Sepsis and meningitis in neonates and young children: Causes, clinical signs and treatment

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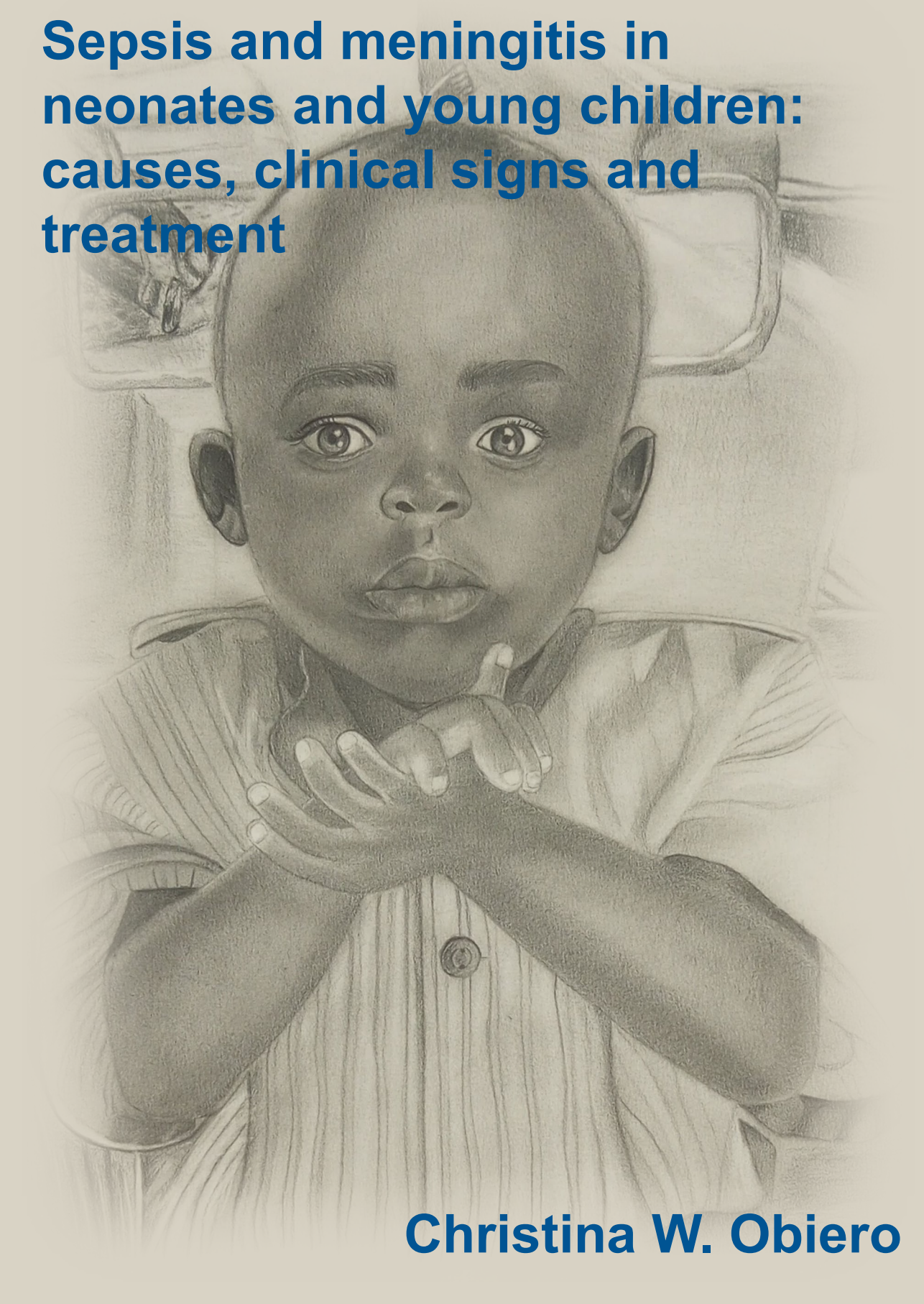

Christina W. Obiero

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Sepsis and meningitis in neonates and young children: causes, clinical signs, and treatment

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
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ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
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geboren te Nairobi

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Faculteit der Geneeskunde

DEDICATION

To my father, Calistus Obimbo Obiero and my mother, Jane Kageha Obiero

To Anyango, Gilyana, Nyabola, Aloum, Udunyi, Wudunyi, Werimo, Lanogwa, Wesonga, Gakenia, Mwende, Muthoni, Aza, Kageha, Zuri, and those who will come after you

To the children of Kenya, to whom the highest attainable standard of healthcare services is a right enshrined in Article 43 of the Constitution of Kenya

TABLE OF CONTENTS

ABBREVIATIONS

AF	Attribution fraction
AGISAR	Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMR	Antimicrobial resistance
ANISA	Aetiology of Neonatal Infections in South Asia
APHL	Association of Public Health Laboratories
AST	Antimicrobial susceptibility test
CBC	Complete blood count
CHAMPS	Child Health and Mortality Prevention Surveillance
CI	Confidence interval
CMV	Cytomegalovirus
CoNS	Coagulase-negative Staphylococcus
CRP	C-reactive protein
CSF	Cerebrospinal fluid
EDTA	Ethylenediamine tetra-acetic acid
EMA	European Medicines Agency
EONS	Early onset neonatal sepsis
ESBL	Extended spectrum beta-lactamase
GBS	Group B Streptococcus
GCLP	Good Clinical Laboratory Practice
HDU	High dependency unit
Hib	*Haemophilus influenzae* type B
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IBI	Invasive bacterial infection
IMCI	Integrated Management of Childhood Illness
KCH	Kilifi County Hospital
KHDSS	Kilifi Health and Demographic Surveillance System
KIDMS	Kilifi Integrated Data Management System
KIPMAT	Kilifi Perinatal and Maternal surveillance
KWTRP	Kenya Medical Research Institute – Wellcome Trust Research Programme
LMIC	Low- and middle-income country
LP	Lumbar puncture
MAR	Maternal admission record
MDR	Multidrug resistance
MRSA	Methicillin-resistant *Staphylococcus aureus*
NPHL	National Public Health Laboratory
NPV	Negative Predictive Value

NSIC	NeoOBS study inclusion criteria
NSS	NeoSEP severity score
OR	Odds ratio
PCR	Polymerase chain reaction
PCT	Procalcitonin
PCV	Pneumococcal conjugate vaccine
PD	Pharmacodynamic
PICU	Paediatric intensive care unit
PPV	Positive Predictive Value
PK	Pharmacokinetic
PROM	Premature rapture of membranes
pSBI	Possible serious bacterial infection
ROC	Receiver operating characteristic curve
rRNA	Ribosomal ribonucleic acid
RT-qPCR	Real-time reverse transcription quantitative polymerase chain reaction
SANISA	Sepsis Aetiology in Neonates in South Africa
SBI	Serious bacterial infection
SERU	Scientific Ethics Review Unit
SOC	Standard-of-care
SOC-F	Standard-of-care plus fosfomycin
SSC	Scientific Steering Committee
TAC	TaqMan Array Cards
TNA	Total nucleic acid
US CDC	United States Centre for Disease Control and Prevention
WHO	World Health Organization

Background

Chapter 1

General introduction

CHILD MORTALITY IN SUB-SAHARAN AFRICA

Reduction of neonatal and under-five mortality to 12 and 25 deaths per 1,000 live births respectively is integral to achieving the third Sustainable Development Goal and there is an urgent need to accelerate progress towards improved health outcomes, particularly in high-disease burden sub-Saharan Africa.[1]

In 2020, 5 million children under the age of five years died globally of which nearly half of the deaths occurred during the first 28 days of life (neonatal period).[2 3] Fifty-three percent of these deaths occurred in sub-Saharan Africa, where the highest under-five mortality rate (76 per 1,000 live births) was reported.[4] Invasive infections, including sepsis and meningitis, are among the leading causes of mortality among young children in sub-Saharan Africa and can be rapidly fatal if inadequately treated.[5] Neonates are particularly vulnerable to life-threatening infection due to immature innate and adaptive immune responses[6] and ongoing physiologic adaptation to postnatal life,[7] and may face underlying risk factors of infection during birth such as prolonged rupture of membranes.[8] Regional disparities in access to quality and cost-effective healthcare within sub-Saharan Africa limit progress towards reduction of child mortality,[9] particularly during the neonatal period.[10] Early diagnosis and treatment of sepsis and meningitis using effective antibiotics is important to help improve outcomes in young children.

The novel Coronavirus pandemic has caused untold disruption in health care services in sub-Saharan Africa and threatens to roll-back gains made during the pre-pandemic era.[11] Stockouts of medication and vaccines, low immunization rates, reduced hospital deliveries, adverse pregnancy outcomes (e.g. low-birth weight and prematurity), and increased neonatal hospitalizations and deaths have been reported.[12 13] Efforts to mitigate the impact of the ongoing pandemic on child health indicators need to prioritize case detection and prompt management of other preventable and treatable diseases.[14]

SEPSIS AND MENINGITIS IN CHILDREN

Definition

Sepsis is a life-threatening clinical syndrome caused by a dysregulated host response to infection which may be complicated with cellular, metabolic or organ dysfunction.[15 16] Sepsis definitions have been modified over time following significant advances in its pathophysiology, management, and

epidemiology.[15] Variabilities in definitions used in paediatric clinical practice and research may contribute to underestimation of sepsis burden.[17] Sepsis definitions such as the recently revised Sepsis-3 definitions[15] were derived and validated in adults and have limited use in children due to differences in aetiology, pathophysiology, comorbidities, and outcomes.[17-19] In addition, most formal definitions are not applicable to resource-limited settings.[20] There is lack of a consensus definition of neonatal sepsis and variable definitions (based on timing [early- versus late-onset], setting [hospital- versus community-acquired], or clinical and/or laboratory criteria [e.g. World Health Organization [WHO] criteria for possible serious bacterial infection [pSBI][21] and European Medicines Agency [EMA] criteria[22]]) have been used for clinical and research purposes.[20] As much as context-specific sepsis definitions are important, a universal case definition will facilitate standardized clinical care and allow comparison of research findings across different settings.

Meningitis is characterised by inflammation of the membranes covering the brain and the spinal cord and is classified based on its aetiology which may be infectious (bacterial, viral, fungal or parasitic) or non-infectious (drug-induced or associated with malignancy or autoimmunity).[23] Bacterial meningitis arises from haematogenous spread of bacteria across the blood brain barrier or from direct localised bacterial invasion of the meninges, and is the commonest cause of central nervous system infection in young children.[24 25] Like sepsis, meningitis is a severe illness that can be rapidly fatal or cause serious sequelae if not promptly detected and treated.[26]

Burden

Forty-two percent of global incident cases of sepsis occurred in children aged less than 5 years in 2017, majority of which were in sub-Saharan Africa and in the neonatal age group.[5] Approximately 3 million neonatal sepsis cases and between 500,000 and 900,000 deaths occur annually globally.[27] Studies on sepsis-related morbidity and mortality rates underestimate the burden of sepsis in sub-Saharan Africa due to lack of quality data and exclusion of community cases.[28] Despite this, the burden of paediatric sepsis cases and deaths globally is higher in low- and middle-income countries (LMICs) than in high-income countries.[5 29 30] One point prevalence survey of severe paediatric sepsis in 128 paediatric intensive care units (PICUs) in 26 countries worldwide included only 3 PICUs in sub-Saharan Africa (South Africa) and found that this region had the highest prevalence of severe sepsis (23% [95% confidence interval (CI) 14-35%])

compared to the other regions (North America 7.7% [6.9–8.5%], Europe 6.2% [5.0–7.6%], Australia/New Zealand 6.8% [4.4–9.8%], Asia 15% [12–20%], and South America 16% [12–21%], *P*<0.001).[31] Nearly three-quarters of children with severe sepsis included in this survey received mechanical ventilation, a quarter died (majority deaths occurred in sub-Saharan Africa and Asia), and 20% of the survivors developed new functional disabilities. This study clearly demonstrates the need for intensive care capacity for critically ill septic children in sub-Saharan Africa. In addition to developing acute disabilities, sepsis is associated with a high risk of readmission,[32] increased healthcare resource utilization and costs,[33] post-discharge mortality,[34] and long-term neurodevelopmental impairment among survivors.[35]

Surveillance systems for meningitis in sub-Saharan Africa are weak and its true burden is also underestimated.[36] In 2019, 2.5 million new cases of meningitis were reported globally of which 51% occurred in children under the age of five years and 48% in sub-Saharan Africa.[37] This analysis included data from multiple sources ranging from disease registries and health service utilization data to national census, and did not always distinguish confirmed meningitis cases from those reported as a clinical diagnosis and lacking CSF results.[38] Two hundred and thirty-six thousand meningitis cases died of which 48% of deaths occurred in children under the age of five years and 61% in sub-Saharan Africa. Although widespread use of conjugate vaccines has led to a significant decline in vaccine-type meningitis incidence in sub-Saharan Africa,[39] case fatality rates remain high, especially for pneumococcal meningitis.[40] Early in the course of disease, meningitis is associated with life-threatening complications such as septic shock, cerebral herniation, subdural effusion or empyema, and vascular thrombosis.[41] Long-term disabling complications in survivors include hydrocephalus, sensorineural hearing loss, visual impairment, cognitive and motor deficits, and epilepsy.[42] Nearly a quarter of sub-Saharan African children with meningitis develop post-discharge neurological sequelae with limited opportunities for long-term care and follow-up.[42] In addition, costs of acute or long-term care of meningitis cases are high.[43]

Aetiology

Staphylococcus aureus (25%), *Klebsiella pneumoniae* (21%), and *Escherichia coli* (10%), are the leading causes of sepsis in neonates and young infants aged <60 days in sub-Saharan Africa, and *Acinetobacter* species (5%) are emerging as important pathogens.[44] In older children, *Streptococcus pneumoniae* (25%), *S.*

aureus (22%), *E. coli* (11%) and non-typhoidal Salmonella (10%) are the leading blood culture isolates[45] with the epidemiology shifting towards predominance of Gram-negative bacteria.[46] Although Coagulase-negative Staphylococci are common culture isolates,[47] they are mostly regarded as contaminants in public hospitals in sub-Saharan Africa where indwelling medical devices and invasive procedures that predispose patients to colonization and subsequent infection are rare.[48 49]

Widespread use of pneumococcal (PCV), *Haemophilus influenzae* type b (Hib) and meningococcal A protein–polysaccharide (MenA [MenAfriVac]) conjugate vaccines over the past two decades[50] has resulted in significant reduction in meningitis cases secondary to these bacteria in sub-Saharan Africa.[36 39] However, *S. pneumoniae* and *Neisseria meningitidis* remain the leading causes of meningitis in children of all age groups. In addition, Group B Streptococcus (GBS; 24%), *S. aureus* (12%), *E. coli* (11%) and *K. pneumoniae* (10%) are responsible for majority of neonatal meningitis cases in sub-Saharan Africa.[44]

Clinical presentation and diagnosis

Children with sepsis or meningitis often present with subtle or atypical signs and symptoms which mimic those seen in other infectious[24 51] or non-infectious life-threatening conditions.[52 53] Non-specific signs and symptoms such as temperature abnormalities, difficulty in feeding, signs of respiratory distress, impaired consciousness and convulsions are often seen in young children suspected to have serious infection.[54] Classic signs of meningeal irritation (i.e. neck stiffness, Kernig's signs and Brudzinski's sign) are rare in young infants aged <12 months with meningitis.[55] Use of clinical signs and symptoms in isolation in identifying children suspected to have sepsis or meningitis may result in misclassification of cases.

