47	18	9	2
Niger	West	3	4,066,000	919.3	37,379	47	17,568	9	1,581
Nigeria	West	3	31,109,000	919.3	285,985	48	137,273	9	12,355
Senegal	West	2	2,493,000	146	3,640	47	1,711	9	154
Sierra Leone	West	3	1,135,000	919.3	10,434	47	4,904	9	441
Togo	West	3	1,162,000	919.3	10,682	47	5,021	9	452
All countries			**186,884,000**		**1,353,172**		**629,334**		**56,158**

[1] MS=Mortality Stratum; AF=Aetiologic fraction (obtained from the literature); CFR=Case fatality ratio (obtained from the literature)
[2] Incidence rates of *S. aureus* disease are per 100,000 children (obtained from the literature)

Appendix 11: Number of cases and deaths of neonatal *S. aureus* septicaemia by country in 2015 (population-based studies)

Country	Region	MS[1]	Live births[2]	*S. aureus* septicaemia Incidence[3]	*S. aureus* NNS[1] cases	CFR[1]	*S. aureus* NNS[1] deaths
Burundi	Central	3	847,000	1.26	1067.22	21	224
Cameroon	Central	3	167,000	1.26	210	21	44
Central African Republic	Central	4	164,000	1.26	207	21	43
Chad	Central	4	630,000	1.26	794	21	167
Congo	Central	3	3,217,000	1.26	4,053	21	851
Democratic Republic of Congo	Central	3	29,000	1.26	37	21	8
Equatorial Guinea	Central	3	266,820	1.26	336	21	71
Gabon	Central	2	51,000	1.26	64	33.3	21
Sao tome or Principe	Central	2	6,000	1.26	8	33.3	3
Comoros	East	3	26,000	1.26	33	21	7
Djibouti	East	3	22,000	1.26	28	21	6
Eritrea	East	2	175,000	1.26	221	33.3	73
Ethiopia	East	2	3,176,000	1.26	4,002	33.3	1,333
Kenya	East	3	1,571,000	0.44	691	21	145
Madagascar	East	2	831,000	1.26	1,047	33.3	349
Mauritius	East	1	14,000	1.26	18	33.3	6
Rwanda	East	2	363,000	1.26	457	33.3	152
Seychelles	East	1	2,000	1.26	3	33.3	1
Somali	East	3	471,000	1.26	593	21	125
South Sudan	East	3	446,000	1.26	562	21	118
Sudan	East	3	1,319,000	1.26	1,662	21	349

[1] MS=Mortality stratum; NN=Neonatal; NNS=Neonatal septicaemia; CFR=Case fatality ratio (obtained from the literature)

[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

[3] Incidence rates of *S. aureus* disease are per 1,000 live births (obtained from the literature)

Appendix 11: Number of cases and deaths of neonatal *S. aureus* septicaemia by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Live births[2]	*S. aureus* septicaemia Incidence[3]	*S. aureus* NNS[1]cases	CFR[1]	*S. aureus* NNS[1] deaths
Tanzania	East	2	2,064,000	1.26	2,601	33.3	866
Uganda	East	3	1,665,000	1.26	2,098	21	441
Algeria	North	2	936,000	1.26	1,179	33.3	393
Egypt	North	2	2,488,000	1.26	3,135	33.3	1,044
Libya	North	1	129,000	1.26	163	33.3	54
Mauritania	North	3	134,000	1.26	169	21	35
Morocco	North	2	699,000	1.26	881	33.3	293
Tunisia	North	1	202,000	1.26	255	33.3	85
Western Sahara	North	2	478,898	1.26	603	33.3	201
Angola	South	4	1,128,000	1.26	1,421	21	298
Botswana	South	2	55,000	1.26	69	33.3	23
Lesotho	South	3	61,000	1.26	77	21	16
Malawi	South	3	665,000	1.26	838	21	176
Mozambique	South	3	1,087,000	1.26	1,370	21	288
Namibia	South	2	72,000	1.26	91	33.3	30
South Africa	South	2	1,111,000	1.26	1,400	33.3	466
Swaziland	South	3	38,000	1.26	48	21	10
Zambia	South	3	645,000	1.26	813	21	171
Zimbabwe	South	2	539,000	1.26	679	33.3	226
Benin	West	3	388,000	1.26	489	21	103
Burkina Faso	West	3	717,000	1.26	903	21	190
Cape Verde	West	1	11,000	1.26	14	33.3	5

[1] MS=Mortality stratum; NN=Neonatal; NNS=Neonatal septicaemia; CFR=Case fatality ratio (obtained from the literature)

[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

[3] Incidence rates of *S. aureus* disease are per 1,000 live births (obtained from the literature)

Appendix 11: Number of cases and deaths of neonatal *S. aureus* septicaemia by country in 2015 (population-based studies) (continued)

Country	Region	MS[1]	Live births[2]	*S. aureus* septicaemia Incidence[3]	*S. aureus* NNS[1] cases	CFR[1]	*S. aureus* NNS[1] deaths
Cote d'Ivoire	West	3	838,000	1.26	1,056	21	222
Gambia	West	3	83,000	3.5	291	18	52
Ghana	West	3	884,000	1.26	1,114	21	234
Guinea	West	3	460,000	1.26	580	21	122
Guinea Bissau	West	4	68,000	1.26	86	21	18
Liberia	West	3	156,000	1.26	197	21	41
Mali	West	3	758,000	1.26	955	21	201
Niger	West	3	983,000	1.26	1,239	21	260
Nigeria	West	3	7,133,000	1.26	8,988	23	2,067
Senegal	West	2	567,000	1.26	714	33.3	238
Sierra Leone	West	3	229,000	1.26	289	21	61
Togo	West	3	256,000	1.26	323	21	68
All countries			**41,521,718**		**51,215**		**13,091**

[1] MS=Mortality stratum; NN=Neonatal; NNS=Neonatal septicaemia; CFR=Case fatality ratio (obtained from the literature)

[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

[3] Incidence rates of *S. aureus* disease are per 1,000 live births (obtained from the literature)

Appendix 11a: Number of cases and deaths of neonatal *S. aureus* septicaemia by country in 2015 (hospital-based studies) (continued)

Country	Region	MS [1]	Live births[2]	All-cause NN[1] septicaemia Incidence[3]	All-cause NNS[1] cases	NN AF[1]	NN *S. aureus* cases	CFR[1]	NN *S. aureus* deaths
Burundi	Central	3	266,820	7.1	1,894	45	852	21	179
Cameroon	Central	3	847,000	7.1	6,014	45	2,706	21	568
Central African Republic	Central	4	164,000	7.1	1,164	45	524	21	110
Chad	Central	4	630,000	7.1	4,473	45	2,013	21	423
Congo	Central	3	167,000	7.1	1,186	45	534	21	112
Democratic Republic of Congo	Central	3	3,217,000	7.1	22,841	45	10,278	21	2,158
Equatorial Guinea	Central	3	29,000	7.1	206	45	93	21	19
Gabon	Central	2	51,000	7.1	362	22	80	33.3	27
Sao tome or Principe	Central	2	6,000	7.1	43	22	9	33.3	3
Comoros	East	3	26,000	7.1	185	45	83	21	17
Djibouti	East	3	22,000	7.1	156	45	70	21	15
Eritrea	East	2	175,000	7.1	1,243	22	273	33.3	91
Ethiopia	East	2	3,176,000	7.1	22,550	22	4,961	33.3	1,652
Kenya	East	3	1,571,000	5.46	8,578	45	3,860	21	811
Madagascar	East	2	831,000	7.1	5,900	22	1,298	33.3	432
Mauritius	East	1	14,000	7.1	99	22	22	33.3	7
Rwanda	East	2	363,000	7.1	2,577	22	567	33.3	189
Seychelles	East	1	2,000	7.1	14	22	3	33.3	1
Somali	East	3	471,000	7.1	3,344	45	1,505	21	316

[1] MS=Mortality stratum; NN=Neonatal; NNS=Neonatal septicaemia; NN AF=Neonatal aetiological fraction (obtained from the literature); CFR=Case fatality ratio (obtained from the literature)

[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

[3] Incidence rates of *S. aureus* disease are per 1,000 live births (obtained from the literature)

Appendix 11a: Number of cases and deaths of neonatal *S. aureus* septicaemia by country in 2015 (hospital-based studies) (continued)

Country	Region	MS [1]	Live births[2]	All-cause NN[1] septicaemia Incidence[3]	All-cause NNS[1] cases	NN AF[1]	NN *S. aureus* cases	CFR[1]	NN *S. aureus* deaths
South Sudan	East	3	446,000	7.1	3,167	45	1,425	21	299
Sudan	East	3	1,319,000	7.1	9,365	45	4,214	21	885
Tanzania	East	2	2,064,000	7.1	14,654	22	3,224	33.3	1,074
Uganda	East	3	1,665,000	7.1	118,212	52	6,147	21	1,291
Algeria	North	2	936,000	7.1	6,646	22	1,462	33.3	487
Egypt	North	2	2,488,000	7.1	17,665	22	3,886	33.3	1,294
Libya	North	1	129,000	7.1	916	22	201	33.3	67
Mauritania	North	3	134,000	7.1	951	45	428	21	90
Morocco	North	2	699,000	7.1	4,963	22	1,092	33.3	364
Tunisia	North	1	202,000	7.1	1,434	22	316	33.3	105
Western Sahara	North	2	478,898	7.1	3,400	22	748	33.3	249
Angola	South	4	1,128,000	7.1	8,009	45	3,604	21	757
Botswana	South	2	55,000	7.1	391	22	86	33.3	29
Lesotho	South	3	61,000	7.1	433	45	195	21	41
Malawi	South	3	665,000	7.1	4,722	45	2,125	21	446
Mozambique	South	3	1,087,000	7.1	7,718	45	3,473	21	729
Namibia	South	2	72,000	7.1	511	22	112	33.3	37
South Africa	South	2	1,111,000	7.1	7,888	22	1,735	33.3	578
Swaziland	South	3	38,000	7.1	270	45	121	21	25
Zambia	South	3	645,000	7.1	4,580	45	2,061	21	433
Zimbabwe	South	2	539,000	7.1	3,827	22	842	33.3	280
Benin	West	3	388,000	7.1	2,755	45	1,240	21	260