Microbiology cultures

Detection of pathogens causing sepsis or meningitis requires a blood or CSF culture. However, most public hospitals in sub-Saharan Africa lack capacity to perform blood and CSF cultures and where available, culture yields are low[46 56 57] due to factors such as suboptimal sample volumes,[58 59] high contamination rates,[46] substandard laboratory practices,[57] and prior antibiotic exposure.[57 60 61] Culture results have a long turnaround time limiting their use in guiding early initiation of treatment in seriously ill children. However, they guide antibiotic prescribing thereby optimizing treatment options and outcomes. In addition to

CSF culture, bacterial meningitis is confirmed by either a positive CSF Gram stain or antigen test and distinguished from other forms of meningitis by predominantly polymorphonuclear leucocytosis, elevated protein and reduced glucose.[62]

Empiric antibiotic treatment guidelines target the known pathogen profiles of sepsis and meningitis based on culture results.[54 63] Appropriate antibiotic prescribing among hospitalised patients is associated with higher treatment success rates, shorter hospital stays, and lower treatment costs, and these outcomes may be influenced by disease severity or the antibiotic sensitivity patterns of pathogens causing infection.[64] Blood and CSF cultures are important for sepsis and meningitis diagnosis respectively and less technically challenging to implement in clinical practice when compared to molecular methods of pathogen detection.[65] In addition to supporting sepsis and meningitis diagnosis in clinical practice through bacterial species identification and antimicrobial susceptibility testing (AST), microbiology cultures contribute towards antibiotic stewardship and infection prevention and control strategies.[66] Microbiology cultures are also an essential component of antimicrobial resistance (AMR) surveillance systems whose success is dependent on quality laboratory practices and management.[66] Phenotypic-based AST methods following blood or CSF cultures remain the key diagnostic tests for the antibiotic susceptibility spectrum of pathogens in most clinical settings given that recently developed rapid molecular-based techniques require further validation.[67] Aseptic sampling techniques, ongoing education of phlebotomists to minimise contamination and ensure adequate sample volume collection, and use of automated continuous-monitoring blood culture systems such as BACTEC (Becton-Dickinson, USA) have been shown to improve blood culture yields.[68] CSF culture yields may be optimised by timing lumbar punctures (LPs) prior to initiation of antibiotics in children without contraindications to the procedure, and training clinicians on implementation of clinical guidelines and on how to safely and efficiently obtain CSF samples.[69 70]

Molecular diagnostics

Advances in molecular techniques over the years have assured rapid detection of a broad range of organisms causing sepsis or severe sepsis-like illness[71-74] and meningitis[75] in children. However, these methods are costly, require trained laboratory staff to implement, have limited specificity with high false-positive rates, and are difficult to incorporate into clinical practice. A Cochrane

systematic review of the diagnostic accuracy of several molecular tests based on microbial genome amplification e.g. polymerase chain reaction (PCR) compared to cultures for neonatal sepsis included 35 studies with an overall mean sensitivity of 90% (95%CI 82-95) and mean specificity of 93% (95%CI 89-96), suggesting that molecular diagnostics have potential benefit in patient management as add-on tests to cultures given the rapid turnaround time of results.[76] Similarly, PCR has been shown to be more sensitive than culture in detection of pathogens causing meningitis in children in both LMICs[77] and high-income settings.[78]

Recently, customised TaqMan Array cards (TAC) have been commercially developed and optimised for diagnosis and surveillance of multiple pathogens in a wide range of specimens using real-time reverse transcription quantitative PCR (RT—qPCR) assays.[79 80] TAC is a 384- well microfluidic array consisting of dried-down individual single plex qPCR reaction vessels containing lyophilized oligonucleotides for amplification of target nucleic acid using 5'-hydrolysis chemistry.[79 80] Each array has 8 individual microfluid channels and can be customized in a single panel to detect up to 48 targets from a single specimen with a variable number of unique assays or replicate PCR reactions. The closed system is simple to use, requiring minimal pipetting steps, which limits potential for cross-contamination or other operator error that may lead to inaccurate results. TAC technology was used in South Asia (Aetiology of Neonatal Infection in South Asia – ANISA)[81] and South Africa (Sepsis Aetiology in Neonates in South Africa – SANISA)[71] to identify the causes of neonatal sepsis. TAC was also used in the Child Health and Mortality Prevention Surveillance (CHAMPS) study to detect pathogens contributing to mortality in children aged <5 years and still births in 7 countries in sub-Saharan Africa and South Asia,[82] and in another study to detect a broad range of pathogens causing meningitis in children aged <5 years in 5 West African countries.[75]In addition to TAC RT-qPCR, metagenomic next generation sequencing methods such as nanopore sequencing have shown promise in the rapid and accurate diagnosis of meningitis in LMICs.[83 84] Recently, the MinION, a nanopore-technology based sequencer of the 16S rRNA gene was deployed in Zambia, and had better performance than conventional culture-based methods in detection of pathogens among 11 patients with meningitis.[84] Concordance between sequencing and culture was observed in 4 of 6 CSF samples, and larger studies are needed to further evaluate the clinical use of this technology for meningitis diagnosis.

Ancillary laboratory tests

Ancillary laboratory tests measuring haematological indices, acute phase reactants and cytokines have been used to improve diagnosis of sepsis and meningitis and monitor disease progression with mixed results. Currently, no biomarker has been adequately validated for detection of bacterial infection within the first critical hours of presentation to hospital during which prompt institution of antibiotics and other supportive measures are crucial for survival.[85] Complete blood count (CBC) parameters including total white cell, absolute neutrophil and platelet counts have been shown to have limited diagnostic accuracy when used to identify infants with invasive bacterial infection in isolation.[86] C-reactive Protein (CRP) has been extensively studied in hospitalised children in high-income countries with variable results of its diagnostic value.[87] Although CRP may be useful in distinguishing patients with bacterial infection from those without bacterial infection, with potential benefit in guiding antibiotic prescribing, its low positive predictive value limits its use in isolation.[87] CRP has also been shown to have poor discrimination in distinguishing children with meningitis from those without meningitis.[88 89] A recent meta-analysis of the performance of procalcitonin (PCT) in diagnosis of sepsis concluded that it had moderate accuracy (sensitivity 85% [95% CI 76-90] and specificity 54% [95%CI 38-70]) among neonates with suspected sepsis at a cut-off of 2.0-2.5 ng/ml; more studies with high methodological quality in neonates and older children are needed.[90] CRP and PCT point-of-care tests are commercially available but have not been adequately evaluated for use in the diagnosis of sepsis and meningitis in young children in sub-Saharan Africa.[91 92] Interleukin-6[93] and neutrophil CD64[94] have been shown to have better diagnostic accuracy for sepsis or meningitis when used in combination with other biomarkers or clinical signs. However, use of these biomarkers has not minimised diagnostic uncertainty given that their levels may be altered by either the presence of other infective (e.g. malaria[92]) or non-infective conditions or the timing of sample collection in relation to the onset of infection. In addition, different analytical methods and cut-off values confound interpretation and comparison of results across populations of patients.[87 91]

Cord blood for diagnosis of Early Onset Neonatal Sepsis (EONS)

A recent systematic review and meta-analysis investigating the diagnostic accuracy of cord blood culture for EONS included 17 studies and found that cord blood culture had a higher pooled sensitivity than peripheral blood culture (43%

[95%CI 13-72%] versus 20% [95%CI 0-41%]) and similar specificity (98% [95%CI 93-100] versus 100% [95%CI 100-100%]).[95] Cord blood has also been reliably used to measure peripheral blood cell indices and inflammatory markers of infection.[96]

Diagnosis of sepsis requires collection of blood samples from sick children, and this is often done through peripheral venepuncture. Sample collection may be associated with local site pain, bleeding, and swelling, and result in sample contamination if the site is poorly prepared. Between 2017-2020, blood culture contamination rates at our research site, the Kilifi County Hospital (KCH) were higher (11% and 8.2% among infants aged <60 days and neonates aged 0-2 days respectively [unpublished data]) than the recommended 3% target.[97] Interventions that have helped reduce blood culture contamination rates include use of standardised protocols supporting aseptic techniques and education packages for staff on the same.[98] Regular training of phlebotomists on aseptic blood sample collection techniques is done at KCH. Optimizing blood sample volumes is also key in improving recovery of microorganisms[97 98] and 1-2ml of blood is recommended among neonates in the presence of low-colony-count sepsis.[58] Although blood culture inoculant volumes ≥1ml are feasible in majority of neonates, inadequate specimen volumes are often obtained in premature neonates where venepuncture can be difficult.[99] Umbilical cord blood can serve as an alternative to peripheral venous blood for diagnosis of EONS.[100 101] Cord blood sampling is simple to perform, minimally invasive, painless, and assures adequate specimen volumes. Cord blood culture,[100 101] PCR[102] and molecular sequencing[103] have been shown to be reliable alternatives to peripheral venous blood culture in the diagnosis of EONS in both asymptomatic[100] and symptomatic neonates, including those with risk factors of infection (e.g. prematurity, premature or prolonged rupture of membranes, foul smelling liquor and maternal fever).[96 101 104 105] Research to generate evidence of the diagnostic utility of cord blood for EONS in our setting is needed.

Clinical guidelines

Lack of microbiology diagnostic capacity in most public hospitals in sub-Saharan Africa means that clinicians rely on clinical guidelines and judgement to identify children with probable sepsis or meningitis and initiate empiric antibiotics. The World Health Organization (WHO)[54] and Kenya national guidelines[106] recommend a blood culture or an LP for CSF analysis, and antibiotics in children presenting with signs and symptoms suggestive of sepsis or meningitis

respectively. These guidelines were derived from limited research that was not based on confirmatory cultures[21] and have not been updated over the past decade to reflect changes in disease epidemiology and patient profiles. For meningitis, two studies conducted at our centre prior to the introduction and widespread use of conjugate vaccines identified clinical signs indicating need for an LP and presumptive treatment for meningitis.[107 108] Existing clinical prediction rules or scores for sepsis, bacteraemia or meningitis aid in detection of children needing prompt initiation of antibiotics and examples of these are shown on Table 1 (addendum). However, these prediction rules and scores have limited use in sub-Saharan Africa as most were developed and validated in high-income countries with lower disease prevalence, target variable patient populations (based on age, clinical appearance, presence of fever, and whether hospitalised or in the outpatient department) and include laboratory criteria not readily available in our setting (e.g., CRP and procalcitonin levels).[109-113] Clinical rules predicting mortality in premature low-birth-weight neonates with late-onset sepsis or critically ill children under intensive care are useful in prioritising management for improved outcomes.[114-117] However, limited use and validation in sub-Saharan Africa underscores the need for further research in this area. We recently conducted an observational study (NeoObs) of the clinical features, treatment and outcomes among infants aged <60 days hospitalised with sepsis across 19 sites in 11 LMICs. Three thousand two hundred and four infants were enrolled between 2018 and 2020, including 197 in Kilifi, Kenya (submitted for publication). Data collected was used to derive a score predicting the risk of mortality in young infants with potential for use in clinical practice and future trials.

Antimicrobial treatment of sepsis and meningitis

Current antibiotic recommendations

Ampicillin/penicillin (or flucloxacillin if Staphylococcal infection is suspected) plus gentamicin are recommended as first-line antibiotics in young infants <60 days old and in children ≥60 days old suspected to have sepsis, and young infants <60 days old suspected to have meningitis; third generation cephalosporins e.g. ceftriaxone and cefotaxime are second-line.[54 106] Ceftriaxone is recommended for first-line treatment of meningitis in children aged ≥60 days suspected to have meningitis; ampicillin/penicillin plus chloramphenicol are alternatives in the absence of significant resistance to these options.[54] Effective treatment of meningitis requires bactericidal

antibiotics which have the ability to penetrate the blood-brain-barrier and achieve adequate CSF concentrations.[24] Prompt recognition of sepsis or meningitis and initiation of appropriate empiric antibiotics is essential for optimal outcomes. Where cultures are available, treatment is tailored according to susceptibility results while monitoring clinical progress. High rates of culture-negative sepsis or meningitis contribute to high antibiotic consumption rates[118] and increase the risk of development of antibiotic resistance. Where cultures are unavailable, as is often the case in sub-Saharan Africa, clinical rules guiding antibiotic initiation may result in under- or overtreatment of hospitalised children.[31] Inappropriate antibiotic use is common and is associated with prolonged hospitalization,[119] readmission, increased healthcare costs,[120] and mortality.[121] In neonates, prolonged antibiotic exposure is associated with invasive fungal infection,[122] perturbation of the infant gut microbiome and resistome[123] and necrotizing enterocolitis.[122 124] Essentially, injudicious use of antibiotics selects for resistant bacterial strains rendering antibiotics ineffective in treating infection.[125]

Antimicrobial resistance

Antimicrobial resistance (AMR) poses an increasing and rapidly evolving threat to child survival globally. In 2019, about 5 million deaths globally were associated with bacterial AMR, including 1.3 million deaths directly attributed to AMR globally; the highest burden of AMR occurred in LMICs.[126] Similar pathogen profiles of sepsis and meningitis in children, and high resistance rates to multiple classes of antibiotics have been reported in sub-Saharan Africa and South Asia.[127-131] Half of the 255,000 deaths in 2019 attributable to AMR in sub-Saharan Africa occurred in children under the age of 5 years.[126] Despite limited availability of quality microbiological data, significant resistance to commonly used antibiotics has been reported in both hospital-acquired and community-acquired infections.[44 46] High resistance rates of both Gram-positive and Gram-negative bacterial isolates to WHO-recommended β-lactams and third generation cephalosporins have been reported in sub-Saharan Africa as shown in a recent review that included 67,451 culture samples of which 8% were positive for a pathogenic species.[132] Increasing incidence of extended spectrum β-lactamase (ESBL) producing Enterobacterales (*E. coli* and *K. pneumoniae*) infection has been reported and this is associated with high case fatality rates and antibiotic resistance rates.[133] Multidrug resistant (MDR) Gram-negative bacteria are becoming a major concern in sub-Saharan Africa; a recent systematic review of 7,056 positive blood cultures of which 67% had Gram-

negative organisms found that 75% of these bacteria had MDR (resistance to ampicillin, chloramphenicol, and cotrimoxazole).[134] Emergence of Carbapenemase-producing Enterobacterales isolated from clinical specimens and hospital environments is of great concern as this limits treatment options for infections caused by MDR bacteria.[135] Changing patterns of bacteria causing sepsis or meningitis secondary to widespread conjugate vaccination, poor infection prevention and control practices and inappropriate antibiotic use contribute to the emergence and spread of AMR, and underscore the need to update current antibiotic treatment guidelines.

Novel antibiotic options in the face of increasing antimicrobial resistance (AMR)

Emergence and spread of AMR challenges drug discovery and efforts to accelerate development of new antimicrobial molecules, targets, and approaches are needed.[136] There are limited novel and effective therapeutic options in the development pipeline with no new classes approved for clinical application over the past 3 decades,[137] and existing alternatives such as piperacillin plus tazobactam and carbapenems are either costly or under-utilised in most settings in sub-Saharan Africa. Several recently developed molecules have been reported to either have low potency against drug-resistant infections or be unsuitable for clinical use due to cytotoxicity.[136] The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) recommends repurposing legacy off-patent antibiotics for the treatment of infection.[113] Alternative antibiotic options for use in children would need to be affordable (off-patent), have minimal toxicities, be licensed or have extensive use for clinical indication in population of interest, and have adequate activity against MDR bacteria with potential for consideration in novel treatment regimens.[138] Available antibiotic options with potential for use in combination regimens for neonatal sepsis in LMICs include amikacin, cefepime, fosfomycin, flomoxef, and tobramycin.

Amikacin and tobramycin are aminoglycosides with activity against Staphylococci and gentamicin-resistant Gram-negative bacteria including *P. aeruginosa*.[138] Monotherapy of both antibiotics would have limited use in treatment of sepsis given their limited efficacy against Gram-positive bacteria. The pharmacokinetic (PK) and pharmacodynamic (PD) profiles of amikacin and tobramycin are known and both are associated with nephro- and ototoxicity although safety data in neonates is limited.[139] Fosfomycin is a phosphoric acid derivative isolated from *Streptomyces* species in 1969,[140]and is bactericidal,

inhibiting phosphoenolpyruvate transferase during cell wall synthesis.[141 142] Fosfomycin has broad spectrum bactericidal activity against both Gram-positive (including methicillin-resistant *S. aureus* [MRSA] and vancomycin-resistant Enterococci spp.) and Gram-negative (including ESBL-producers) bacteria.[143] Several studies have reported fosfomycin efficacy in treating MDR infections.[144] Intravenous fosfomycin presents a significant sodium load[145] and has variable dosing range (100-400mg/kg/d),[146] while oral fosfomycin preparations contain a large amount of fructose[147] and lack dosing regimens.[148] Limited fosfomycin safety and PK data exists in neonates.[149-153] Flomoxef is an oxacephem class β-lactam antibiotic with high levels of activity against Gram-positive (including MRSA) and Gram-negative bacteria (including anaerobes), and low resistance rates have been reported.[138 154 155] Despite available PK/PD data, high clinical and microbiological success rates,[156] and an acceptable safety profile in neonates with mild changes in platelet counts and liver enzymes,[155 157-159] flomoxef is currently licenced in South Asia only, and extensively used in Japan where it was first synthesised.[138 154] Cefepime is a fourth-generation cephalosporin, has broad spectrum of activity against both Gram-positive and Gram-negative bacteria, [160] but no activity against anaerobes.[161] Although cefepime has been widely used among neonates and has a good safety profile,[138] it has been shown to have limited effectiveness against certain ESBL Enterobacteriales,[162] similar to tobramycin.[163] Further clinical evaluation of these antibiotics is needed to assess their potential for use in vulnerable populations with limited empiric antibiotic options in the face of increasing AMR.

CHILD HEALTH SERVICES IN SUB-SAHARAN AFRICA

Public health facilities in sub-Saharan Africa are mostly government-run and located in rural or densely populated urban areas. Quality of health services is mostly poor but varies between and within countries based on availability of essential medicines and diagnostics, level of staffing and equipment, user fees, and accessibility.[164 165] A recent retrospective study of a longitudinal cohort of 256,031 children born between 1995 and 2016 in 7 countries (Kenya, Uganda, Tanzania, Rwanda, Namibia, Ghana and Senegal) in sub-Saharan Africa used standardized surveys and parametric survival models to estimate the effect of health service access, quality, and cost on infant and child survival.[166] This study found that proportions of facilities that had doctors and charged fees for sick child visits, delivery and immunization varied by country. Proportion of health care providers with training on WHO Integrated Management of Childhood

Illness (IMCI) guidelines varied by country as well and Senegal had the highest levels of IMCI training (average 20% trained per facility). The overall risk of child mortality declined over time, and improved survival was associated with higher ratio of health facilities to population density and lower cost of care.

Public health services in Kenya have similar characteristics to those available in most sub-Saharan countries. Following the promulgation of a new constitution in Kenya in 2020, essential health service delivery was devolved from the central government to the county level in 2013. Figure 1 shows the hierarchical structure of the healthcare system in Kenya.[167] All levels of care are run by county governments with the exception of Level 6 hospitals which are managed by the national government. Significant regional disparities in availability of equipped, affordable and accessible health facilities exist.[168] The Kenya National Public Health Laboratory (NPHL) in collaboration with the United States Centres for Disease Control and Prevention (US CDC) and the Association of Public Health Laboratories (APHL) recently conducted laboratory capacity mapping in the 47 counties in Kenya and found that most laboratories scored poorly in policy management, equipment management, data management, quality and biosafety/biosecurity, and zoonotic testing and surveillance.[169] There were only 21 blood culture machines in the country including those located in research laboratories. Hence, detection of pathogens causing sepsis and surveillance of AMR in most hospitals is not possible. Eight laboratories linked to 8 Level 5 hospitals reported CSF culture for isolation and identification of meningococcal meningitis while 1 laboratory reported rapid antigen testing for the same. The mapping exercise did not report on laboratory capacity to test for other pathogens that cause meningitis. Key areas that were identified as those needing urgent attention include workforce capacity, testing of priority diseases, referral networks for strengthening priority diseases, safety/biosafety, quality, and surveillance.

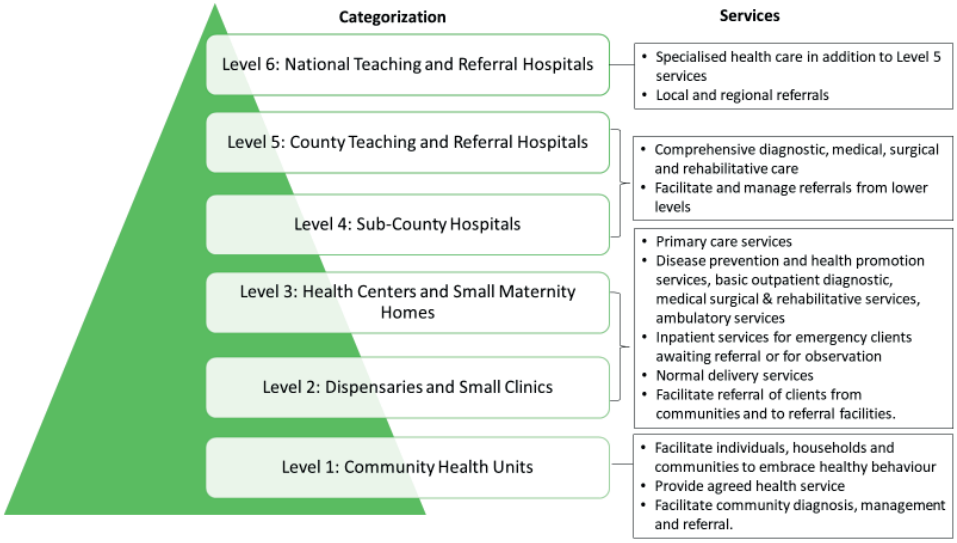


Figure 1. Categorization of health facilities in Kenya

Child health services in Kenya are characterised by basic resources constraints (e.g. lack of oxygen, stockouts of essential drugs, and absence of consultant paediatricians or medical officers) and poor service delivery (e.g. poor documentation, inadequate or incorrect prescribing of medication,[170] and high patient:care-giver ratios resulting in missed care and low rates of task completion[171]) which contribute to an increased risk of mortality.[172] Adherence to paediatric management guidelines is also variable across different hospitals and children are rarely fully investigated to establish definitive diagoses.[173] Targeted interventions to improve hospital care for children are urgently needed. A cluster randomised trial conducted in 8 rural secondary-level Kenyan hospitals compared a multifaceted intervention (evidence-based guidelines, training, job aides e.g. structures paediatric admission records, local facilitation, external supervision, and face-to-face feedback) to a partial intervention (provision of guidelines without training on their use, didactic training, job aides, and written feedback) and found that the former intervention was associated with improved implementation of WHO treatment guidelines and clinical care as it resulted in higher completion of admission assessment tasks and uptake of guideline recommended therapeutic practices.[174] In addition, the proportion of children receiving inappropriate doses of drugs was lower in the 4 intervention hospitals than in the 4 control hospitals. This study showed that

improved delivery of care and outcomes can be attained by implementation of simple strategies targeting deficiencies in knowledge, skills and organization of care.

RATIONALE FOR THIS THESIS

There is a paucity of data on the aetiology of sepsis and meningitis in neonates and young children in sub-Saharan Africa due to limited diagnostic laboratory resources and Kenya is not an exception to this. As a result, clinicians often rely on recognition of a set of simple clinical signs and symptoms at admission to decide on initiation and duration of antibiotics. The use of such clinical decision rules to guide antibiotic use or identify children at high risk of poor outcomes may contribute to development of AMR as neonates and young children often present to hospital with subtle and non-specific signs and symptoms.

Similarly, the decision to step up, step down or stop antibiotics rarely benefits from reliable cultures or ancillary investigations such as CBC. Irrational antibiotic use (both over- and under-use) in the face of rising AMR and limited therapeutic options in the development pipeline threatens child survival in sub-Saharan Africa.

This thesis aimed to address several related questions faced in the diagnosis and management of sepsis and meningitis in neonates and young children in resource-limited settings. The overarching research objectives of this thesis are:

1. To investigate the causes of early-onset neonatal sepsis (EONS)
2. To validate clinical decision rules predicting serious bacterial infection (SBI; sepsis and/or meningitis) and mortality in hospitalised neonates and young children
3. To investigate the safety and pharmacokinetic profile of fosfomycin in neonates hospitalised with clinical sepsis.

REFERENCES

1. Strong K, Noor A, Aponte J, et al. Monitoring the status of selected health related sustainable development goals: methods and projections to 2030. *Glob Health Action* 2020;13(1):1846903. doi: 10.1080/16549716.2020.1846903 [published Online First: 2020/12/01]
2. United Nations Inter-agency Group for Child Mortality Estimation (UN IGME), 'Levels & Trends in Child Mortality: Report 2021, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation', United Nations Children's Fund, New York, 2021.
3. Paulson KR, Kamath AM, Alam T, et al. Global, regional, and national progress towards Sustainable Development Goal 3.2 for neonatal and child health: all-cause and cause-specific mortality findings from the Global Burden of Disease Study 2019. *The Lancet* 2021;398(10303):870-905. doi: 10.1016/s0140-6736(21)01207-1
4. United Nations Inter-agency Group for Child Mortality Estimation (UNIGME) LTiCMR, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation', United Nations Children's Fund, New York, 2020.
5. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020;395(10219):200-11. doi: 10.1016/S0140-6736(19)32989-7 [published Online First: 2020/01/20]
6. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Biol Sci* 2015;282(1821):20143085. doi: 10.1098/rspb.2014.3085 [published Online First: 2015/12/25]
7. Morton SU, Brodsky D. Fetal Physiology and the Transition to Extrauterine Life. *Clinics in perinatology* 2016;43(3):395-407. doi: 10.1016/j.clp.2016.04.001 [published Online First: 2016/08/16]
8. Ocviyanti D, Wahono WT. Risk Factors for Neonatal Sepsis in Pregnant Women with Premature Rupture of the Membrane. *J Pregnancy* 2018;2018:4823404. doi: 10.1155/2018/4823404 [published Online First: 2018/11/08]
9. Rutherford ME, Mulholland K, Hill PC. How access to health care relates to under-five mortality in sub-Saharan Africa: systematic review. *Tropical medicine & international health : TM & IH* 2010;15(5):508-19. doi: 10.1111/j.1365-3156.2010.02497.x [published Online First: 2010/03/30]
10. Sarrassat S, Meda N, Badolo H, et al. Distance to care, care seeking and child mortality in rural Burkina Faso: findings from a population-based cross-sectional survey. *Tropical medicine & international health : TM & IH* 2019;24(1):31-42. doi: 10.1111/tmi.13170 [published Online First: 2018/10/23]
11. Ekwebelem OC, Ofielu ES, Nnorom-Dike OV, et al. Threats of COVID-19 to Achieving United Nations Sustainable Development Goals in Africa. *Am J Trop Med Hyg* 2020;104(2):457-60. doi: 10.4269/ajtmh.20-1489 [published Online First: 2020/12/18]
12. Burt JF, Ouma J, Lubyayi L, et al. Indirect effects of COVID-19 on maternal, neonatal, child, sexual and reproductive health services in Kampala, Uganda. *BMJ Glob Health* 2021;6(8):e006102. doi: 10.1136/bmjgh-2021-006102 [published Online First: 2021/08/29]
13. das Neves Martins Pires PH, Macaringue C, Abdirazak A, et al. Covid-19 pandemic impact on maternal and child health services access in Nampula, Mozambique: a mixed methods research. *BMC Health Serv Res* 2021;21(1):860. doi: 10.1186/s12913-021-06878-3 [published Online First: 2021/08/25]
14. Coker M, Folayan MO, Michelow IC, et al. Things must not fall apart: the ripple effects of the COVID-19 pandemic on children in sub-Saharan Africa. *Pediatric research*