[1] MS=Mortality stratum; NN=Neonatal; NNS=Neonatal septicaemia; NN AF=Neonatal aetiological fraction (obtained from the literature); CFR=Case fatality ratio (obtained from the literature)

[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

[3] Incidence rates of *S. aureus* disease are per 1,000 live births (obtained from the literature)

Appendix 11a: Number of cases and deaths of neonatal *S. aureus* septicaemia by country in 2015 (hospital-based studies) (continued)

Country	Region	MS[1]	Live births[2]	All-cause NN[1] septicaemia Incidence[3]	All-cause NNS[1] cases	NN AF[1]	NN *S. aureus* cases	CFR[1]	NN *S. aureus* deaths
Burkina Faso	West	3	717,000	7.1	5,091	45	2,291	21	481
Cape Verde	West	1	11,000	7.1	78	22	17	33.3	6
Cote d'Ivoire	West	3	838,000	7.1	5,950	45	2,677	21	562
Gambia	West	3	83,000	9	747	45	336	18	61
Ghana	West	3	884,000	7.1	6,276	45	2,824	21	593
Guinea	West	3	460,000	7.1	3,266	45	1,470	21	309
Guinea Bissau	West	4	68,000	7.1	483	45	217	21	46
Liberia	West	3	156,000	7.1	1,108	45	498	21	105
Mali	West	3	758,000	7.1	5,382	45	2,422	21	509
Niger	West	3	983,000	7.1	6,979	45	3,141	21	660
Nigeria	West	3	7,133,000	7.1	50,644	40	20,258	23	4,659
Senegal	West	2	567,000	7.1	4,026	22	886	33.3	295
Sierra Leone	West	3	229,000	7.1	1,626	45	732	21	154
Togo	West	3	256,000	7.1	1,818	45	818	21	172
All countries			**41,521,718**		**292,385**		**107,056**		**25,561**

[1] MS=Mortality stratum; NN=Neonatal; NNS=Neonatal septicaemia; NN AF=Neonatal aetiological fraction (obtained from the literature); CFR=Case fatality ratio (obtained from the literature)

[2] United Nations Children's Fund (UNICEF). The State of the World's Children New York, USA: Division of Communication, UNICEF, 2017.

[3] Incidence rates of *S. aureus* disease are per 1,000 live births (obtained from the literature)

Appendix 12

Staphylococcus aureus Bacteremia in Children of Rural Areas of The Gambia, 2008–2015

Aderonke Odutola, Christian Bottomley, Syed A. Zaman, Jodi Lindsay, Muhammed Shah, Ilias Hossain, Malick Ndiaye, Chidebere D.I. Osuorah, Yekini Olatunji, Henry Badji, Usman N.A. Ikumapayi, Ahmad Manjang, Rasheed Salaudeen, Lamin Ceesay, Momodou Jasseh, Richard A. Adegbola, Tumani Corrah, Philip C. Hill, Brian M. Greenwood, Grant A. Mackenzie

Staphylococcus aureus bacteremia is a substantial cause of childhood disease and death, but few studies have described its epidemiology in developing countries. Using a population-based surveillance system for pneumonia, sepsis, and meningitis, we estimated *S. aureus* bacteremia incidence and the case-fatality ratio in children <5 years of age in 2 regions in the eastern part of The Gambia during 2008–2015. Among 33,060 children with suspected pneumonia, sepsis, or meningitis, we performed blood culture for 27,851; of 1,130 patients with bacteremia, 198 (17.5%) were positive for *S. aureus*. *S. aureus* bacteremia incidence was 78 (95% CI 67–91) cases/100,000 person-years in children <5 years of age and 2,080 (95% CI 1,621–2,627) cases/100,000 person-years in neonates. Incidence did not change after introduction of the pneumococcal conjugate vaccine. The case-fatality ratio was 14.1% (95% CI 9.6%–19.8%). Interventions are needed to reduce the *S. aureus* bacteremia burden in The Gambia, particularly among neonates.

In 2016, invasive bacterial diseases accounted for one quarter of the 5.6 million childhood deaths worldwide (*1*). Most invasive bacterial diseases occur in sub-Saharan Africa and other low- and middle-income countries (*2*). Deaths caused by these diseases outnumber those caused by malaria among children <5 years of age (*3*). The main bacteria implicated in invasive bacterial diseases has been *Streptococcus pneumoniae* and *Haemophilus influenzae* (*4*). However, after the widespread use of conjugate vaccines against *H. influenzae* type b (Hib) and *S. pneumoniae*, Hib disease has decreased considerably (*5*), and vaccine-serotype pneumococcal disease is declining (*6*). The decreased disease incidence associated with these pathogens has led to *Staphylococcus aureus* becoming a relatively more common cause of invasive bacterial disease (*7*). The clinical spectrum of *S. aureus* disease ranges from life-threatening invasive diseases, such as septicemia, pneumonia, osteomyelitis, endocarditis, meningitis, and brain abscess, to less severe skin and soft tissue infections. *S. aureus* bacteremia is often used as a marker of invasive *S. aureus* disease (*8*).

In high-income countries, *S. aureus* bacteremia is the second most common cause of neonatal sepsis, after group B *Streptococcus* (*9*). From the 1970s through the 2000s, the incidence of *S. aureus* bacteremia among children <16 years of age increased in several countries (*10*), probably because of the increased use of central venous catheters and other factors (*10*). In the 2010s, the incidence of *S. aureus* bacteremia remained stable (*11*) or decreased (*10*).

In Africa, *S. aureus* bacteremia is a common cause of invasive bacterial disease in children. Before the introduction of the Hib vaccine and pneumococcal conjugate vaccine (PCV), population-based studies in Kenya and Mozambique showed that *S. aureus* was the most common gram-positive pathogen among neonates with sepsis (*4*,*12*). Also, hospital-based studies showed *S. aureus* to be the most common cause of invasive bacterial disease in children <3 months of age in The Gambia (*13*) and one of the main causes of invasive bacterial disease in children <5 years of age in Nigeria (*14*).

Few population-based studies have been conducted in sub-Saharan Africa on the incidence of *S. aureus* bacteremia. In South Africa, a population-based study of children <13 years of age in an area with a high HIV prevalence

Author affiliations: London School of Hygiene and Tropical Medicine, London, UK (A. Odutola, C. Bottomley, S.A. Zaman, B.M. Greenwood, G.A. Mackenzie); Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine, Banjul, The Gambia (A. Odutola, S.A. Zaman, M. Shah, I. Hossain, M. Ndiaye, C.D.I. Osuorah, Y. Olatunji, H. Badji, U.N.A. Ikumapayi, A. Manjang, R. Salaudeen, M. Jasseh, R.A. Adegbola, T. Corrah, G.A. Mackenzie); St. George's University of London, London (J. Lindsay); King Fahad Medical City, Riyadh, Saudi Arabia (A. Manjang); Ministry of Health and Social Welfare, Banjul (L. Ceesay); University of Otago, Dunedin, New Zealand (P.C. Hill); Murdoch Children's Research Institute, Parkville, Victoria, Australia (G.A. Mackenzie)

DOI: https://doi.org/10.3201/eid2504.180935

indicated an incidence of 26 cases/100,000 person-years (*15*). A study in Kenya involving children admitted to a secondary healthcare facility showed an incidence of 27 cases/100,000 person-years in children <5 years of age; the highest incidence was in infants (89 cases/100,000 person-years) (*4*). However, variation in the age groups studied and methods used preclude direct comparison of these studies (*4*,*12*,*13*,*15*). After introduction of the Hib vaccine and before the introduction of PCV, a hospital-based study in The Gambia reported that *S. aureus* was the most common cause of bacteremia (*16*).

Given the paucity of population-based data on the epidemiology of *S. aureus* bacteremia in sub-Saharan Africa, we studied the incidence, clinical characteristics, case-fatality rate, and risk factors for *S. aureus* bacteremia in young children in a rural region of The Gambia. We also explored the association of *S. aureus* bacteremia with the introduction of PCV.

Methods

Study Site and Population

Surveillance for septicemia, pneumonia, and meningitis was performed among children ≥2 months of age residing in Basse in the Upper River Region of The Gambia through the Basse Health and Demographic Surveillance System (BHDSS) (Figure 1). We established the BHDSS in 2007, and the population in this surveillance area (≈179,000 persons in 2015, 19% <5 years of age) is enumerated every 4 months. The BHDSS is served by 5 satellite clinics and the Basse Health Centre (Basse, The Gambia), a primary and secondary healthcare facility with 25 beds to care for children. During 2011–2015, surveillance was extended to include all residents <5 years of age, and a similar surveillance was set up in the adjacent district of Fuladu West for all residents <5 years of age during a similar time range (2012–2014) through the Fuladu West Health and Demographic Surveillance System (FWHDSS; Figure 1). The population in Fuladu West is enumerated annually (population 92,464 in 2014, 18% <5 years of age). The FWHDSS is served by Bansang Hospital (Bansang, The Gambia) and 2 satellite clinics. Every resident in the areas surveilled by the BHDSS and FWHDSS was assigned a unique identifier.