2021;89(5):1078-86. doi: 10.1038/s41390-020-01174-y [published Online First: 2020/09/25]
15. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama* 2016;315(8):801-10. doi: 10.1001/jama.2016.0287 [published Online First: 2016/02/24]
16. Goldstein B, Giroir B, Randolph A, et al. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies* 2005;6(1):2-8. doi: 10.1097/01.PCC.0000149131.72248.E6 [published Online First: 2005/01/08]
17. Menon K, Schlapbach LJ, Akech S, et al. Pediatric Sepsis Definition-A Systematic Review Protocol by the Pediatric Sepsis Definition Taskforce. *Crit Care Explor* 2020;2(6):e0123. doi: 10.1097/CCE.0000000000000123 [published Online First: 2020/07/23]
18. Prusakowski MK, Chen AP. Pediatric Sepsis. *Emerg Med Clin North Am* 2017;35(1):123-38. doi: 10.1016/j.emc.2016.08.008 [published Online First: 2016/12/03]
19. Emr BM, Alcamo AM, Carcillo JA, et al. Pediatric Sepsis Update: How Are Children Different? *Surg Infect (Larchmt)* 2018;19(2):176-83. doi: 10.1089/sur.2017.316 [published Online First: 2018/02/03]
20. McGovern M, Giannoni E, Kuester H, et al. Challenges in developing a consensus definition of neonatal sepsis. *Pediatric research* 2020;88(1):14-26. doi: 10.1038/s41390-020-0785-x [published Online First: 2020/03/04]
21. Young Infants Clinical Signs Study G. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *Lancet* 2008;371(9607):135-42. doi: 10.1016/S0140-6736(08)60106-3 [published Online First: 2008/01/15]
22. Tuzun F, Ozkan H, Cetinkaya M, et al. Is European Medicines Agency (EMA) sepsis criteria accurate for neonatal sepsis diagnosis or do we need new criteria? *PLoS One* 2019;14(6):e0218002. doi: 10.1371/journal.pone.0218002 [published Online First: 2019/06/07]
23. Mount HR, Boyle SD. Aseptic and Bacterial Meningitis: Evaluation, Treatment, and Prevention. *Am Fam Physician* 2017;96(5):314-22. [published Online First: 2017/09/20]
24. Kim KS. Acute bacterial meningitis in infants and children. *The Lancet Infectious diseases* 2010;10(1):32-42. doi: 10.1016/S1473-3099(09)70306-8 [published Online First: 2010/02/05]
25. Leib SL, Tauber MG. Pathogenesis of bacterial meningitis. *Infect Dis Clin North Am* 1999;13(3):527-48, v-vi. doi: 10.1016/s0891-5520(05)70093-3 [published Online First: 1999/09/02]
26. Roine I, Pelkonen T, Bernardino L, et al. Factors affecting time to death from start of treatment among children succumbing to bacterial meningitis. *The Pediatric infectious disease journal* 2014;33(8):789-92. doi: 10.1097/INF.0000000000000350 [published Online First: 2014/04/16]
27. Popescu CR, Cavanagh MMM, Tembo B, et al. Neonatal sepsis in low-income countries: epidemiology, diagnosis and prevention. *Expert review of anti-infective therapy* 2020;18(5):443-52. doi: 10.1080/14787210.2020.1732818 [published Online First: 2020/02/20]
28. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, et al. The global burden of paediatric and neonatal sepsis: a systematic review. *Lancet Respir Med* 2018;6(3):223-30. doi: 10.1016/S2213-2600(18)30063-8 [published Online First: 2018/03/07]

29. Tan B, Wong JJ, Sultana R, et al. Global Case-Fatality Rates in Pediatric Severe Sepsis and Septic Shock: A Systematic Review and Meta-analysis. *JAMA pediatrics* 2019;173(4):352-62. doi: 10.1001/jamapediatrics.2018.4839 [published Online First: 2019/02/12]
30. Fleischmann C, Reichert F, Cassini A, et al. Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis. *Archives of disease in childhood* 2021;106(8):745-52. doi: 10.1136/archdischild-2020-320217 [published Online First: 2021/01/24]
31. Weiss SL, Fitzgerald JC, Pappachan J, et al. Global epidemiology of pediatric severe sepsis: the sepsis prevalence, outcomes, and therapies study. *Am J Respir Crit Care Med* 2015;191(10):1147-57. doi: 10.1164/rccm.201412-2323OC [published Online First: 2015/03/04]
32. Prout AJ, Talisa VB, Carcillo JA, et al. Epidemiology of Readmissions After Sepsis Hospitalization in Children. *Hosp Pediatr* 2019;9(4):249-55. doi: 10.1542/hpeds.2018-0175 [published Online First: 2019/03/03]
33. Odetola FO, Gebremariam A. Resource Use and Outcomes for Children Hospitalized With Severe Sepsis or Septic Shock. *J Intensive Care Med* 2021;36(1):89-100. doi: 10.1177/0885066619885894 [published Online First: 2019/11/12]
34. Wiens MO, Kumbakumba E, Larson CP, et al. Postdischarge mortality in children with acute infectious diseases: derivation of postdischarge mortality prediction models. *BMJ open* 2015;5(11):e009449. doi: 10.1136/bmjopen-2015-009449 [published Online First: 2015/11/27]
35. Seale AC, Blencowe H, Zaidi A, et al. Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. *Pediatric research* 2013;74 Suppl 1(Suppl 1):73-85. doi: 10.1038/pr.2013.207 [published Online First: 2013/12/25]
36. Zunt JR, Kassebaum NJ, Blake N, et al. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology* 2018;17(12):1061-82. doi: 10.1016/s1474-4422(18)30387-9
37. Global Health Data Exchange IHME Data. 2020 Accessed on 27 September 2021. Available online: https://ghdx.healthdata.org/ [
38. Diseases GBD, Injuries C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020;396(10258):1204-22. doi: 10.1016/S0140-6736(20)30925-9 [published Online First: 2020/10/19]
39. 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. *The Lancet Global health* 2018;6(7):e744-e57. doi: 10.1016/S2214-109X(18)30247-X [published Online First: 2018/06/16]
40. Boisier P, Mainassara HB, Sidikou F, et al. Case-fatality ratio of bacterial meningitis in the African meningitis belt: we can do better. *Vaccine* 2007;25 Suppl 1:A24-9. doi: 10.1016/j.vaccine.2007.04.036 [published Online First: 2007/05/25]
41. Saez-Llorens X, McCracken GH, Jr. Bacterial meningitis in children. *Lancet* 2003;361(9375):2139-48. doi: 10.1016/S0140-6736(03)13693-8 [published Online First: 2003/06/27]
42. Ramakrishnan M, Ulland AJ, Steinhardt LC, et al. Sequelae due to bacterial meningitis among African children: a systematic literature review. *BMC medicine* 2009;7:47. doi: 10.1186/1741-7015-7-47 [published Online First: 2009/09/16]
43. de Broucker G, Sim SY, Brenzel L, et al. Cost of Nine Pediatric Infectious Illnesses in Low- and Middle-Income Countries: A Systematic Review of Cost-of-Illness

Studies. *Pharmacoeconomics* 2020;38(10):1071-94. doi: 10.1007/s40273-020-00940-4 [published Online First: 2020/08/05]
44. 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. *The Lancet Infectious diseases* 2019;19(11):1219-34. doi: 10.1016/S1473-3099(19)30414-1 [published Online First: 2019/09/17]
45. Droz N, Hsia Y, Ellis S, et al. Bacterial pathogens and resistance causing community acquired paediatric bloodstream infections in low- and middle-income countries: a systematic review and meta-analysis. *Antimicrobial resistance and infection control* 2019;8:207. doi: 10.1186/s13756-019-0673-5 [published Online First: 2020/01/02]
46. Dramowski A, Cotton MF, Rabie H, et al. Trends in paediatric bloodstream infections at a South African referral hospital. *BMC pediatrics* 2015;15:33. doi: 10.1186/s12887-015-0354-3 [published Online First: 2015/04/18]
47. Zelellw DA, Dessie G, Worku Mengesha E, et al. A Systemic Review and Meta-analysis of the Leading Pathogens Causing Neonatal Sepsis in Developing Countries. *BioMed research international* 2021;2021:6626983. doi: 10.1155/2021/6626983 [published Online First: 2021/07/02]
48. Seale AC, Obiero CW, Jones KD, et al. Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya? *The Pediatric infectious disease journal* 2017;36(11):1073-78. doi: 10.1097/INF.0000000000001699 [published Online First: 2017/07/22]
49. Obiero CW, Seale AC, Jones K, et al. Should first-line empiric treatment strategies cover coagulase-negative staphylococcal infections in severely malnourished or HIV-infected children in Kenya? *PLoS One* 2017;12(8):e0182354. doi: 10.1371/journal.pone.0182354 [published Online First: 2017/08/09]
50. International Vaccine Access Center. View-hub Report: Global Vaccine Introduction and Implementation. March 2021. Available at: https://view-hub.org. Accessed 21 June 2021.
51. Berkley JA, Mwangi I, Mellington F, et al. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM : monthly journal of the Association of Physicians* 1999;92(3):151-7. [published Online First: 1999/05/18]
52. Wynn JL. Defining neonatal sepsis. *Current opinion in pediatrics* 2016;28(2):135-40. doi: 10.1097/MOP.0000000000000315 [published Online First: 2016/01/15]
53. Hermansen CL, Mahajan A. Newborn Respiratory Distress. *Am Fam Physician* 2015;92(11):994-1002. [published Online First: 2016/01/14]
54. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed: World Health Organization, 2013:412.
55. Valmari P, Peltola H, Ruuskanen O, et al. Childhood bacterial meningitis: initial symptoms and signs related to age, and reasons for consulting a physician. *European journal of pediatrics* 1987;146(5):515-8. doi: 10.1007/BF00441607 [published Online First: 1987/09/01]
56. Hamer DH, Darmstadt GL, Carlin JB, et al. Etiology of bacteremia in young infants in six countries. *The Pediatric infectious disease journal* 2015;34(1):e1-8. doi: 10.1097/INF.0000000000000549 [published Online First: 2014/11/13]
57. Kisame R, Najjemba R, van Griensven J, et al. Blood Culture Testing Outcomes among Non-Malarial Febrile Children at Antimicrobial Resistance Surveillance Sites in Uganda, 2017-2018. *Trop Med Infect Dis* 2021;6(2):71. doi: 10.3390/tropicalmed6020071 [published Online First: 2021/06/03]
58. Schelonka RL, Chai MK, Yoder BA, et al. Volume of blood required to detect common neonatal pathogens. *The Journal of pediatrics* 1996;129(2):275-78. doi: 10.1016/s0022-3476(96)70254-8 [published Online First: 1996/08/01]

59. Connell TG, Rele M, Cowley D, et al. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics* 2007;119(5):891-6. doi: 10.1542/peds.2006-0440 [published Online First: 2007/05/03]
60. Paisley JW, Lauer BA. Pediatric blood cultures. *Clin Lab Med* 1994;14(1):17-30. [published Online First: 1994/03/01]
61. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 2001;108(5):1169-74. [published Online First: 2001/11/06]
62. Shahan B, Choi EY, Nieves G. Cerebrospinal Fluid Analysis. *Am Fam Physician* 2021;103(7):422-28. [published Online First: 2021/04/01]
63. Ministry of Health RoK. Basic Paediatric Protocols 4th ed, 2016.
64. Adorka M, Honore MK, Lubbe M, et al. The Impact of Appropriate Antibiotic Prescribing on Treatment Evaluation Parameters. *J Public Health Afr* 2013;4(1):e2. doi: 10.4081/jphia.2013.e2 [published Online First: 2013/07/02]
65. Umutesi G, Velin L, Muwanguzi M, et al. Strengthening Antimicrobial Resistance Diagnostic Capacity in Rural Rwanda: A Feasibility Assessment. *Ann Glob Health* 2021;87(1):78. doi: 10.5334/aogh.3416 [published Online First: 2021/08/26]
66. Malania L, Wagenaar I, Karatuna O, et al. Setting up laboratory-based antimicrobial resistance surveillance in low- and middle-income countries: lessons learned from Georgia. *Clin Microbiol Infect* 2021;27(10):1409-13. doi: 10.1016/j.cmi.2021.05.027 [published Online First: 2021/05/28]
67. Gajic I, Kabic J, Kekic D, et al. Antimicrobial Susceptibility Testing: A Comprehensive Review of Currently Used Methods. *Antibiotics (Basel)* 2022;11(4):427. doi: 10.3390/antibiotics11040427 [published Online First: 2022/04/24]
68. Gonzalez MD, Chao T, Pettengill MA. Modern Blood Culture: Management Decisions and Method Options. *Clin Lab Med* 2020;40(4):379-92. doi: 10.1016/j.cll.2020.07.001 [published Online First: 2020/10/31]
69. Rub DM, Dhudasia MB, Healy T, et al. Role of microbiological tests and biomarkers in antibiotic stewardship. *Seminars in perinatology* 2020;44(8):151328. doi: 10.1016/j.semperi.2020.151328 [published Online First: 2020/11/08]
70. Schulga P, Grattan R, Napier C, et al. How to use... lumbar puncture in children. *Arch Dis Child Educ Pract Ed* 2015;100(5):264-71. doi: 10.1136/archdischild-2014-307600 [published Online First: 2015/06/25]
71. Velaphi SC, Westercamp M, Moleleki M, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. *PLoS One* 2019;14(4):e0214077. doi: 10.1371/journal.pone.0214077 [published Online First: 2019/04/11]
72. Águeda S, Leitão A, Rocha G, et al. Viral Infections in a Neonatal Intensive Care Unit. *Pediat Therapeut* 2013;3(154):2161-0665.1000154.
73. Ahmad S, Dalwai A, Al-Nakib W. Frequency of enterovirus detection in blood samples of neonates admitted to hospital with sepsis-like illness in Kuwait. *Journal of medical virology* 2013;85(7):1280-5. doi: 10.1002/jmv.23604
74. Venkatesh M, Flores A, Luna RA, et al. Molecular microbiological methods in the diagnosis of neonatal sepsis. *Expert review of anti-infective therapy* 2010;8(9):1037-48. doi: 10.1586/eri.10.89 [published Online First: 2010/09/08]
75. Kwambana-Adams BA, Liu J, Okoi C, et al. Etiology of Pediatric Meningitis in West Africa Using Molecular Methods in the Era of Conjugate Vaccines against Pneumococcus, Meningococcus, and Haemophilus influenzae Type b. *The American Journal of Tropical Medicine and Hygiene* 2020;103(2):696-703. doi: 10.4269/ajtmh.19-0566 [published Online First: 2020/05/28]

76. Pammi M, Flores A, Versalovic J, et al. Molecular assays for the diagnosis of sepsis in neonates. *The Cochrane database of systematic reviews* 2017;2(2):CD011926. doi: 10.1002/14651858.CD011926.pub2 [published Online First: 2017/02/27]
77. Boula A, Senghore M, Ngoh R, et al. Hospital-based Surveillance Provides Insights Into the Etiology of Pediatric Bacterial Meningitis in Yaounde, Cameroon, in the Post-Vaccine Era. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2019;69(Suppl 2):S148-S55. doi: 10.1093/cid/ciz506 [published Online First: 2019/09/11]
78. Papavasileiou K, Papavasileiou E, Tzanakaki G, et al. Acute bacterial meningitis cases diagnosed by culture and PCR in a children's hospital throughout a 9-Year period (2000-2008) in Athens, Greece. *Mol Diagn Ther* 2011;15(2):109-13. doi: 10.2165/11587690-000000000-00000 [published Online First: 2011/04/02]
79. Lin B, Diaz MH, Waller JL, et al. Optimization of Multiple Pathogen Detection Using the TaqMan Array Card: Application for a Population-Based Study of Neonatal Infection. *PLoS ONE* 2013;8(6):e66183. doi: 10.1371/journal.pone.0066183 [published Online First: 2013/06/28]
80. Diaz MH, Waller JL, Theodore MJ, et al. Development and Implementation of Multiplex TaqMan Array Cards for Specimen Testing at Child Health and Mortality Prevention Surveillance Site Laboratories. *Clinical Infectious Diseases* 2019;69(Supplement_4):S311-S21. doi: 10.1093/cid/ciz571 [published Online First: 2019/10/11]
81. Saha SK, Schrag SJ, El Arifeen S, et al. Causes and incidence of community-acquired serious infections among young children in south Asia (ANISA): an observational cohort study. *Lancet* 2018;392(10142):145-59. doi: 10.1016/S0140-6736(18)31127-9
82. Taylor AW, Blau DM, Bassat Q, et al. Initial findings from a novel population-based child mortality surveillance approach: a descriptive study. *The Lancet Global Health* 2020;8(7):e909-e19. doi: 10.1016/s2214-109x(20)30205-9
83. Hong NTT, Nghia HDT, Thanh TT, et al. Cerebrospinal fluid MinION sequencing of 16S rRNA gene for rapid and accurate diagnosis of bacterial meningitis. *J Infect* 2020;80(4):469-96. doi: 10.1016/j.jinf.2019.12.011 [published Online First: 2020/01/01]
84. Nakagawa S, Inoue S, Kryukov K, et al. Rapid sequencing-based diagnosis of infectious bacterial species from meningitis patients in Zambia. *Clin Transl Immunology* 2019;8(11):e01087. doi: 10.1002/cti2.1087 [published Online First: 2019/11/12]
85. Shetty A, Macdonald SP, Keijzers G, et al. Review article: Sepsis in the emergency department - Part 2: Investigations and monitoring. *Emerg Med Australas* 2018;30(1):4-12. doi: 10.1111/1742-6723.12924 [published Online First: 2018/01/18]
86. Cruz AT, Mahajan P, Bonsu BK, et al. Accuracy of Complete Blood Cell Counts to Identify Febrile Infants 60 Days or Younger With Invasive Bacterial Infections. *JAMA pediatrics* 2017;171(11):e172927. doi: 10.1001/jamapediatrics.2017.2927 [published Online First: 2017/09/12]
87. Escadafal C, Incardona S, Fernandez-Carballo BL, et al. The good and the bad: using C reactive protein to distinguish bacterial from non-bacterial infection among febrile patients in low-resource settings. *BMJ Global Health* 2020;5(5):e002396. doi: 10.1136/bmjgh-2020-002396
88. Durrani NUR, Dutta S, Rochow N, et al. C-reactive protein as a predictor of meningitis in early onset neonatal sepsis: a single unit experience. *Journal of Perinatal Medicine* 2020;48(8):845-51. doi: 10.1515/jpm-2019-0420 [published Online First: 2020/08/10]

89. Sturgeon JP, Zanetti B, Lindo D. C-Reactive Protein (CRP) levels in neonatal meningitis in England: an analysis of national variations in CRP cut-offs for lumbar puncture. *BMC pediatrics* 2018;18(1):380. doi: 10.1186/s12887-018-1354-x [published Online First: 2018/12/05]
90. Pontrelli G, De Crescenzo F, Buzzetti R, et al. Accuracy of serum procalcitonin for the diagnosis of sepsis in neonates and children with systemic inflammatory syndrome: a meta-analysis. *BMC infectious diseases* 2017;17(1):302. doi: 10.1186/s12879-017-2396-7 [published Online First: 2017/04/26]
91. Teggert A, Datta H, Ali Z. Biomarkers for Point-of-Care Diagnosis of Sepsis. *Micromachines (Basel)* 2020;11(3):286. doi: 10.3390/mi11030286 [published Online First: 2020/03/14]
92. Bottieau E, Declercq S, De Weggheleire A, et al. Point of care biomarkers to guide antibiotic prescription for acute febrile illness in sub-Saharan Africa: promises and caveats. *Open Forum Infect Dis* 2020;7(8):ofaa260-ofaa60. doi: 10.1093/ofid/ofaa260
93. Sun B, Liang LF, Li J, et al. A meta-analysis of interleukin-6 as a valid and accurate index in diagnosing early neonatal sepsis. *Int Wound J* 2019;16(2):527-33. doi: 10.1111/iwj.13079 [published Online First: 2019/02/09]
94. Song Y, Chen Y, Dong X, et al. Diagnostic value of neutrophil CD64 combined with CRP for neonatal sepsis: A meta-analysis. *Am J Emerg Med* 2019;37(8):1571-76. doi: 10.1016/j.ajem.2019.05.001 [published Online First: 2019/05/16]
95. Dierikx TH, van Kaam A, de Meij TGJ, et al. Umbilical cord blood culture in neonatal early-onset sepsis: a systematic review and meta-analysis. *Pediatric research* 2021 doi: 10.1038/s41390-021-01792-0 [published Online First: 2021/10/30]
96. Greer R, Safarulla A, Koeppel R, et al. Can Fetal Umbilical Venous Blood Be a Reliable Source for Admission Complete Blood Count and Culture in NICU Patients? *Neonatology* 2019;115(1):49-58. doi: 10.1159/000491993 [published Online First: 2018/10/10]
97. Wilson ML, Mitchell M, Morris A, et al. Principles and procedures for blood cultures; approved guideline. *CLSI document M47-A Clinical and Laboratory Standards Institute, Wayne, PA* 2007
98. Allen E, Cavallaro A, Keir AK. A Quality Improvement Initiative to Reduce Blood Culture Contamination in the Neonatal Unit. *Pediatr Qual Saf* 2021;6(3):e413-e13. doi: 10.1097/pq9.0000000000000413
99. Woodford EC, Dhudasia MB, Puopolo KM, et al. Neonatal blood culture inoculant volume: feasibility and challenges. *Pediatric research* 2021;90(5):1086-92. doi: 10.1038/s41390-021-01484-9 [published Online First: 2021/04/08]
100. Hansen A, Forbes P, Buck R. Potential substitution of cord blood for infant blood in the neonatal sepsis evaluation. *Biol Neonate* 2005;88(1):12-8. doi: 10.1159/000083946 [published Online First: 2005/02/16]
101. Meena J, Charles MV, Ali A, et al. Utility of cord blood culture in early onset neonatal sepsis. *Australas Med J* 2015;8(8):263-7. doi: 10.4066/AMJ.2015.2460 [published Online First: 2015/09/24]
102. Hassanein SM, El-Farrash RA, Hafez HM, et al. Cord blood interleukin-6 and neonatal morbidities among preterm infants with PCR-positive Ureaplasma urealyticum. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* 2012;25(10):2106-10. doi: 10.3109/14767058.2012.678435 [published Online First: 2012/04/12]
103. Midan DA, Abo El Fotoh WMM, El Shalakany AH. The potential role of incorporating real-time PCR and DNA sequencing for amplification and detection of 16S rRNA gene signatures in neonatal sepsis. *The journal of maternal-fetal & neonatal*

medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet 2017;30(12):1476-83. doi: 10.1080/14767058.2016.1219994 [published Online First: 2016/08/04]
104. Meena R, Meena KK, Athwani V, et al. Umbilical Cord Blood Culture in Diagnosis of Early Onset Neonatal Sepsis. *Indian journal of pediatrics* 2020;87(10):793-97. doi: 10.1007/s12098-020-03345-5 [published Online First: 2020/05/30]
105. Kalathia MB, Shingala PA, Parmar PN, et al. Study of Umbilical Cord Blood Culture in Diagnosis of Early-onset Sepsis Among Newborns with High-risk Factors. *Journal of clinical neonatology* 2013;2(4):169-72. doi: 10.4103/2249-4847.123092 [published Online First: 2014/01/10]
106. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
107. Berkley JA, Versteeg AC, Mwangi I, et al. Indicators of acute bacterial meningitis in children at a rural Kenyan district hospital. *Pediatrics* 2004;114(6):e713-9. doi: 10.1542/peds.2004-0007 [published Online First: 2004/12/03]
108. Mwaniki MK, Talbert AW, Njuguna P, et al. Clinical indicators of bacterial meningitis among neonates and young infants in rural Kenya. *BMC infectious diseases* 2011;11:301. doi: 10.1186/1471-2334-11-301 [published Online First: 2011/11/03]
109. Aronson PL, McCulloh RJ, Tieder JS, et al. Application of the Rochester Criteria to Identify Febrile Infants With Bacteremia and Meningitis. *Pediatric emergency care* 2019;35(1):22-27. doi: 10.1097/PEC.0000000000001421 [published Online First: 2018/02/07]
110. Aronson PL, Shabanova V, Shapiro ED, et al. A Prediction Model to Identify Febrile Infants </=60 Days at Low Risk of Invasive Bacterial Infection. *Pediatrics* 2019;144(1):e20183604. doi: 10.1542/peds.2018-3604 [published Online First: 2019/06/07]
111. Bachur RG, Harper MB. Predictive model for serious bacterial infections among infants younger than 3 months of age. *Pediatrics* 2001;108(2):311-6. doi: 10.1542/peds.108.2.311 [published Online First: 2001/08/03]
112. Kuppermann N, Dayan PS, Levine DA, et al. A Clinical Prediction Rule to Identify Febrile Infants 60 Days and Younger at Low Risk for Serious Bacterial Infections. *JAMA pediatrics* 2019;173(4):342-51. doi: 10.1001/jamapediatrics.2018.5501 [published Online First: 2019/02/19]
113. Nigrovic LE, Malley R, Kuppermann N. Meta-analysis of bacterial meningitis score validation studies. *Archives of disease in childhood* 2012;97(9):799-805. doi: 10.1136/archdischild-2012-301798 [published Online First: 2012/07/06]
114. Wynn JL, Polin RA. A neonatal sequential organ failure assessment score predicts mortality to late-onset sepsis in preterm very low birth weight infants. *Pediatric research* 2020;88(1):85-90. doi: 10.1038/s41390-019-0517-2 [published Online First: 2019/08/09]
115. Fleiss N, Coggins SA, Lewis AN, et al. Evaluation of the Neonatal Sequential Organ Failure Assessment and Mortality Risk in Preterm Infants With Late-Onset Infection. *JAMA Netw Open* 2021;4(2):e2036518. doi: 10.1001/jamanetworkopen.2020.36518 [published Online First: 2021/02/05]
116. Zeigler AC, Ainsworth JE, Fairchild KD, et al. Sepsis and Mortality Prediction in Very Low Birth Weight Infants: Analysis of HeRO and nSOFA. *Am J Perinatol* 2021 doi: 10.1055/s-0041-1728829 [published Online First: 2021/05/11]
117. Niederwanger C, Varga T, Hell T, et al. Comparison of pediatric scoring systems for mortality in septic patients and the impact of missing information on their predictive power: a retrospective analysis. *PeerJ* 2020;8:e9993. doi: 10.7717/peerj.9993 [published Online First: 2020/10/22]

118. Klingenberg C, Kornelisse RF, Buonocore G, et al. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Frontiers in pediatrics* 2018;6:285. doi: 10.3389/fped.2018.00285
119. Yehualaw A, Taferre C, Bantie AT, et al. Appropriateness and Pattern of Antibiotic Prescription in Pediatric Patients at Adigart General Hospital, Tigray, Ethiopia. *BioMed research international* 2021;2021:6640892. doi: 10.1155/2021/6640892 [published Online First: 2021/05/04]
120. Atif ML, Sadaoui F, Bezzaoucha A, et al. Prolongation of hospital stay and additional costs due to nosocomial bloodstream infection in an Algerian neonatal care unit. *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* 2008;29(11):1066-70. doi: 10.1086/591858 [published Online First: 2008/10/02]
121. Karam G, Chastre J, Wilcox MH, et al. Antibiotic strategies in the era of multidrug resistance. *Critical care (London, England)* 2016;20(1):136. doi: 10.1186/s13054-016-1320-7 [published Online First: 2016/06/23]
122. Esaiassen E, Fjalstad JW, Juvet LK, et al. Antibiotic exposure in neonates and early adverse outcomes: a systematic review and meta-analysis. *The Journal of antimicrobial chemotherapy* 2017;72(7):1858-70. doi: 10.1093/jac/dkx088 [published Online First: 2017/04/04]
123. Gasparrini AJ, Crofts TS, Gibson MK, et al. Antibiotic perturbation of the preterm infant gut microbiome and resistome. *Gut Microbes* 2016;7(5):443-9. doi: 10.1080/19490976.2016.1218584 [published Online First: 2016/07/30]
124. Silverman MA, Konnikova L, Gerber JS. Impact of Antibiotics on Necrotizing Enterocolitis and Antibiotic-Associated Diarrhea. *Gastroenterol Clin North Am* 2017;46(1):61-76. doi: 10.1016/j.gtc.2016.09.010 [published Online First: 2017/02/07]
125. Vink J, Edgeworth J, Bailey SL. Acquisition of MDR-GNB in hospital settings: a systematic review and meta-analysis focusing on ESBL-E. *The Journal of hospital infection* 2020;106(3):419-28. doi: 10.1016/j.jhin.2020.09.006 [published Online First: 2020/09/13]
126. Antimicrobial Resistance C. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022;399(10325):629-55. doi: 10.1016/S0140-6736(21)02724-0 [published Online First: 2022/01/24]
127. Sands K, Carvalho MJ, Portal E, et al. Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. *Nat Microbiol* 2021;6(4):512-23. doi: 10.1038/s41564-021-00870-7 [published Online First: 2021/03/31]
128. Fox-Lewis A, Takata J, Miliya T, et al. Antimicrobial Resistance in Invasive Bacterial Infections in Hospitalized Children, Cambodia, 2007-2016. *Emerg Infect Dis* 2018;24(5):841-51. doi: 10.3201/eid2405.171830 [published Online First: 2018/04/18]
129. Iroh Tam PY, Musicha P, Kawaza K, et al. Emerging Resistance to Empiric Antimicrobial Regimens for Pediatric Bloodstream Infections in Malawi (1998-2017). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2019;69(1):61-68. doi: 10.1093/cid/ciy834 [published Online First: 2018/10/03]
130. Wen SCH, Ezure Y, Rolley L, et al. Gram-negative neonatal sepsis in low- and lower-middle-income countries and WHO empirical antibiotic recommendations: A systematic review and meta-analysis. *PLoS Med* 2021;18(9):e1003787. doi: 10.1371/journal.pmed.1003787 [published Online First: 2021/09/29]
131. Dharmapalan D, Shet A, Yewale V, et al. High Reported Rates of Antimicrobial Resistance in Indian Neonatal and Pediatric Blood Stream Infections. *Journal of*