The conjugate vaccine for Hib was introduced into the Gambian National Programme on Immunization in 1997, and the vaccine for pneumococcus was introduced in 2009. The 7-valent PCV (PCV7) was replaced by the 13-valent vaccine (PCV13) in 2011. In 2012, vaccine coverage for the third dose of the diphtheria-pertussis-tetanus vaccine in these regions surveilled was 81.7% (*17*). In The Gambia, transmission of *Plasmodium falciparum* is largely restricted to the short rainy season during July–November (*18*).

Surveillance Procedures

During May 12, 2008–December 31, 2015, nurses screened all children 2–59 months of age who arrived at a health center participating in the surveillance and who had a unique identifier for septicemia, pneumonia, and meningitis, according to standardized criteria (also referred to as referral surveillance) (*19*). Children who were admitted and children who were treated as outpatients were screened. Children who screened positive were referred to clinicians who used standardized criteria for assessment and investigation (*19*). Data collected included age, sex, anthropometric measurements, signs and symptoms, and suspected diagnosis. Blood was collected for culturing, and depending on clinical presentation, cerebrospinal fluid, lung aspirate, or pleural fluid samples were have also been collected for conventional microbiological

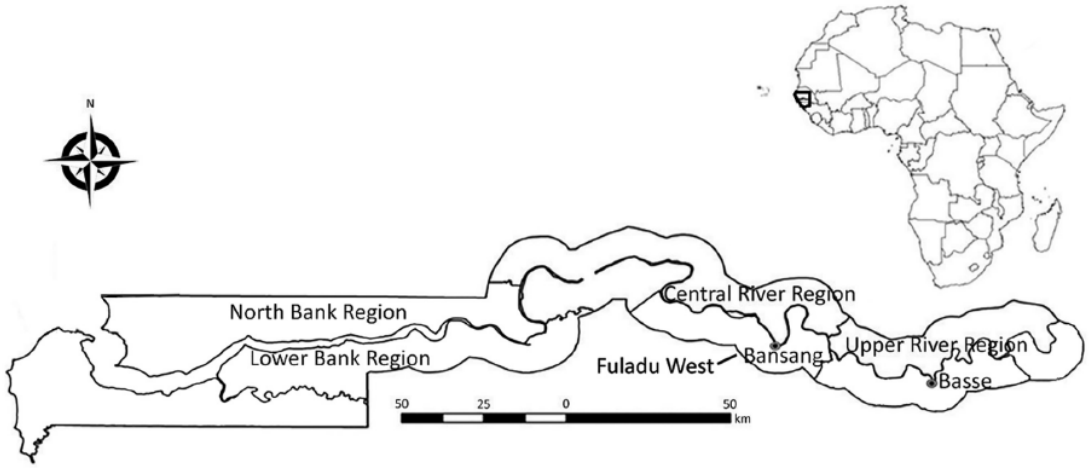


Figure 1. Regions surveilled for *Staphylococcus aureus* bacteremia among children <5 years of age through the Basse and Fuladu West Health and Demographic Surveillance Systems, The Gambia, 2008–2015. Inset indicates location of The Gambia in Africa.

tests (*6*). Rapid diagnostic tests for malaria (ICT Malaria *P.f.* Antigen; ICT Diagnostics, http://www.ictdiagnostics.co.za) were routinely performed during the rainy season and at other times at the discretion of the clinician.

During March 1, 2011–December 31, 2015, surveillance was expanded in the BHDSS to include all children 0–59 months of age who were admitted with an acute medical problem from whom a blood sample was collected for culture (also referred to as admission surveillance). During September 12, 2011–December 31, 2014, a similar admission surveillance was conducted for children 0–59 months of age admitted with an acute medical problem using the FWHDSS. All *S. aureus* bacteremia cases were linked to the Health and Demographic Surveillance System databases by using the unique identifier.

Laboratory Methods

We collected 1–3-mL blood samples from all patients with suspected septicemia, pneumonia, or meningitis; inoculated blood samples into BACTEC bottles (Becton Dickinson, https://www.bd.com); and incubated them in an automated BACTEC 9050 Blood Culture System (Becton Dickinson) for a maximum of 5 days. We subcultured positive cultures on blood agar plates and confirmed isolates as *S. aureus* by using catalase and coagulase tests. We classified cultures that grew *Bacillus* spp., *Corynebacterium* spp., and coagulase-negative *Staphylococcus* as contaminated. We used standard methods to investigate other body fluid samples collected for microbiological tests (*20*). We used disc diffusion methods to determine antimicrobial drug susceptibility according to the Clinical and Laboratory Standards Institute guidelines (*21*). We categorized all *S. aureus* isolates resistant to cefoxitin as methicillin-resistant.

We defined *S. aureus* bacteremia cases as clinically suspected cases of septicemia, pneumonia, meningitis, osteomyelitis, septic arthritis, pyomyositis, or abscess identified by using standardized criteria (*19*) in patients from whom *S. aureus* was isolated from their blood.

Statistical Methods

We used referral and admission surveillance data for statistical analyses. The unique identifier assigned to every patient enabled us to avoid duplication of data in our data set. We used the referral surveillance data to investigate trends in incidence because these data covered a longer period (2008–2015) than the admissions surveillance data (2011–2015). We used both the admission and referral surveillance data to estimate age-specific incidence and the case-fatality ratio (CFR).

We obtained incidence estimates by dividing the number of *S. aureus* bacteremia cases by the number of person-years at risk using the estimated midyear population. To account for the shorter period of observation in 2008 (May 12–December 31), we calculated person-years at risk as the midyear population multiplied by 234/365. We calculated incidence in neonates using 2 methods, first as cases per 1,000 live births and second as cases per 100,000 person-years. We defined the neonatal period as the time from birth to 28 days of age.

With the referral surveillance data, we assessed trends in incidence over time and variation in incidence before (pre-PCV period, May 12, 2008–May 11, 2010) and after (PCV13 period, January 1, 2013–December 31, 2015) the introduction of PCV7 using Poisson regression with robust error variance to allow for overdispersion. To account for the increased rate of eligible patients requiring blood culture over time, we adjusted the number of *S. aureus* bacteremia cases of each age group and year by multiplying by the ratio of the annual rate of eligible children enrolled over the mean rate of eligible children enrolled during the entire study period (*6*). For the denominators of the pre-PCV and PCV13 periods, we used the average of the corresponding midyear populations indicated by the BHDSS.

We defined CFR as the number of patients with *S. aureus* bacteremia who died before discharge divided by the total number of patients with *S. aureus* bacteremia. We identified potential risk factors for death before discharge using logistic regression, although surveillance was not designed to assess risk factors. We generated weight-for-age and weight-for-height z-scores using the 2006 World Health Organization child growth standards (https://www.who.int/childgrowth/standards/technical_report). We considered children with weight-for-age z-scores <3 SDs from the median weight-for-age as severely underweight and weight-for-height z-scores <3 SDs from the median weight-for-height as severely stunted. We performed analyses using Stata 14.0 (https://www.stata.com/stata14) and considered p values <0.05 as the criterion for statistical significance.

Ethics Statement

Ethics approval for this study was granted by The Gambia Government/Medical Research Council Joint Ethics Committee and the London School of Hygiene and Tropical Medicine Ethics Committee. We obtained written informed consent from the parents or guardians of all patients.

Results

In total, 33,060 children met the criteria for investigation, and 27,851 (84.2%) blood samples were collected and cultured (Figure 2). Contaminants grew in the cultures of 2,539 (9.1%) blood samples; these samples were excluded from analysis because contamination can mask an *S. aureus* bacteremia diagnosis.

Bacteremia

Bacteremia was identified in 1,130 children 0–59 months of age (Table 1). *S. aureus* was isolated in 198 (17.5%)

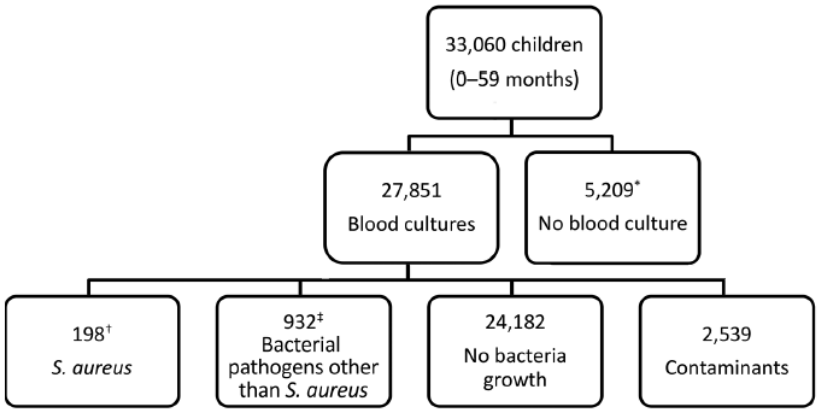


Figure 2. Flowchart of participants included and excluded in study of *Staphylococcus aureus* bacteremia incidence in children <5 years of age, The Gambia, 2008–2015. Participants were identified through the Basse and Fuladu West Health and Demographic Surveillance Systems. In total, 521 cases were identified through referral surveillance and 418 through admission surveillance. *Reasons for not having blood culture done included unsuccessful venipuncture (n = 487), declined consent for venipuncture (n = 416), declined consent to join study (n = 249), and unknown (n = 4,057). †In total, 76 children were identified through referral surveillance and 122 through admission surveillance. ‡Seven patients had polymicrobial bacteremia (*S. aureus* and a second bacterial pathogen).

children with bacteremia (76 identified through referral surveillance and 122 admission surveillance) and was the most common cause of bacteremia in neonates (46.4%, 84/181). Pathogens other than *S. aureus* were isolated from 932 children: *S. pneumoniae* (35.0%, n = 326), *Salmonella* spp. (15.1%, n = 141), and *Escherichia* spp. (10.7%, n = 100). In 7 children with bacteremia, *S. aureus* and a second bacterial pathogen were isolated.

Patient Characteristics

Using the combined admission and referral surveillance data, we found that 18.2% (4,541/24,885) of all patients were severely underweight and 10.9% (2,658/24,405) were severely stunted; 18.3% (4,183/22,902) of patients were admitted in the 2 weeks before disease onset. Antimicrobial drug use in the week before onset of signs and symptoms was uncommon. Most patients had fever (≥37.5°C) and tachypnea (Table 1).

Among patients with *S. aureus* bacteremia, a diagnosis of suspected septicemia was made in 56.2%, suspected pneumonia in 28.4%, and suspected meningitis in 6.7%. The median duration of hospital stay was 5 (interquartile range 2–6) days (Table 1).

Cough and difficult breathing were experienced more often by patients without bacteremia or with bacteremia caused by other pathogens than by patients with *S. aureus* bacteremia (Table 1). *S. aureus* bacteremia patients were more likely to have a diagnosis of suspected septicemia or other focal sepsis and less likely to have a diagnosis of suspected pneumonia than patients without bacteremia or with bacteremia caused by other pathogens (Table 1).

Incidence and Risk Factors for *S. aureus* Bacteremia

Using the combined referral and admission surveillance data (2011–2015 in BDHSS and 2012–2014 in FWDHSS), we found the incidence of *S. aureus* bacteremia to be 78 (95% CI 67–91) cases/100,000 person-years in children 0–59 months of age. The incidence was highest among neonates (2,080 [95% CI 1,621–2,627] cases/100,000 person-years, 3.5 [95% CI 2.9–4.7] cases/1,000 live births) and decreased in older age groups (Table 2). Incidence of *S. aureus* bacteremia in the 1–11-month age group was 133 (95% CI 99–174) cases/100,000 person-years, and incidence in the 1–4-year age group was 27 (95% CI 20–36) cases/100,000 person-years. Among the 84 *S. aureus* bacteremia cases in neonates, 13 (15.5%) presented in the first week of life and 35 (41.7%) in the second. The incidence of *S. aureus* bacteremia was higher in the wet season than in the dry season (Table 2).

Trends in Incidence of *S. aureus* Bacteremia

Using referral surveillance data (2008–2015 in BDHSS), we found the mean annual incidence of *S. aureus* bacteremia in children 2–59 months of age to be 22.3 (95% CI 16.7–29.2) cases/100,000 person-years. The incidence did not change over this period (p value for trend = 0.28), although PCV vaccination coverage increased during this period (Figure 3).

Using the referral surveillance data, we observed that 9 cases (10 cases after enrollment rate adjustment) of *S. aureus* bacteremia occurred in the pre-PCV period and 26 cases (23 cases after enrollment rate adjustment) in the PCV13 period. The crude *S. aureus* bacteremia incidence was 16 cases/100,000 person-years in the pre-PCV period and 26 cases/100,000 person-years in the PCV13 period (incidence rate ratio 1.6, 95% CI 0.8–3.5; p = 0.19). With the increasing size of the population and after adjusting for increased enrollment of eligible children over time, no significant increase in *S. aureus* bacteremia incidence

Table 1. Characteristics of patients <5 years of age with suspected pneumonia, septicemia, or meningitis with or without *Staphylococcus aureus* bacteremia identified through 2 surveillance systems, The Gambia, 2008–2015*

Patient characteristic	Patients with *S. aureus* bacteremia, n = 198	Patients with bacteremia caused by other pathogen, n = 932	Patients without bacteremia, n = 24,182
Age, mo			
<1	84/198 (42.4)	97/932 (10.4)	1,911/24,177 (7.9)
1–11	61/198 (30.8)	310/932 (33.3)	8,675/24,177 (35.9)
12–23	33/198 (16.7)	265/932 (28.4)	7,505/24,177 (31.0)
24–59	20/198 (10.1)	260/932 (27.9)	6,086/24,177 (25.2)
Sex			
M	97/198 (49.0)	532/932 (57.1)	13,740/24,177 (56.8)
F	101/198 (51.0)	400/932 (42.9)	10,437/24,177 (43.2)
Severely stunted†	20/109 (18.3)	216/884 (24.4)	3,425/21,736 (15.8)
Mid-upper arm circumference <11 cm	81/198 (40.9)	184/932 (19.7)	3,080/24,182 (12.7)
Admitted in previous 2 weeks	31/162 (19.1)	157/843 (18.6)	3,995/21,897 (18.2)
Hospital stay, d, median (IQR)	5 (2–6)	4 (3–6)	3 (2–4)
Disease onset during wet season‡	97/198 (49.0)	335/932 (35.9)	10,335/24,171 (42.8)
Died	28/198 (14.1)	161/932 (17.3)	860/24,182 (3.6)
Symptoms			
Cough	103/198 (52.0)	675/928 (72.7)	19,523/24,148 (80.8)
Difficult breathing	89/197 (45.2)	535/927 (57.7)	14,280/24,102 (59.2)
Prostration	29/197 (14.7)	147/918 (16.0)	1,602/23,906 (6.7)
Diarrhea	38/190 (20.0)	271/861 (31.5)	5,798/22,772 (25.5)
Convulsion	8/198 (4.0)	72/927 (7.8)	1,174/24,127 (4.9)
Signs			
Lower chest wall in-drawing	164/198 (82.8)	732/927 (79.0)	17,856/24,129 (74.0)
Meningism	1/192 (0.5)	34/867 (3.9)	174/22,841 (0.8)
Altered level of consciousness	124/193 (64.2)	407/873 (46.6)	9,590/23,518 (40.8)
Axillary temperature			
<36.5°C	18/198 (9.1)	79/932 (8.5)	2,405/24,182 (9.9)
36.5°C–37.5°C	40/198 (20.2)	147/932 (15.8)	6,819/24,182 (28.2)
>37.5°C	140/198 (70.7)	706/932 (75.7)	14,958/24,182 (61.9)
Pulse rate, beats/min§			
Increased for age	84/198 (42.4)	621/932 (66.6)	15,107/24,182 (62.5)
Respiratory rate, breaths/min¶			
Increased for age	128/198 (64.6)	682/932 (73.2)	17,157/24,177 (71.0)
Oxygen saturation <92%	33/198 (16.7)	116/932 (12.4)	2,140/24,182 (8.8)
Suspected diagnosis#			
Septicemia	109/194 (56.2)	434/896 (48.4)	8,549/23,068 (37.1)
Pneumonia	55/194 (28.4)	347/896 (38.8)	13,244/23,068 (57.4)
Meningitis	13/194 (6.7)	96/896 (10.7)	718/23,068 (3.1)
Other focal sepsis	17/194 (8.8)	19/896 (2.1)	557/23,068 (2.4)
Malaria positivity**	14/131 (10.7)	84/723 (11.6)	3,276/21,626 (15.1)

*Values are no. patients/total no. in category (%) except as indicated. Surveillance data are from the Basse Health and Demographic Surveillance System and the Fuladu West Health and Demographic Surveillance System. IQR, interquartile range.
†Defined as weight-for-height z-score <3 SDs from median weight-for-height for the corresponding age group. We calculated weight-for-height using z-scores from the 2006 World Health Organization child growth standards in Stata 14.0 (https://www.stata.com/stata14). Neonates were not included in weight-for-height measurements.
‡The wet season occurs during July–November and the dry season during December–June.
§The reference ranges for pulse rates were 70–190 beats/min for children <1 month of age, 80–160 beats/min for children 1–11 months of age, 80–130 beats/min for children 1–2 years of age, 80–120 beats/min for children 3–4 years of age, 75–115 beats/min for children 5–6 years of age, 70–110 beats/min for children 7–9 years of age, and 60–100 beats/min for children >10 years of age.
¶Increased respiratory rate was defined as >60 breaths/min for children <2 months of age, >50 breaths/min for children 2–12 months of age, >40 breaths/min for children >1–5 years of age.
#Surveillance diagnosis was categorized into mutually exclusive groups in order of severity; meningitis was considered more severe than septicemia, which was considered more severe than pneumonia.
**Malaria was tested using a rapid diagnostic test (ICT Malaria *P.f.* Antigen, ICT Diagnostics, http://www.ictdiagnostics.co.za).

was found between the pre-PCV (18 cases/100,000 person-years) and the PCV13 (23 cases/100,000 person-years) periods (incidence rate ratio 1.3, 95% CI 0.6–2.7; p = 0.49).