the Pediatric Infectious Diseases Society 2017;6(3):e62-e68. doi: 10.1093/jpids/piw092 [published Online First: 2017/03/25]
132. Williams PCM, Isaacs D, Berkley JA. Antimicrobial resistance among children in sub-Saharan Africa. *The Lancet Infectious diseases* 2018;18(2):e33-e44. doi: 10.1016/S1473-3099(17)30467-X [published Online First: 2017/10/17]
133. Blomberg B, Jureen R, Manji KP, et al. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. *Journal of clinical microbiology* 2005;43(2):745-9. doi: 10.1128/JCM.43.2.745-749.2005 [published Online First: 2005/02/08]
134. Le Doare K, Bielicki J, Heath PT, et al. Systematic Review of Antibiotic Resistance Rates Among Gram-Negative Bacteria in Children With Sepsis in Resource-Limited Countries. *Journal of the Pediatric Infectious Diseases Society* 2015;4(1):11-20. doi: 10.1093/jpids/piu014 [published Online First: 2015/09/26]
135. Manenzhe RI, Zar HJ, Nicol MP, et al. The spread of carbapenemase-producing bacteria in Africa: a systematic review. *The Journal of antimicrobial chemotherapy* 2015;70(1):23-40. doi: 10.1093/jac/dku356 [published Online First: 2014/09/28]
136. Shinu P, Mouslem AKA, Nair AB, et al. Progress Report: Antimicrobial Drug Discovery in the Resistance Era. *Pharmaceuticals (Basel)* 2022;15(4):413. doi: 10.3390/ph15040413 [published Online First: 2022/04/24]
137. Liu Y, Tong Z, Shi J, et al. Drug repurposing for next-generation combination therapies against multidrug-resistant bacteria. *Theranostics* 2021;11(10):4910-28. doi: 10.7150/thno.56205 [published Online First: 2021/03/24]
138. Darlow CA, da Costa RMA, Ellis S, et al. Potential Antibiotics for the Treatment of Neonatal Sepsis Caused by Multidrug-Resistant Bacteria. *Paediatr Drugs* 2021 doi: 10.1007/s40272-021-00465-z [published Online First: 2021/08/27]
139. Kent A, Turner MA, Sharland M, et al. Aminoglycoside toxicity in neonates: something to worry about? *Expert review of anti-infective therapy* 2014;12(3):319-31. doi: 10.1586/14787210.2014.878648 [published Online First: 2014/01/25]
140. Hendlin D, Stapley EO, Jackson M, et al. Phosphonomycin, a new antibiotic produced by strains of streptomyces. *Science* 1969;166(3901):122-3. doi: 10.1126/science.166.3901.122
141. Raz R. Fosfomycin: an old--new antibiotic. *Clin Microbiol Infect* 2012;18(1):4-7. doi: 10.1111/j.1469-0691.2011.03636.x [published Online First: 2011/09/15]
142. Kahan FM, Kahan JS, Cassidy PJ, et al. The mechanism of action of fosfomycin (phosphonomycin). *Ann N Y Acad Sci* 1974;235(0):364-86. doi: 10.1111/j.1749-6632.1974.tb43277.x [published Online First: 1974/05/10]
143. Falagas ME, Kastoris AC, Kapaskelis AM, et al. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. *The Lancet Infectious diseases* 2010;10(1):43-50. doi: 10.1016/S1473-3099(09)70325-1 [published Online First: 2010/02/05]
144. Cassir N, Rolain JM, Brouqui P. A new strategy to fight antimicrobial resistance: the revival of old antibiotics. *Front Microbiol* 2014;5(551):551. doi: 10.3389/fmicb.2014.00551 [published Online First: 2014/11/05]
145. Infectopharm Arzneimittel und Consilium GmbH . Fomicyt Package Insert. Heppenheim, Germany: Infectopharm; 2015.
146. Traunmuller F, Popovic M, Konz KH, et al. A reappraisal of current dosing strategies for intravenous fosfomycin in children and neonates. *Clinical pharmacokinetics* 2011;50(8):493-503. doi: 10.2165/11592670-000000000-00000 [published Online First: 2011/07/12]

147. Koletzko B, Goulet O, Hunt J, et al. 1. Guidelines on Paediatric Parenteral Nutrition of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Clinical Nutrition and Metabolism (ESPEN), Supported by the European Society of Paediatric Research (ESPR). *J Pediatr Gastroenterol Nutr* 2005;41 Suppl 2:S1-87. doi: 10.1097/01.mpg.0000181841.07090.f4 [published Online First: 2005/10/29]
148. European Medicines Agency. Product Information as approved by the CHMP on 26 March 2020, pending endorsement by the European Commission 2020 [Available from: https://www.ema.europa.eu/en/documents/referral/fosfomycin-article-31-referral-annex-iii_en.pdf accessed 18th June 2020.
149. Li G, Standing JF, Bielicki J, et al. The Potential Role of Fosfomycin in Neonatal Sepsis Caused by Multidrug-Resistant Bacteria. *Drugs* 2017;77(9):941-50. doi: 10.1007/s40265-017-0745-x [published Online First: 2017/05/01]
150. Guggenbichler JP, Kienel G, Frisch H. [Fosfomycin, a new antibiotic drug (author's transl)]. *Padiatr Padol* 1978;13(4):429-36. [published Online First: 1978/01/01]
151. Molina MA, Olay T, Quero J. Pharmacodynamic data on fosfomycin in underweight infants during the neonatal period. *Chemotherapy* 1977;23 Suppl 1:217-22. doi: 10.1159/000222051 [published Online First: 1977/01/01]
152. Bergan T. Degree of absorption, pharmacokinetics of fosfomycin trometamol and duration of urinary antibacterial activity. *Infection* 1990;18 Suppl 2:S65-9. doi: 10.1007/bf01643430 [published Online First: 1990/01/01]
153. Bergan T, Thorsteinsson SB, Albini E. Pharmacokinetic profile of fosfomycin trometamol. *Chemotherapy* 1993;39(5):297-301. doi: 10.1159/000239140 [published Online First: 1993/09/01]
154. Ito M, Ishigami T. The meaning of the development of flomoxef and clinical experience in Japan. *Infection* 1991;19 Suppl 5:S253-7. doi: 10.1007/BF01645536 [published Online First: 1991/01/01]
155. Iwai N, Nakamura H, Miyazu M, et al. [Laboratory and clinical evaluations of flomoxef sodium in neonates]. *Jpn J Antibiot* 1991;44(11):1265-85. [published Online First: 1991/11/01]
156. Darlow CA, Hope W. Flomoxef for neonates: extending options for treatment of neonatal sepsis caused by ESBL-producing Enterobacterales. *The Journal of antimicrobial chemotherapy* 2022;77(3):711-18. doi: 10.1093/jac/dkab468 [published Online First: 2021/12/31]
157. Sato H, Narita A, Matsumoto K, et al. [Studies of flomoxef in neonates]. *Jpn J Antibiot* 1991;44(11):1250-8. [published Online First: 1991/11/01]
158. Kuno K, Nakao Y, Hayakawa F, et al. [Bacteriological and clinical studies of flomoxef in the field of pediatrics]. *Jpn J Antibiot* 1987;40(8):1393-406. [published Online First: 1987/08/01]
159. Fujita K, Murono K, Saijyo M, et al. [Flomoxef in neonates and young infants; clinical efficacy, pharmacokinetic evaluation and effect on the intestinal bacterial flora]. *Jpn J Antibiot* 1991;44(11):1216-27. [published Online First: 1991/11/01]
160. Kessler RE, Bies M, Buck RE, et al. Comparison of a new cephalosporin, BMY 28142, with other broad-spectrum beta-lactam antibiotics. *Antimicrob Agents Chemother* 1985;27(2):207-16. doi: 10.1128/AAC.27.2.207 [published Online First: 1985/02/01]
161. Sanders CC. Cefepime: the next generation? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1993;17(3):369-79. [published Online First: 1993/09/01]
162. Kotapati S, Kuti JL, Nightingale CH, et al. Clinical implications of extended spectrum beta-lactamase (ESBL) producing Klebsiella species and Escherichia coli on cefepime effectiveness. *J Infect* 2005;51(3):211-7. doi: 10.1016/j.jinf.2005.01.005 [published Online First: 2005/10/19]

163. Haidar G, Alkroud A, Cheng S, et al. Association between the Presence of Aminoglycoside-Modifying Enzymes and In Vitro Activity of Gentamicin, Tobramycin, Amikacin, and Plazomicin against Klebsiella pneumoniae Carbapenemase- and Extended-Spectrum-beta-Lactamase-Producing Enterobacter Species. *Antimicrob Agents Chemother* 2016;60(9):5208-14. doi: 10.1128/AAC.00869-16 [published Online First: 2016/06/15]
164. Kruk ME, Chukwuma A, Mbaruku G, et al. Variation in quality of primary-care services in Kenya, Malawi, Namibia, Rwanda, Senegal, Uganda and the United Republic of Tanzania. *Bulletin of the World Health Organization* 2017;95(6):408-18. doi: 10.2471/BLT.16.175869 [published Online First: 2017/06/13]
165. McKinnon B, Harper S, Kaufman JS, et al. Removing user fees for facility-based delivery services: a difference-in-differences evaluation from ten sub-Saharan African countries. *Health policy and planning* 2015;30(4):432-41. doi: 10.1093/heapol/czu027 [published Online First: 2014/05/13]
166. Simmons RA, Anthopolos R, O'Meara WP. Effect of health systems context on infant and child mortality in sub-Saharan Africa from 1995 to 2015, a longitudinal cohort analysis. *Sci Rep* 2021;11(1):16263. doi: 10.1038/s41598-021-95886-8 [published Online First: 2021/08/13]
167. The Kenya Gazette. Notice No. 786. The Medical Practitioners and Dentists Act (Cap 253). Available online http://dc.sourceafrica.net/documents/119977-Kenya-Gazette-Notice-No-786-Categorization-of.html; accessed 2Mar2022.
168. Ministry of Health. Kenya Health Policy 2014-2030. Accessed on 9 November 2021. Available online: http://publications.universalhealth2030.org/uploads/kenya_health_policy_2014_to_2030.pdf
169. Ministry of Health. Kenya Laboratory Capacity Mapping Report. February 2020. Available online https://mapping.nphl.go.ke/wp-content/uploads/2018/01/Kenya_Laboratory_Capacity_Report_February_2020.pdf; accesed 3 Mar 2022.
170. Gathara D, Opiyo N, Wagai J, et al. Quality of hospital care for sick newborns and severely malnourished children in Kenya: a two-year descriptive study in 8 hospitals. *BMC Health Serv Res* 2011;11:307. doi: 10.1186/1472-6963-11-307 [published Online First: 2011/11/15]
171. Gathara D, Serem G, Murphy GAV, et al. Missed nursing care in newborn units: a cross-sectional direct observational study. *BMJ Qual Saf* 2020;29(1):19-30. doi: 10.1136/bmjqs-2019-009363 [published Online First: 2019/06/07]
172. Opondo C, Allen E, Todd J, et al. Association of the Paediatric Admission Quality of Care score with mortality in Kenyan hospitals: a validation study. *The Lancet Global health* 2018;6(2):e203-e10. doi: 10.1016/S2214-109X(17)30484-9 [published Online First: 2018/02/02]
173. Ayieko P, Ogero M, Makone B, et al. Characteristics of admissions and variations in the use of basic investigations, treatments and outcomes in Kenyan hospitals within a new Clinical Information Network. *Archives of disease in childhood* 2016;101(3):223-9. doi: 10.1136/archdischild-2015-309269 [published Online First: 2015/12/15]
174. Ayieko P, Ntoburi S, Wagai J, et al. A multifaceted intervention to implement guidelines and improve admission paediatric care in Kenyan district hospitals: a cluster randomised trial. *PLoS Med* 2011;8(4):e1001018. doi: 10.1371/journal.pmed.1001018 [published Online First: 2011/04/13]

Chapter 2

Research setting

KILIFI COUNTY HOSPITAL

Kilifi County, Kenya is located 3 degrees South of the equator on the Indian Ocean coast, covers an area of 12,540 km^2 and has an estimated population of 1.5 million people.[1] Sixty-three percent of the residents live in rural areas and majority are dependent on subsistence farming.[2] The county has 5 public hospitals and 138 primary health facilities[3] and less than half of these facilities offer routine laboratory services.[2] Kilifi County Hospital (KCH), the primary government-run referral hospital in the county, provides comprehensive inpatient and outpatient healthcare services to adults and children. In 2019, under-five mortality rate in Kilifi county (43 deaths per 1,000 live births)[4] was similar to the Kenya national rate of 42 deaths per 1,000 live births,[5] and neonatal disorders were the second-leading causes of death and disability in the county.[4] Sepsis and meningitis are among the leading causes of child mortality in the county and considerable burden of neurological impairment in young children following neonatal insults has been observed.[6] Childhood vaccines are provided free of charge at all government-run health facilities and vaccine coverage in Kilifi (82%) is higher than the national average of 68% (including conjugate vaccines).[7 8] Significant decline in invasive *H. influenzae* type b[9] and *S. pneumoniae*[10] disease and nasopharyngeal carriage in young children in Kilifi has been reported. Kilifi has experienced a decline in malaria prevalence and case fatality, and changes in clinical spectrum following decades-long use of effective prevention and treatment strategies.[11] Proportion of health facility deliveries and neonatal admissions have increased over the past 3 decades,[12-14] especially after the abolishment of maternity user fees in Kenya (Free Maternity Service policy in 2013[13] subsequently revised to the Linda Mama policy in 2017[14]). On average, approximately 6,000 neonates are now born at KCH annually. More effort is needed to ensure that sick children are appropriately diagnosed and treated in order to improve child survival in Kilifi County.[7 15 16]

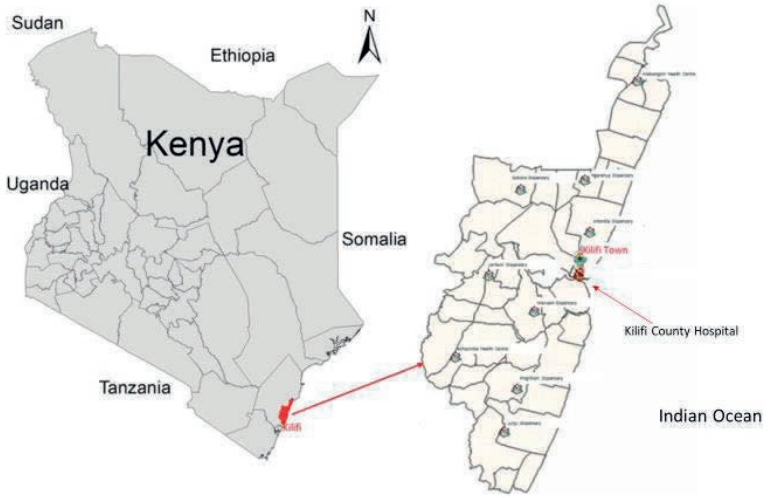


Figure 2. A map showing location of Kilifi County Hospital (KCH) within Kilifi County[17]

The studies described in this thesis include children hospitalised at KCH during the respective study periods. The paediatric unit consists of a 72-bed general paediatric ward, a neonatal unit with capacity for 41 neonates (9 incubators, 22 cots and 10 radiant warmers), and a separate high dependency unit (HDU) staffed and run by the Kenya Medical Research Institute – Wellcome Trust Research Programme (KWTRP). The HDU has limited capacity for invasive treatment and procedures and consists of a 6-bed main ward and 2 separate warm rooms with a total capacity of 15 neonates (4 incubators and 11 cots). Approximately 1,100 critically ill children are admitted to the HDU annually and this comprises 30% of children hospitalised at KCH each year (unpublished data). Close monitoring of patients, training of clinical staff, senior oversight, and reliable diagnostic services provided by KWTRP laboratories means that higher level of care is provided to patients at KCH paediatric unit compared to other public hospitals in Kenya. The KWTRP laboratories in Kilifi have implemented Good Clinical Laboratory Practice (GCLP), are accredited by Qualogy® and are quality assured by the UK National External Quality Assessment service.