CFRs and Risk Factors Associated with Fatality

In total, 28 deaths occurred among 198 *S. aureus* bacteremia patients before discharge (CFR 14.1%, 95% CI 9.4%–20.4%) (Table 1). In comparison, the CFR among patients without bacteremia was 3.6% (95% CI 3.3%–3.8%) and among patients with bacteremia with other pathogens 17.2% (95% CI 14.7%–20.1%). The *S. aureus* bacteremia CFR did not vary by year (p value for trend = 0.75) or age group (p value for trend = 0.99). Deaths associated with *S. aureus* bacteremia most often occurred on the day of admission (71.4%, 20/28). During 2011–2015, *S. aureus* bacteremia deaths accounted for 7.0% (12/171) of all deaths in neonates and 3.6% (24/662) of all deaths in children <5 years of age. The risk factors associated with death from *S. aureus* bacteremia

Table 2. Factors associated with *Staphylococcus aureus* bacteremia in children <5 years of age identified through 2 surveillance systems, The Gambia, 2011–2015*

Variable	No. cases/no. person-years at risk	Incidence, cases/100,000 person-years	Incidence rate ratio (95% CI)	p value
Age, mo				
24–59	18/128,994	14.0	1	
12–23	29/44,433	65.3	4.70 (2.6–8.4)	
1–11	53/39,969	132.6	9.50 (5.6–16.2)	
<1	70/3,367	2079.0	148.99 (88.8–250.1)	<0.001
Sex				
M	82/107,515	76.3	1	
F	88/109,248	80.6	1.06 (0.8–1.4)	0.72
Season				
Dry	85/144,508	58.8	1	
Wet	85/72,255	117.6	2.00 (1.5–2.7)	<0.001

*Surveillance data are from the Basse Health and Demographic Surveillance System and the Fuladu West Health and Demographic Surveillance System

were prostration at clinical presentation and musculoskeletal swelling with or without tenderness (Table 3).

Treatment and Susceptibility of Isolates

Among *S. aureus* bacteremia patients identified through referral surveillance, 17.1% (13/76) received initial empiric therapy with cloxacillin, 23.7% (18/76) ampicillin, 31.6% (24/76) penicillin, and 50.0% (38/76) gentamicin; 50 (65.8%) of these patients received >1 antimicrobial drug. The mortality rate did not differ by empiric therapy. Among the 193 *S. aureus* isolates tested, 3.1% were methicillin-resistant (Table 4).

Discussion

We estimated the incidence and CFR of *S. aureus* bacteremia in a rural part of The Gambia using surveillance data over a 5-year period and evaluated trends in incidence over an 8-year period. The incidence was high, particularly among neonates (3.5 cases/1,000 live births), but did not increase with time (Figure 3). The CFR (14.1%) was substantial (Table 1).

The observed incidence of *S. aureus* bacteremia in The Gambia among children 0–59 months of age (78.4 cases/100,000 person-years) was higher than that for industrialized countries (6.5–42.0 cases/100,000 person-years) (*22*,*23*) and some countries of Africa (*4*) and Asia (*24*,*25*), although lower than that reported for Mozambique (*12*). *S. aureus* bacteremia incidence was reported to be 27 cases/100,000 person-years in children <5 years in Kenya (*4*) and 101 cases/100,000 person-years in Mozambique (*12*). In Thailand, a study that reviewed national hospital-based data on bacteremia reported a *S. aureus* bacteremia incidence of 2.5 cases/100,000 person-years (*24*), whereas a population-based study in Bangladesh that focused on children 1–59 months of age with respiratory symptoms reported an incidence of 9.9 cases/100,000 person-years (*25*). The differences in incidence among studies are likely related to the different selection criteria used in the various studies, nutritional status of the patients, presence of concurrent medical conditions, or high levels of antimicrobial drug use without a prescription, especially in Asia (*26*).

During 2008–2015, we found no trend in *S. aureus* bacteremia incidence in The Gambia. Researchers in industrialized countries have shown an increase in the proportion of all bacteremia cases caused by *S. aureus* after the introduction of PCV (*27*). However, our data do not support an association between *S. aureus* bacteremia incidence and the introduction of PCV. Further studies in different settings could help confirm this finding.

The incidence of *S. aureus* bacteremia was highest in neonates, 8 times that reported in Kenya (*4*), and *S. aureus* was the most common cause of bacteremia in this age group. This finding is similar to those of other studies conducted in Africa (*12*,*14*), where *S. aureus* was responsible for 39.0%–56.2% of isolates recovered from neonates. *S. aureus* carriage, which is likely a prerequisite for disease, is also highest during the neonatal period, higher than the carriage of *S. pneumoniae* and *H. influenzae* (*28*). In addition to high rates of acquisition of *S. aureus*

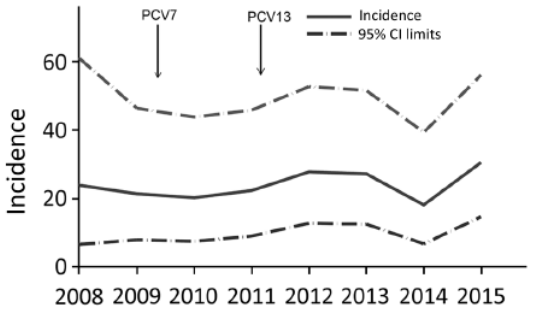


Figure 3. Unadjusted annual incidence of *Staphylococcus aureus* bacteremia (cases/100,000 person-years) in children 2–59 months of age, Basse, The Gambia, 2008–2015. Cases were identified by referral surveillance through the Basse Health and Demographic Surveillance System. Arrows indicate introduction of PCV7 and PCV13. PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine.

Table 3. Sociodemographic and clinical parameters associated with death from *Staphylococcus aureus* bacteremia among children <5 years of age identified through 2 surveillance systems, The Gambia, 2008–2015*

Parameter	Deaths/persons at risk (%)	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)†	p value
Age, mo					
<1	13/84 (15.5)	Referent		Referent	
1–11	8/61 (13.1)	0.8 (0.3–2.1)		0.9 (0.4–2.6)	
12-23	4/33 (12.1)	0.8 (0.2–2.5)		1.3 (0.4–4.6)	
24-59	3/20 (15.0)	1.0 (0.3–3.8)	0.96‡	1.1 (0.3–4.6)	0.96
Sex					
M	16/97 (16.5)	Referent			
F	12/101 (11.9)	0.7 (0.3–1.5)	0.35		
Severely stunted§					
No	20/150 (13.3)	Referent			
Yes	5/41 (12.2)	0.9 (0.3–2.6)	0.85		
Axillary temperature					
36.5°C–37.5°C	4/18 (22.2)	Referent			
<36.5°C	4/40 (10.0)	0.4 (0.1–1.8)			
>37.5°C	20/140 (14.3)	0.6 (0.2–2.0)	0.48		
Pulse rate, beats/min¶					
Within reference ranges	13/114 (11.4)	Referent			
Increased for age	15/84 (17.9)	1.7 (0.8–3.8)	0.20		
Respiratory rate, breaths/min#					
Within reference ranges	8/70 (11.4)	Referent			
Increased for age	20/128 (15.6)	1.4 (0.6–3.5)	0.41		
Need for oxygen supplementation					
No	21/165 (12.7)	Referent			
Yes	7/33 (21.2)	1.9 (0.7–4.8)	0.22		
Season					
Dry	18/101 (17.8)	Referent			
Wet	10/97 (10.3)	0.5 (0.2–1.2)	0.13		
Cough					
No	13/95 (13.7)	Referent			
Yes	15/103 (14.6)	1.1 (0.5–2.4)	0.86		
Difficult breathing					
No	14/108 (13.0)	Referent			
Yes	14/89 (15.7)	1.3 (0.6–2.8)	0.58		
Prostration					
No	17/168 (10.1)	Referent		Referent	
Yes	11/29 (37.9)	5.4 (2.2–13.4)	0.0004	5.7 (2.2–14.8)	0.01
Admitted in previous 2 weeks					
No	20/131 (15.3)	Referent			
Yes	2/31 (6.5)	0.4 (0.1–1.7)	0.16		

*Surveillance data are from the Basse Health and Demographic Surveillance System and the Fuladu West Health and Demographic Surveillance System. OR, odds ratio.
†Adjusted for age only.
‡p value for trend.
§Defined as weight-for-height z-score <3 SDs from median weight-for-height for the corresponding age group. We calculated weight-for-height using z-scores from the 2006 World Health Organization child growth standards in Stata 14.0 (https://www.stata.com/stata14). Neonates were not included in weight-for-height measurements.
¶The reference ranges for pulse rates were 70–190 beats/min for children <1 month of age, 80–160 beats/min for children 1–11 months of age, 80–130 beats/min for children 1–2 years of age, 80–120 beats/min for children 3–4 years of age, 75–115 beats/min for children 5–6 years of age, 70–110 beats/min for children 7–9 years of age, and 60–100 beats/min for children >10 years of age.
#Increased respiratory rate was defined as >60 breaths/min for children <2 months of age, >50 breaths/min for children 2–12 months of age, >40 breaths/min for children >1–5 years of age.

during the neonatal period, other reasons for the high risk for *S. aureus* bacteremia might include an immature immune system (*29*).

In our study, only 16% of the *S. aureus* bacteremia cases in neonates presented within the first week of life, unlike for group B *Streptococcus* disease, where 80% of parents seek treatment for their neonates within this period (*30*). Reasons for the difference in timing of treatment might relate to the age at and source of *S. aureus* acquisition.

We found *S. aureus* bacteremia to be more common during the wet season. This seasonal variation might relate to *S. aureus* colonization (a prerequisite for disease), which is highest during the wet season (*31*), or seasonal differences in the incidence of viral infections, which are known to disrupt mucosal epithelium, thereby encouraging *S. aureus* invasion (*32*). In a study of US adults (*33*), the peak incidence of *S. aureus* infection occurred during the winter months and coincided with the peak incidence of viral infections. In Africa, the incidence of viral infections usually peaks during the wet season (*34*), coinciding with the peak *S. aureus* bacteremia incidence.

7. Waters D, Jawad I, Ahmad A, Lukšić I, Nair H, Zgaga L, et al. Aetiology of community-acquired neonatal sepsis in low and middle income countries. J Glob Health. 2011;1:154–70.
8. Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. J Antimicrob Chemother. 2005;56:455–62. http://dx.doi.org/10.1093/jac/dki266
9. Stoll BJ, Hansen NI, Sánchez PJ, Faix RG, Poindexter BB, Van Meurs KP, et al.; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues. Pediatrics. 2011;127:817–26. http://dx.doi.org/10.1542/peds.2010-2217
10. Cobos-Carrascosa E, Soler-Palacín P, Nieves Larrosa M, Bartolomé R, Martín-Nalda A, Antoinette Frick M, et al. *Staphylococcus aureus* bacteremia in Children: changes during eighteen years. Pediatr Infect Dis J. 2015;34:1329–34. http://dx.doi.org/10.1097/INF.0000000000000907
11. Mejer N, Westh H, Schønheyder HC, Jensen AG, Larsen AR, Skov R, et al.; Danish Staphylococcal Bacteraemia Study Group. Stable incidence and continued improvement in short term mortality of *Staphylococcus aureus* bacteraemia between 1995 and 2008. BMC Infect Dis. 2012;12:260. http://dx.doi.org/10.1186/1471-2334-12-260
12. Sigaúque B, Roca A, Mandomando I, Morais L, Quintó L, Sacarlal J, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J. 2009;28:108–13. http://dx.doi.org/10.1097/INF.0b013e318187a87d
13. Mulholland EK, Ogunlesi OO, Adegbola RA, Weber M, Sam BE, Palmer A, et al. Etiology of serious infections in young Gambian infants. Pediatr Infect Dis J. 1999;18(Suppl):S35–41. http://dx.doi.org/10.1097/00006454-199910001-00007
14. Uzodimma CC, Njokanma F, Ojo O, Falase M, Ojo T. Bacterial isolates from blood cultures of children with suspected sepsis in an urban hospital in Lagos: a prospective study using BACTEC blood culture system. Internet J Pediatr Neonatol. 2013;16:1623.
15. Groome MJ, Albrich WC, Wadula J, Khoosal M, Madhi SA. Community-onset *Staphylococcus aureus* bacteraemia in hospitalised African children: high incidence in HIV-infected children and high prevalence of multidrug resistance. Paediatr Int Child Health. 2012;32:140–6. http://dx.doi.org/10.1179/1465328111Y.0000000044
16. Hill PC, Onyeama CO, Ikumapayi UN, Secka O, Ameyaw S, Simmonds N, et al. Bacteraemia in patients admitted to an urban hospital in West Africa. BMC Infect Dis. 2007;7:2. http://dx.doi.org/10.1186/1471-2334-7-2
17. Scott S, Odutola A, Mackenzie G, Fulford T, Afolabi MO, Jallow YL, et al. Coverage and timing of children's vaccination: an evaluation of the expanded programme on immunisation in The Gambia. PLoS One. 2014;9:e107280. http://dx.doi.org/10.1371/journal.pone.0107280
18. Mwesigwa J, Okebe J, Affara M, Di Tanna GL, Nwakanma D, Janha O, et al. On-going malaria transmission in The Gambia despite high coverage of control interventions: a nationwide cross-sectional survey. Malar J. 2015;14:314. http://dx.doi.org/10.1186/s12936-015-0829-6
19. Mackenzie GA, Plumb ID, Sambou S, Saha D, Uchendu U, Akinsola B, et al. Monitoring the introduction of pneumococcal conjugate vaccines into West Africa: design and implementation of a population-based surveillance system. PLoS Med. 2012;9:e1001161. http://dx.doi.org/10.1371/journal.pmed.1001161
20. Adegbola RA, Falade AG, Sam BE, Aidoo M, Baldeh I, Hazlett D, et al. The etiology of pneumonia in malnourished and well-nourished Gambian children. Pediatr Infect Dis J. 1994;13:975–82. http://dx.doi.org/10.1097/00006454-199411000-00008
21. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard—twelfth edition (M02-A12). Wayne (PA): The Institute; 2015.
22. Frederiksen MS, Espersen F, Frimodt-Møller N, Jensen AG, Larsen AR, Pallesen LV, et al. Changing epidemiology of pediatric *Staphylococcus aureus* bacteremia in Denmark from 1971 through 2000. Pediatr Infect Dis J. 2007;26:398–405. http://dx.doi.org/10.1097/01.inf.0000261112.53035.4c
23. Asgeirsson H, Gudlaugsson O, Kristinsson KG, Vilbergsson GR, Heiddal S, Haraldsson A, et al. Low mortality of *Staphylococcus aureus* bacteremia in Icelandic children: nationwide study on incidence and outcome. Pediatr Infect Dis J. 2015;34:140–4. http://dx.doi.org/10.1097/INF.0000000000000485
24. Kanoksil M, Jatapai A, Peacock SJ, Limmathurotsakul D. Epidemiology, microbiology and mortality associated with community-acquired bacteremia in northeast Thailand: a multicenter surveillance study. PLoS One. 2013;8:e54714. http://dx.doi.org/10.1371/journal.pone.0054714
25. Arifeen SE, Saha SK, Rahman S, Rahman KM, Rahman SM, Bari S, et al. Invasive pneumococcal disease among children in rural Bangladesh: results from a population-based surveillance. Clin Infect Dis. 2009;48(Suppl 2):S103–13. http://dx.doi.org/10.1086/596543
26. Zellweger RM, Carrique-Mas J, Limmathurotsakul D, Day NPJ, Thwaites GE, Baker S, et al.; Southeast Asia Antimicrobial Resistance Network. A current perspective on antimicrobial resistance in Southeast Asia. J Antimicrob Chemother. 2017; 72:2963–72. http://dx.doi.org/10.1093/jac/dkx260
27. Greenhow TL, Hung YY, Herz A. Bacteremia in Children 3 to 36 months old after introduction of conjugated pneumococcal vaccines. Pediatrics. 2017;139:e20162098. http://dx.doi.org/10.1542/peds.2016-2098
28. Peacock SJ, Justice A, Griffiths D, de Silva GD, Kantzanou MN, Crook D, et al. Determinants of acquisition and carriage of *Staphylococcus aureus* in infancy. J Clin Microbiol. 2003; 41:5718–25. http://dx.doi.org/10.1128/JCM.41.12.5718-5725.2003
29. Power Coombs MR, Kronforst K, Levy O. Neonatal host defense against Staphylococcal infections. Clin Dev Immunol. 2013;2013:826303. http://dx.doi.org/10.1155/2013/826303
30. Trijbels-Smeulders M, de Jonge GA, Pasker-de Jong PC, Gerards LJ, Adriaanse AH, van Lingen RA, et al. Epidemiology of neonatal group B streptococcal disease in the Netherlands before and after introduction of guidelines for prevention. Arch Dis Child Fetal Neonatal Ed. 2007;92:F271–6. http://dx.doi.org/10.1136/adc.2005.088799
31. Bojang A, Kendall L, Usuf E, Egere U, Mulwa S, Antonio M, et al. Prevalence and risk factors for *Staphylococcus aureus* nasopharyngeal carriage during a PCV trial. BMC Infect Dis. 2017;17:588. http://dx.doi.org/10.1186/s12879-017-2685-1
32. Wang X, Zhang N, Glorieux S, Holtappels G, Vaneechoutte M, Krysko O, et al. Herpes simplex virus type 1 infection facilitates invasion of *Staphylococcus aureus* into the nasal mucosa and nasal polyp tissue. PLoS One. 2012;7:e39875. http://dx.doi.org/10.1371/journal.pone.0039875
33. Lewis SS, Walker VJ, Lee MS, Chen L, Moehring RW, Cox CE, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* pneumonia in community hospitals. Infect Control Hosp Epidemiol. 2014;35:1452–7. http://dx.doi.org/10.1086/678594
34. Breiman RF, Cosmas L, Njenga M, Williamson J, Mott JA, Katz MA, et al. Severe acute respiratory infection in children in a densely populated urban slum in Kenya, 2007–2011. BMC Infect Dis. 2015;15:95. http://dx.doi.org/10.1186/s12879-015-0827-x

Address for correspondence: Aderonke Odutola, Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine, Disease Control and Elimination Theme, 20 Atlantic Rd, Fajara, PO Box 273, Banjul, The Gambia; email: ceemee10@yahoo.ca

Appendix 13

London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
United Kingdom
Switchboard: +44 (0)20 7636 8636

www.lshtm.ac.uk

Observational / Interventions Research Ethics Committee

Dr Aderonke Odutola
LSHTM

21 February 2017

Dear Aderonke,

Study Title: Acquisiton of Staphylococcus aureus to neonates in rural Gambia

LSHTM Ethics Ref: 11677

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Investigator CV	CV_SyedZaman_June2015	30/06/2015	1.0
Investigator CV	CV C Bottomley25.04.16	25/04/2016	1.0
Investigator CV	Publications Bottomley_MRC	29/06/2016	1.0
Investigator CV	CV_Odutola_LSHTM_2016_Jul_31	31/07/2016	1.0
Investigator CV	Curriculum Vitae_gm_v3	01/08/2016	3.0
Information Sheet	S aureus_Transmission_ICD Adult_v1 0	06/08/2016	1.0
Information Sheet	S aureus_Transmission_ICD Adult-HCW_v1 0	06/08/2016	1.0
Information Sheet	S aureus_Transmission_ICD Child_v1 0	06/08/2016	1.0
Protocol / Proposal	Sensitization Form_2016_Aug_14	14/08/2016	1.0
Protocol / Proposal	SCC Application form_Odutola_2016_Aug_22_v1 0	22/08/2016	1.0
Local Approval	SCC 1510v1.1_Odutola_Approved_20Sep16	20/09/2016	1.0
Information Sheet	S aureus_Transmission_ICD Adult_v3 0	04/10/2016	3.0
Information Sheet	S aureus_Transmission_ICD Adult-HCW_v3 0	04/10/2016	3.0
Information Sheet	S aureus_Transmission_ICD Child_v3 0	04/10/2016	3.0
Local Approval	SCC 1510_Odutola_Approved_25Oct.16	25/10/2016	1.0
Covering Letter	Response to LSHTM EC_	10/02/2017	1.0

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project

by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 14

The Gambia Government/MRC Joint
ETHICS COMMITTEE

C/o MRC Unit: The Gambia, Fajara
P.O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496513
Tel: +220 – 4495442-6 Ext. 2308
Email: ethics@mrc.gm

25 October 2016

Dr Aderonke Odutola
MRC Unit The Gambia
Disease Control & Elimination Theme

Dear Dr Odutola,

SCC 1510, Acquisition of Staphylococcus aureus in neonates in rural Gambia

Thank you for submitting your response letter dated 24 October 2016 addressing the issues raised by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 30 September 2016.