Children presenting for admission at KCH are reviewed by qualified and trained clinicians who obtain medical history from parents/guardians and perform physical examination. Routine investigations performed at admission as clinically indicated include CBC, microscopy for malaria parasites, renal function

tests, blood gas analysis (in seriously ill children), blood culture (BACTEC™ Plus Aerobic/F bottles and BACTEC™ 9050 instrument, BD, USA), and CSF culture (including chemistry, microscopy and antigen test).[18 19] Systematic HIV-1 testing has been performed since 2007 using the national rapid diagnostic test serial algorithm as previously described.[20] Demographic, clinical and laboratory data are collected on a standard proforma and systematically entered real time into a password-protected database (Kilifi Integrated Data Management System [KIDMS]). Empiric antimicrobials are prescribed according to WHO[21] and Kenya national paediatric guidelines.[22]

SURVEILLANCE PLATFORMS WITHIN KILIFI COUNTY

Kilifi Health and Demographic Surveillance System (KHDSS)

The KHDSS (Figure 3) was established in 2000 by the KWTRP and covers an area of 891 km^2 with an approximate population of 300,000 residents.[23] KWTRP conducts medical research within the KHDSS and monitors pregnancies, births, migration events, and deaths through quarterly home visits. KCH is nested within the KHDSS and serves most of its residents, providing linkage between hospital morbidity and community surveillance. KHDSS residents are linked to KCH via KIDMS for continuous monitoring of the incidence and prevalence of disease. KHDSS also serves as a sampling frame for research done by KWTRP including the evaluation of the impact of interventions such as vaccines.[10] A recent analysis of mortality within KHDSS between 2003 and 2018 showed majority (39%) of under-five deaths occurred during the neonatal period.[24] Overall under-five mortality was shown to have a steep decline between 2003-2008 followed by a gradual decline in subsequent years, demonstrating the impact of life-saving interventions on child survival. However, the slow decline over the past few years compounded by an increase in the burden of mortality attributed to the neonatal period suggests that more efforts are needed to improve outcomes in this vulnerable group.

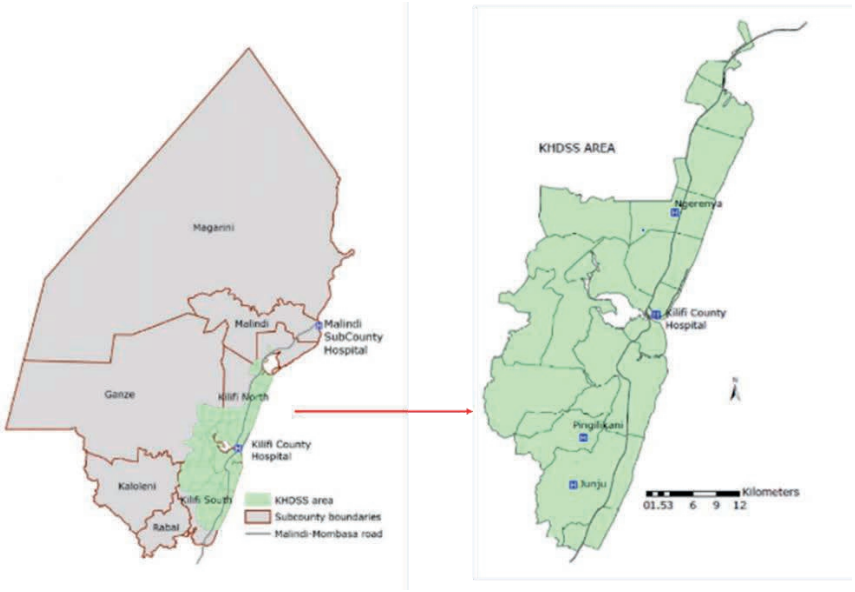


Figure 3. Kilifi Health and Demographic Surveillance System (KHDSS) area within Kilifi county

Paediatric ward surveillance at the Kilifi County Hospital (KCH)

KWTRP has carried out longitudinal clinical surveillance of invasive bacterial infections (IBI) in children hospitalised at KCH since 1998.[18] All children (except those admitted for elective procedures or observation following minor trauma) have a blood sample for blood culture collected at admission for routine care and surveillance for bacteraemia. Clinical and laboratory data obtained through surveillance conducted in the KCH paediatric unit has provided evidence of the changing epidemiology of community and hospital-acquired bacteraemia and AMR in our setting.[18 25-27] Susceptibility of systematically collected and archived culture isolates to fosfomycin have also been tested *in vitro* and results showed that fosfomycin monotherapy was highly active against invasive Gram-negative isolates, including 90% of Enterobacteriales and 96% of *Pseudomonas* spp.[28] All 247 isolates were susceptible to a fosfomycin plus amikacin combination, indicating the potential use of this combination in future combinations for treatment of sepsis in children.

Kilifi Perinatal and Maternal (KIPMAT) surveillance

KIPMAT is a ward-based surveillance embedded into routine clinical care to evaluate the potential and risk factors of maternal and infant morbidity and mortality (ClinicalTrials.gov Identifier: NCT01757028).[29] Between 1st January 2011 and 30th September 2021, pregnant mothers presenting in labour at the KCH maternity ward underwent structured physical examination following registration and medical history taking. Data on key variables such as antenatal history and examination, delivery details, and maternal and neonatal outcomes (Addendum 3) were systematically collected on a structured maternal admission record (MAR). Pathogens and risk factors of interest included GBS, human immunodeficiency virus (HIV), malaria, maternal nutrition, and anaemia. Follow-up through linkage to KHDSS data was done for maternal readmissions, child admissions and survival during the first 2 years of life. A CBC, random blood glucose, a rapid test for malaria infection and a urine dipstick were done as routine investigations for clinical care, and rapid HIV testing was offered if not tested during antenatal visits, according to national guidelines.[30] Additional samples collected for research purposes include vaginal and rectal swabs for GBS carriage, maternal blood (via venepuncture), cord blood and placental biopsy to assess immune responses to infection, characterise infections using molecular methods, and determine other factors affecting foetal growth and maturity. We used cord blood samples collected and archived under KIPMAT between March 2011 and March 2016 to investigate the pathogens causing EONS using TaqMan PCR.

OUTLINE OF THIS THESIS

Chapter 1 introduces this thesis by giving a detailed description of the burden, causes, clinical presentation and antimicrobial treatment of sepsis and meningitis in sub-Saharan Africa. Key gaps and priorities in research and clinical practice are highlighted, and the research objectives of this thesis are listed. **Chapter 2** describes, KCH, which serves as the study site of research covered in this thesis and concludes with a brief description of the thesis outline. Description of the study site is vital to understanding of the health care service delivery gaps prevalent in most public health facilities in Kenya, and the importance of this research in generating evidence that can be used to improve outcomes in these settings.

Part I of this thesis explores the use of a customised multiplex TaqMan Array Card (TAC) polymerase chain reaction (PCR) platform to identify bacterial and

viral causes of EONS (**Chapter 3**). I compared pathogens detected in cord blood samples between neonates who developed possible serious bacterial infection (pSBI) in the first 48 hours of life (cases: survived and died) and neonates remaining well (controls). Cord blood samples were obtained at delivery under KIPMAT.

Part II describes re-validation of clinical indicators of meningitis in young infants aged <60 days (**Chapter 4**) and children aged between 60 days and 13 years (**Chapter 5**) given changes in disease epidemiology and patient profile following widespread conjugate vaccination. I performed retrospective analyses of clinical and microbiological data to investigate the performance of signs included in current meningitis guidelines in distinguishing children with meningitis from those without meningitis. **Chapter 6** describes the clinical and microbiological profile of serious bacterial infection (SBI [bacteraemia and/or meningitis]) and validation of the performance of the NeoObs clinical and laboratory criteria in distinguishing infants aged <60 days with SBI from those without SBI. These signs were used to screen and identify infants at high risk of serious infection in the NeoObs study (ClinicalTrials.gov ID. NCT03721302). In addition, I validated the NeoSEP severity score against mortality.

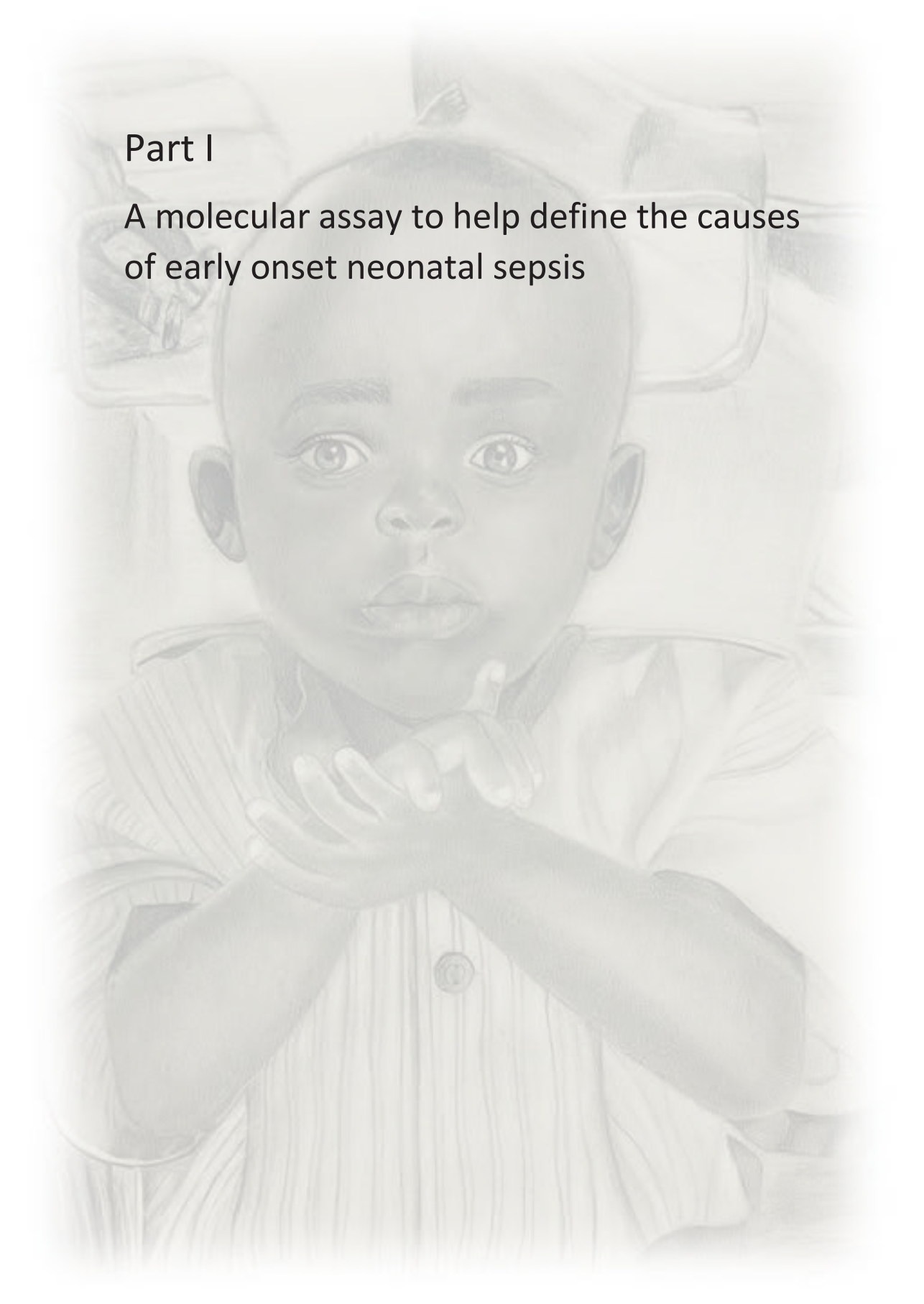
Part III examines the safety and PK profile of fosfomycin for neonatal sepsis in the context of rising antimicrobial resistance (**Chapter 7**). In this trial, we enrolled neonates aged ≤28 days hospitalised with clinical sepsis and randomised to receive either standard-of-care (SOC) antibiotics only or SOC plus fosfomycin (SOC-F).

The last chapter of this thesis summarises and discusses the main findings (**Chapter 8**) of this research and their implications on future perspectives. The chapter ends with concluding remarks on the diagnosis and management of neonates and young children hospitalised with sepsis and/or meningitis in rural Kenya.