Your responses are quite satisfactory. This proposal has now received full ethical approval.

With best wishes,

Yours sincerely,

[redacted]

Mr Malamin Sonko
Chairman Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- EC reply letter – 16 October 2016
- Response letter – 24 October 2016
- SCC application form - 19 October 2016
- SCC approval letter – 20 September 2016
- SCC application form, version 1.1 – 9 September 2016
- Response letter – 14 September 20136
- Informed Consent Documents (adult/child), version 1.1 – 9 September 2016
- Informed Consent Document (HCW), version 1.1 –9 September 2016
- CRFs, version 1.0 – 14 August 2016

Appendix 15: PARTICIPANT INFORMATION SHEET

Version 1.0 Date 06 Aug 2016

Study Title: Transmission of *Staphylococcus aureus* to neonates in rural Gambia

SCC:	1510	Protocol:	**Transmission of *Staphylococcus aureus* to neonates in rural Gambia**

Sponsor & Funder: Medical Research Council Unit, The Gambia

What is informed consent?

You are invited to take part in a research study. Participating in a research study is not the same as getting regular medical care. The purpose of regular medical care is to improve one's health. The purpose of a research study is to gather information. It is your choice to take part and you can stop any time.
Before you decide you need to understand all information about this study and what it will involve. Please take time to read the following information or get the information explained to you in your language. Listen carefully and feel free to ask if there is anything that you do not understand. Ask for it to be explained until you are satisfied. You may also wish to consult your spouse, family members or others before deciding to take part in the study.

If you decide to join the study, you will need to sign or thumbprint a consent form saying you agree to be in the study.

Why is this study being done?

A leading cause of death of newborns in Gambia is severe infection. Studies have shown the bacteria *Staphylococcus aureus* is the major cause of these infections. *S. aureus* infections are difficult to treat, and children often stay in hospital some time. The *S. aureus* bacteria inhabits the nose, umbilicus, hands and groin of children and adults, from where it is spreads to other parts of the body to cause disease. How do these newborn babies get to carry the bugs? Babies are free of this and other organisms at birth but quickly acquire this organism within hours of delivery. It is thought that they get it from the birth canal during delivery, the person who conducts delivery, equipment used during delivery or from other family members. We do not know which of these is the most important source of *S. aureus* being spread to newborns. This study plans to find out the main source of *S. aureus* being spread to newborns. If we know how the babies get this bug, then we can plan to block the route through which they get it and therefore reduce infections due to this bug.

The results of the study will be made available to you.

What does this study involve?

To take part in this study, one of our field staff will approach and tell you the details of the study. If you agree to take part in the study, you will be asked to sign/thumbprint the consent form in the presence of a witness who will also sign the same form. When you are in labour, the nurse will

insert a soft cotton swab into your vagina (vagina swab) and another one into your nose (nasal swab) for a few seconds and remove. Nasal swabs will also be taken from your baby, one the other caregiver of your baby and two of the under-five children in your house at birth. One week after birth, nasal swabs will be taken from your baby. The field staff will ask you questions about you and your child(ren) and s/he will document your answers in a questionnaire. To take the nasal swabs the field staff will insert a soft cotton swab into your or your child/ward's nose for a few seconds and then remove. To take vaginal swab, the nurse will insert a soft cotton swab into your vagina, rotate and remove. Thereafter, the swab will be put in a small container with liquid and taken to the MRC laboratory for testing.

In case the investigator discovers you or your child(ren) is(are) sick and decides that you or your child(ren) cannot participate in the study because of that, you or your child(ren) will be sent to the appropriate health facility to receive immediate care.

If the research study needs to be stopped, you will be informed, and you and your child will have the normal medical care.

What will happen to the samples taken in this study?

All swabs taken will be transported to the MRC laboratory where they will be processed. Some of bacteria we get from the swabs will be sent to the United Kingdom for further tests which cannot be done here in MRC.

What harm or discomfort can you expect in the study?

This process should not cause you or your child(ren) any harm but you will experience some temporary discomfort when the swab is inserted into the nose.

What benefits can you expect in the study?

Although there may be no direct benefit to the child but result of the study may help us plan ways of reducing the transmission and carriage of the organism by newborns. Also, all newborns will be examined during each home visit and if any sign of illness is noticed the baby will be referred to the clinic if required.

Will you be compensated for participating in the study?

You will not get paid for participation, but you will get either transport by MRC or get the costs for the transport reimbursed.

What happens if you refuse to participate in the study or change your mind later?

You are free to participate or not in the study and you have the right to stop participating at any time without giving a reason. This will not affect the medical care that you would normally receive.

In case you decide to withdraw your participation during the study, any information already generated from the samples until the time of withdrawal will be used and samples already collected, for which you have given consent, will also be analysed and data used. The study doctor may also ask for tests for your safety.
Should any new information become available during the study that may affect your participation, you will be informed as soon as possible.

If you are injured in the study what compensation will be available?

We will be responsible to provide for treatment caused by procedures of the research study.
If medical treatment is required as an emergency, please refer to your health centre or clinic and contact the field worker who gave his/her telephone number to you or contact Dr Aderonke Odutola on 5668751 Ext 130 or Demba Njie 7628945.

How will personal records remain confidential and who will have access to it?

All information that is collected about you in the course of the study will be kept strictly confidential. Your personal information will only be available to the study team members and might be seen by some rightful persons from the Ethics Committee, Government authorities and sponsor.

Who should you contact if you have questions?

If you have any queries or concerns, you can contact Dr Aderonke Odutola on 5668751 Ext 130 or Demba Njie 7628945 and you can always call the personal numbers of the study staff given to you. Please feel free to ask any question you might have about the research study.

Who has reviewed this study?

This study has been reviewed and approved by a panel of scientists at the Medical Research Council and the Gambia Government/MRC Joint Ethics Committee, which consists of scientists and lay persons to protect your rights and wellbeing.

CONSENT FORM

Participant Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|

_____

(Printed name of participant)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I

- confirm that my choice to participate is entirely voluntarily,
- confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,
- understand that I grant access to data about me to authorised persons described in the information sheet,
- have received sufficient time to consider taking part in this study,
- agree to take part in this study.

Tick as appropriate

I agree for my samples to be shipped outside of The Gambia Yes ☐ No ☐

I agree to further research on my samples as described in the information sheet Yes ☐ No ☐

Participant's signature/ thumbprint* ☐

_____

Date (dd/mmm/yyyy)

Time (24hr)

Printed name of witness* _____

Printed name of person obtaining consent _____

I attest that I have explained the study information accurately in _____ to and was understood to the best of my knowledge by, the participant. He/she has freely given consent to participate *in the presence of the above-named witness (where applicable).

Signature of person obtaining consent

_____

Date (dd/mmm/yyyy)

Time (24hr)

** Only required if the participant is unable to read or write.*

Appendix 16: Supplemental tables for Chapter 4

Supplemental Table 4.1: Characteristics of the 227 mothers

Characteristics	n (%)
Age group in years	
17-24	64 (28.2)
25-30	109 (48.0)
>31	54 (23.8)
Ethnic group	
Sarahule	105 (46.3)
Fula	57 (25.1)
Others[a]	65 (28.6)
Educational level	
None	80 (35.2)
Arabic school	94 (41.4)
Primary and above	53 (23.4)
Occupation	
Unemployed	200 (88.1)
Employed[b]	27 (11.9)
Marital status	
Married	227 (100.0)
Number of newborn's siblings	
0-3	101 (44.5)
4-5	87 (38.3)
>5	39 (17.2)
Hospitalised in the past six months	
Yes	12 (5.3)
Antibiotic use within the previous six months	
Yes	12 (5.3)
No	205 (90.3)
Unsure	10 (4.4)
Smoking status	
Yes	2 (0.9)
Nose picking	
Yes	121 (53.3)
Current skin disease, e.g. dermatitis or skin ulcers	
Yes	10 (4.4)
Daily contact with animals	
Yes	147 (64.8)

[a] Other ethnic groups include Mandinka, Wollof, Jola

[b] Teacher, farmer, childcare attendant, accountant

Supplemental Table 4.2: Obstetric history of the 227 pregnant women

Characteristics	n (%)
Mode of delivery	
Vaginal delivery	227 (100.0)
Rupture of membranes ≥18 hours	
Yes	0 (0.0)
History of dysuria or UTI[1] one week before delivery	
Yes	12 (5.3)
History of vaginal discharge one week before delivery	
Yes	12 (5.3)
History of vaginal bleeding one week before delivery	
Yes	14 (6.2)
Peri-partum fever within one week of delivery	
Yes	95 (41.9)
Foul-smelling liquor	
Yes	35 (15.4)
Place of delivery	
Home	41 (18.1)
Health Centre	186 (81.9)
Health care talk on cord care	
Yes	90 (39.7)
Admitted during this pregnancy	
Yes	14 (6.2)
Nurse/TBA[1] washed hands before taking delivery	
Yes	198 (87.2)
No	11 (4.9)
Don't know	18 (7.9)
Nurse/TBA wore gloves before taking delivery	
Yes	203 (89.4)
No	21 (9.3)
Don't know	3 (1.3)

[1] UTI=Urinary tract infection; TBA=traditional birth attendant

Supplemental Table 4.3: Comparison of characteristics of deliveries by midwives and traditional birth attendants

Characteristics	TBA n/N (%)	Midwives n/N (%)	p-value[1]
Travelled in the previous two weeks			
No	31/41 (75.6)	142/186 (76.3)	
Yes	10/41 (24.4)	44/186 (23.7)	0.92
Nose picking			
No	11/41 (35.7)	71/186 (38.2)	
Yes	30/41 (64.3)	115/186 (61.8)	0.18
Hands washed before delivery			
No	5/41 (12.2)	5/186 (2.7)	
Yes	36/41 (87.8)	181/186 (97.3)	0.01
Current episode of cough or cold			
No	26/41 (36.6)	139/186 (74.7)	
Yes	15/41 (63.4)	47/186 (25.3)	0.15
Current skin lesion			
No	41/41 (100.0)	185/186 (99.5)	
Yes	0/41 (0.0)	1/186 (0.5)	-
Antibiotic use in the past six months			
No	35/41 (85.3)	132/186 (71.0)	
Yes	6/41 (14.7)	54/186 (29.0)	0.07

[1] Logistic regression with robust standard error was used to determine the p-value. Clustering was by

Supplemental Table 4.4: Characteristics of household children

Characteristics	n/N (%)[1]
Age (months)	
Median (IQR) [2]	38 (29, 50)
Sex	
Male	233/438 (53.2)
Bed shared with newborn	
Yes	32/434 (7.4)
Cough or cold since day 0 visit	
Yes	54/434 (12.4)
Antibiotic use since day 0 visit	
Yes	10/434 (2.3)

1 Data for 18 children were missing

[2] IQR=Interquartile range

Supplemental Table 4.5: Characteristics of caregivers

Characteristics	n/N (%)
Age (years)	
Median (IQR) [1]	54.5 (44, 65)
Sex	
Female	208/219 (95.0)
Ethnic group	
Sarahule	94/213 (44.1)
Fula	57/213 (26.8)
Others [2]	62/213 (29.1)
Residence	
Same house as newborn	124/212 (58.5)
Another house	88/212 (41.5)
Bed shared with newborn	
Yes	8/212 (3.8)
Cough or cold since day 0 visit	
Yes	29/212 (13.7)
Antibiotic use since day 0 visit	
Yes	7/212 (3.3)

[1] IQR=Interquartile range

[2] Other ethnic groups include Mandinka, Wollof, Jola

Supplemental Table 4.6: Obstetrics factors potentially associated with *S. aureus* carriage at birth

Characteristics	Cases/Persons at risk	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio (95%CI)	p-value
History of dysuria or UTI[1] one week before delivery					
No	20/215 (9.3)	1		1	
Yes	2/12 (16.7)	1.95 (0.40 – 9.53)	0.44	1.78 (0.34 – 9.22)	0.51
History of vaginal discharge one week before delivery					
No	1/12 (8.3)	1		1	
Yes	21/215 (9.8)	0.84 (0.10 – 6.83)	0.87	0.79 (0.10 – 6.57)	0.82
History of vaginal bleeding one week before delivery					
No	21/213 (9.9)	1		1	
Yes	1/14 (7.1)	0.70 (0.09 – 5.65)	0.73	0.77 (0.09 – 6.32)	0.80
Peri-partum fever within one week of delivery					
No	9/132 (6.8)	1		1	
Yes	13/95 (13.7)	2.17 (0.89 – 5.30)	0.09	2.26 (0.91 – 5.61)	0.08
Foul-smelling liquor					
No	17/192 (8.9)	1		1	
Yes	5/35 (14.3)	1.72 (0.59 – 5.00)	0.34	1.54 (0.51 – 4.71)	0.46
Place of delivery					
Home	5/41 (12.2)	1		1	
Health Centre	17/186 (9.1)	0.72 (0.25 – 2.09)	0.56	0.78 (0.26 – 2.31)	0.66
Health care talk on cord care					
No	11/137 (8.0)	1		1	
Yes	11/90 (12.2)	1.60 (0.66 – 3.85)	0.30	1.82 (0.72 – 4.60)	0.21
Admitted during this pregnancy					
No	22/213 (10.3)	-	-	-	-
Yes	0/14 (0.0)				
Nurse/TBA[1] washed hands before taking delivery					
No	3/11 (27.3)	1		1	
Yes	18/198 (9.1)	0.27 (0.06 – 1.10)		0.34 (0.05 – 1.04)	
Don't know	1/18 (5.6)	0.16 (0.01 – 1.75)	0.20	0.13 (0.01 – 1.56)	0.16
Nurse/TBA wore gloves before taking delivery					
No	4/21 (19.1)	1		1	
Yes	17/203 (8.4)	0.39 (0.12 – 1.29)		1.72 (0.52 – 5.63)	
Don't know	1/3 (33.3)	2.13 (0.15 – 29.7)	0.19	1.87 (0.59 – 5.92)	0.21

[1] UTI=Urinary tract infection; TBA=traditional birth attendant

Supplemental Table 4.7: Maternal demographic factors associated with *S. aureus* carriage acquisition in newborns between birth and one week

Characteristics	Carriage acquisition (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95%CI)	p-value
Age group in years					
17-24	48/64 (75.0)	1		1	
25-30	74/109 (67.9)	0.71 (0.35 – 1.41)		0.70 (0.33 – 1.39)	
>31	41/54 (75.9)	1.05 (0.45 – 2.44)	0.45	1.09 (0.42 – 2.44)	0.42
Ethnic group					
Sarahule	77/105 (73.3)	1		1	
Fula	40/57 (70.2)	0.86 (0.42 – 1.75)		0.90 (0.44 – 2.17)	
Others[2]	46/65 (70.8)	0.88 (0.44 – 1.75)	0.89	0.84 (0.46 – 2.09)	0.96
Educational level					
None	52/80 (65.0)	1		1	
Arabic school	71/94 (75.5)	1.66 (0.86 – 3.21)		1.67 (0.81 – 3.35)	
Primary and above	40/53 (75.5)	1.66 (0.76 – 3.60)	0.25	1.74 (0.71 – 3.64)	0.25
Occupation					
Unemployed	143/200 (71.5)	1		1	
Employed[3]	20/27 (74.1)	1.14 (0.46 – 2.84)	0.78	0.98 (0.43 – 3.17)	0.98
Number of children excluding newborn					
0-3	79/101 (78.2)	1		1	
4-5	55/87 (63.2)	0.48 (0.25 – 0.91)		0.45 (0.20 – 1.02)	
>5	29/39 (74.4)	0.81 (0.34 – 1.91)	0.07	0.67 (0.22 – 2.06)	0.13
Hospitalised in the past six months					
No	154/215 (71.6)	1		1	
Yes	9/12 (75.0)	1.19 (0.31 – 4.54)	0.80	1.22 (0.31 – 4.75)	0.78
Antibiotic use within the previous six months					
Yes	146/205 (71.2)	1		1	
No	9/12 (75.0)	1.21 (0.32 – 4.64)		1.18 (0.30 – 4.66)	
Unsure	8/10 (80.0)	1.62 (0.33 – 7.84)	0.80	1.64 (0.33 – 8.14)	0.81
Nose picking					
No	76/106 (71.7)	1		1	
Yes	87/121 (71.9)	1.01 (0.57 – 1.80)	0.97	1.06 (0.59 – 1.92)	0.85
Current skin disease					
No	156/217 (71.9)	1		1	
Yes	7/10 (70.0)	0.91 (0.23 – 3.64)	0.90	0.97 (0.24 – 3.97)	0.97
Daily contact with animals					
No	62/80 (77.5)	1		1	
Yes	101/147 (68.7)	0.64 (0.34 – 1.20)	0.16	0.65 (0.34 – 1.25)	0.19

[1] Adjusted for maternal age, maternal educational status, and ethnicity

[2] Other ethnic groups include Mandinka, Wollof, Jola

[3] Teacher, farmer, childcare attendant, accountant

Supplemental Table 4.8: Characteristics of household children potentially associated with *S. aureus* carriage acquisition in newborns between birth and one week

Characteristics	Carriage acquisition (%)	Unadjusted Odds Ratio (95% CI)	p-value	Adjusted Odds Ratio[1] (95% CI)	p-value
Bed shared with newborn					
No	144/195 (73.9)	1		1	
Yes	18/29 (62.1)	0.58 (0.26 – 1.31)	0.20	0.57 (0.25 – 1.32)	0.20
Cough or cold since the last visit					
No	126/179 (70.4)	1		1	
Yes	36/45 (80.0)	1.68 (0.76 – 3.74)	0.19	1.67 (0.73 – 3.81)	0.21
Antibiotic use since the last visit					
No	154/215 (71.6)	1		1	
Yes	8/9 (88.9)	3.17 (0.39 – 25.90)	0.22	3.23 (0.39 – 27.04)	0.22

[1] Adjusted for maternal age, maternal educational status, and ethnicity