REFERENCES

1. Kenya National Bureau of Statistics. 2019 Kenya Population and Housing Census Volume I: Population by County and Sub-County. November 2019. Accessed at https://www.knbs.or.ke/?wpdmpro=2019-kenya-population-and-housing-census-volume-i-population-by-county-and-sub-county# on 20th August 2021.
2. County Government of Kilifi. County Integrated Development Plan 2018–2022. Kilifi 2018. Accessed at https://www.kilifi.go.ke/lib.php?com=5&res_id=1202 on 19 August 2021.
3. County Government of Kilifi. Accessed on 19 February 2022; https://kilifi.go.ke/ova_dep/health-services/ [
4. Institute for Health Metrics and Evaluation. Accessed on 19 February 2022; https://www.healthdata.org/kenya-kilifi [
5. United Nations Inter-agency Group for Child Mortality Estimation (UNIGME) LTiCMR, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation', United Nations Children's Fund, New York, 2020.
6. Mung'ala-Odera V, Meehan R, Njuguna P, et al. Prevalence and risk factors of neurological disability and impairment in children living in rural Kenya. *Int J Epidemiol* 2006;35(3):683-8. doi: 10.1093/ije/dyl023 [published Online First: 2006/02/24]
7. Kenya Demographic and Health Survey 2014. December 2015. Accessed at https://dhsprogram.com/pubs/pdf/FR308/FR308.pdf on 24th August 2021.
8. Adetifa IMO, Karia B, Mutuku A, et al. Coverage and timeliness of vaccination and the validity of routine estimates: Insights from a vaccine registry in Kenya. *Vaccine* 2018;36(52):7965-74. doi: 10.1016/j.vaccine.2018.11.005 [published Online First: 2018/11/13]
9. Hammitt LL, Crane RJ, Karani A, et al. Effect of Haemophilus influenzae type b vaccination without a booster dose on invasive H influenzae type b disease, nasopharyngeal carriage, and population immunity in Kilifi, Kenya: a 15-year regional surveillance study. *The Lancet Global health* 2016;4(3):e185-94. doi: 10.1016/S2214-109X(15)00316-2 [published Online First: 2016/02/09]
10. Hammitt LL, Etyang AO, Morpeth SC, et al. Effect of ten-valent pneumococcal conjugate vaccine on invasive pneumococcal disease and nasopharyngeal carriage in Kenya: a longitudinal surveillance study. *Lancet* 2019;393(10186):2146-54. doi: 10.1016/S0140-6736(18)33005-8 [published Online First: 2019/04/20]
11. Njuguna P, Maitland K, Nyaguara A, et al. Observational study: 27 years of severe malaria surveillance in Kilifi, Kenya. *BMC medicine* 2019;17(1):124. doi: 10.1186/s12916-019-1359-9 [published Online First: 2019/07/10]
12. Mwaniki MK, Gatakaa HW, Mturi FN, et al. An increase in the burden of neonatal admissions to a rural district hospital in Kenya over 19 years. *BMC public health* 2010;10:591. doi: 10.1186/1471-2458-10-591 [published Online First: 2010/10/12]
13. Lang'at E, Mwanri L, Temmerman M. Effects of implementing free maternity service policy in Kenya: an interrupted time series analysis. *BMC Health Serv Res* 2019;19(1):645. doi: 10.1186/s12913-019-4462-x [published Online First: 2019/09/08]
14. Orangi S, Kairu A, Malla L, et al. Impact of free maternity policies in Kenya: an interrupted time-series analysis. *BMJ Glob Health* 2021;6(6):e003649. doi: 10.1136/bmjgh-2020-003649 [published Online First: 2021/06/11]
15. Macharia PM, Giorgi E, Thuranira PN, et al. Sub national variation and inequalities in under-five mortality in Kenya since 1965. *BMC public health* 2019;19(1):146. doi: 10.1186/s12889-019-6474-1 [published Online First: 2019/02/06]

16. Deribew A, Ojal J, Karia B, et al. Under-five mortality rate variation between the Health and Demographic Surveillance System (HDSS) and Demographic and Health Survey (DHS) approaches. *BMC public health* 2016;16(1):1118. doi: 10.1186/s12889-016-3786-2 [published Online First: 2016/10/26]
17. Wambui KM, Musenge E. A space-time analysis of recurrent malnutrition-related hospitalisations in Kilifi, Kenya for children under-5 years. *BMC Nutr* 2019;5:32. doi: 10.1186/s40795-019-0296-5 [published Online First: 2020/03/11]
18. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *The New England journal of medicine* 2005;352(1):39-47. doi: 10.1056/NEJMoa040275 [published Online First: 2005/01/07]
19. Berkley JA, Mwangi I, Ngetsa CJ, et al. Diagnosis of acute bacterial meningitis in children at a district hospital in sub-Saharan Africa. *Lancet* 2001;357(9270):1753-7. doi: 10.1016/S0140-6736(00)04897-2 [published Online First: 2001/06/14]
20. Obiero CW, Seale AC, Jones K, et al. Should first-line empiric treatment strategies cover coagulase-negative staphylococcal infections in severely malnourished or HIV-infected children in Kenya? *PLoS One* 2017;12(8):e0182354. doi: 10.1371/journal.pone.0182354 [published Online First: 2017/08/09]
21. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed: World Health Organization, 2013:412.
22. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
23. Scott JA, Bauni E, Moisi JC, et al. Profile: The Kilifi Health and Demographic Surveillance System (KHDSS). *Int J Epidemiol* 2012;41(3):650-7. doi: 10.1093/ije/dys062 [published Online First: 2012/05/01]
24. Otiende M, Bauni E, Nyaguara A, et al. Mortality in rural coastal Kenya measured using the Kilifi Health and Demographic Surveillance System: a 16-year descriptive analysis. *Wellcome Open Research* 2021;6(327) doi: 10.12688/wellcomeopenres.17307.1
25. Aiken AM, Mturi N, Njuguna P, et al. Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study. *Lancet* 2011;378(9808):2021-27. doi: 10.1016/S0140-6736(11)61622-X [published Online First: 2011/12/03]
26. Seale AC, Davies MR, Anampiu K, et al. Invasive Group A Streptococcus Infection among Children, Rural Kenya. *Emerg Infect Dis* 2016;22(2):224-32. doi: 10.3201/eid2202.151358 [published Online First: 2016/01/27]
27. Talbert AW, Mwaniki M, Mwarumba S, et al. Invasive bacterial infections in neonates and young infants born outside hospital admitted to a rural hospital in Kenya. *The Pediatric infectious disease journal* 2010;29(10):945-9. doi: 10.1097/INF.0b013e3181dfca8c [published Online First: 2010/04/27]
28. Williams PCM, Waichungo J, Gordon NC, et al. The potential of fosfomycin for multi-drug resistant sepsis: an analysis of in vitro activity against invasive paediatric Gram-negative bacteria. *Journal of medical microbiology* 2019;68(5):711-19. doi: 10.1099/jmm.0.000973 [published Online First: 2019/04/18]
29. Seale AC, Barsosio HC, Koech AC, et al. Embedding surveillance into clinical care to detect serious adverse events in pregnancy. *Vaccine* 2015;33(47):6466-8. doi: 10.1016/j.vaccine.2015.07.086 [published Online First: 2015/08/10]
30. National AIDS and STI Control Programme, Ministry of Health, Kenya. National HTS Training Manual in Kenya. Nairobi: NASCOP; 2018. Assessed on 22 February 2022 at https://www.nascop.or.ke/hiv-testing-services/.



Part I

A molecular assay to help define the causes of early onset neonatal sepsis

Chapter 3

Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR

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Wellcome Open Res 2022;7:3.

ABSTRACT

Background Early onset neonatal sepsis (EONS) typically begins prior to, during or soon after birth and may be rapidly fatal. There is paucity of data on the aetiology of EONS in sub-Saharan Africa due to limited diagnostic capacity in this region, despite the associated significant mortality and long-term neurological impairment.

Methods We compared pathogens detected in cord blood samples between neonates admitted to hospital with possible serious bacterial infection (pSBI) in the first 48 hours of life (cases) and neonates remaining well (controls). Cord blood was systematically collected at Kilifi County Hospital (KCH) from 2011-2016, and later tested for 21 bacterial, viral and protozoal targets using multiplex PCR via TaqMan Array Cards (TAC).

Results Among 603 cases (101 [17%] of whom died), 179 (30%) tested positive for ≥1 target and 37 (6.1%) tested positive for multiple targets. *Klebsiella oxytoca*, *Escherichia coli/Shigella* spp., *Pseudomonas aeruginosa,* and *Streptococcus pyogenes* were commonest. Among 300 controls, 79 (26%) tested positive for ≥1 target, 11 (3.7%) were positive for multiple targets, and *K. oxytoca* and *P. aeruginosa* were most common. Cumulative odds ratios across controls:cases (survived):cases (died) were *E. coli/Shigella* spp. 2.6 (95%CI 1.6-4.4); *E. faecalis* 4.0 (95%CI 1.1-15); *S. agalactiae* 4.5 (95%CI 1.6-13); *Ureaplasma* spp. 2.9 (95%CI 1.3-6.4); Enterovirus 9.1 (95%CI 2.3-37); and *Plasmodium* spp. 2.9 (95%CI 1.4-6.2). Excluding *K. oxytoca* and *P. aeruginosa* as likely contaminants, aetiology was attributed in 9.4% (95%CI 5.1-13) cases using TAC. Leading pathogen attributions by TAC were *E. coli/Shigella* spp. (3.5% (95%CI 1.7-5.3)) and *Ureaplasma* spp. (1.7% (95%CI 0.5-3.0)).

Conclusions Cord blood sample may be useful in describing EONS pathogens at birth, but more specific tests are needed for individual diagnosis. Careful sampling of cord blood using aseptic techniques is crucial to minimize contamination. In addition to culturable bacteria, *Ureaplasma* and Enterovirus were causes of EONS.

INTRODUCTION

Forty-one percent of global neonatal deaths occur in sub-Saharan Africa[1] and the risk of dying is highest in the first week of life[2]. Infection is a leading cause of neonatal mortality, accounting for ~37% of deaths in sub-Saharan Africa[3], and is associated with long-term neurological impairment[4]. Early-onset neonatal sepsis (EONS) is often due to maternal transmission of pathogens[5] prior to, during, or soon after birth, and can be rapidly fatal. Neonatal sepsis lacks a consensus definition and the reference point for EONS is variable, based on the timing of onset of symptoms or sampling of a positive culture[6], i.e., occurring within the first 48 hours[7 8], 72 hours[9] or seven days[10] of life. Most research conducted in developing countries has focused on culturable bacterial pathogens, with *Klebsiella* spp*., Escherichia coli* and *Staphylococcus aureus* identified as leading causes of EONS.[11 12] Group B Streptococcus (GBS) has been variably implicated in EONS, and may be underestimated due to its rapid fatality and surveillance methodology[13-15]. There are limited published data on viruses such as Herpes Simplex Virus (HSV)[16] and Cytomegalovirus (CMV)[17] as causes of EONS in this setting.

Blood culture is the gold standard diagnostic test for EONS, despite low sensitivity[18 19]. One to two millilitres of blood volume is recommended to improve microorganism recovery[20], but smaller volumes are often obtained from sick neonates. Intrapartum antimicrobials[21], presence of fastidious organisms and culture contamination may also contribute to low culture yields. Lack of availability of microbiology facilities, lengthy turn-around times[22] and high rates of culture-negative sepsis contribute to antibiotic consumption[19], exacerbating antimicrobial resistance[23], affecting the gut microbiota[24], and potentially missing important non-culturable organisms.

Nucleic acid amplification techniques can detect a broad range of pathogens[25] with up to 90% sensitivity and 93% specificity compared to microbial culture in some studies[22]. Recently, a custom TaqMan Array Card (TAC) approach based on quantitative reverse-transcription polymerase chain reaction (RT-qPCR)[26] was applied to neonatal blood and respiratory samples in South Asia and South Africa[27 28]. Causal attribution of organisms identified in blood and respiratory samples in EONS using latent class modelling was 23% in South Asia[28] and 27% in South Africa[27]. Bacteria were predominant and *Ureaplasma* spp. was identified as a significant pathogen in these studies. However, healthy controls were not sampled in identical circumstances to cases in South Asia (cases were

recruited from study health facilities while controls were identified from the community using an automated algorithm; controls were older than cases at sample collection)[28] whilst in South Africa both cases and controls were recruited from the study hospital[27].

Cord blood provides a potential opportunity for early pathogen detection prior to the clinical onset of infection, and with adequate sample volumes[29]. Biomarkers in cord blood may correlate with peripheral blood parameters including total and differential white blood cell counts[30], and acute phase reactants such as C-reactive protein, serum amyloid A, haptoglobin[31], interleukin-6 and procalcitonin[32]. Culture, PCR and sequencing have identified pathogenic bacteria and correlate with acute phase reactants in cord blood[33 34]. However, cord blood contamination may easily occur[29 33 35].

We hypothesized that pathogens detected in cord blood using a molecular technique would be associated with subsequent admission and death with suspected EONS. In a nested case control study of cord blood samples systematically collected at birth, we selected neonates hospitalized within 48 hours of life with possible serious bacterial infection (pSBI) and a random set of neonates who were sampled identically and remained well.

MATERIALS AND METHODS

Study design and participants

We performed a retrospective case-control study of cord blood samples obtained at delivery at Kilifi County Hospital (KCH) within a systematic clinical surveillance of maternal and neonatal adverse events (clinicaltrials.gov NCT01757028)[36]. KCH serves a mostly rural population along the Kenyan coast. About half of all admissions to the neonatal ward are from the KCH maternity department, where there are ~4000 deliveries per year. Hospital deliveries and neonatal admissions have increased since maternity user fees were abolished (Free Maternity Service policy, 2013)[37]. Maternal clinical data and cord blood samples were obtained and analysed during the surveillance (clinicaltrials.gov NCT01757028)[36] and stored for future research. Data were collected using a standardized maternal admission record[36]. Cord blood samples were obtained by trained clinicians using standard aseptic techniques and universal safety precautions. After delivery of the neonate and the placenta, the umbilical cord was swabbed using 70% isopropyl alcohol and spirit, double clamped and cut. Approximately 10 ml of venous cord blood was collected using either a sterile

18-gauge needle (preferred) or 5Fr gastric tube and a syringe into ethylenediamine tetra-acetic acid (EDTA) tubes (BD Diagnostics, USA), centrifuged within an hour of collection; plasma and cell pellet aliquots were then frozen separately at -80°C.

Neonates born between March 2011 and March 2016 who were resident of the Kilifi Health and Demographic Surveillance System (KHDSS)[38] and had cord blood samples available were considered for this analysis. Cases were defined as neonates hospitalized within 48 hours of life with one or more features of possible serious bacterial infection (pSBI): history of difficulty feeding, history of convulsions, movement only when stimulated, respiratory rate of ≥60 breaths/min, severe chest indrawing, and a temperature of ≥37.5°C, or ≤35.5°C[39]. Cases were further categorized as those who died during hospitalization, and those who survived and discharged home well. None of the cases were readmitted following the initial hospital admission, including during the first 48 hours of life. Unmatched controls were randomly drawn from neonates who had cord blood samples taken in identical circumstances, did not have pSBI, were discharged home well after delivery, and survived for at least 60 days without hospitalization, determined using the KHDSS household census.

Clinical data for hospitalised neonates was systematically collected at admission using standard proforma and entered in real-time on a database within a surveillance for invasive bacterial disease[38 40]. Routine laboratory investigations for all admissions for clinical care included blood culture (BACTEC Peds Plus/F bottles and BACTEC 9050 instrument, Becton Dickinson, UK) and cerebrospinal fluid (CSF) culture where indicated, as previously described[41]. *Bacillus* spp., Coagulase-negative *Staphylococci* (CoNS), Coryneforms, *Micrococcus* spp., and viridans *Streptococci* were considered clinically non-significant blood culture isolates in this context.

The Kenya Medical Research Institute Scientific Steering Committee (KEMRI SSC) approved collection of cord blood samples and clinical data (SSC 1778 and 1433). Written informed consent for collection and use of samples and data for research was obtained from all participants' parents/guardians. This retrospective analysis was approved by the KEMRI Scientific Ethics Review Unit (SERU 3007). All studies were conducted according to relevant guidelines and regulations.

Study size

We estimated that 600 pSBI cases and 300 controls would give 90% power and 5% alpha for minimum proportions pathogens detected in 5% of pSBI cases and 1% in healthy controls.

Total nucleic acid extraction

Stored cord blood plasma and pellets were mixed after thawing and total nucleic acid (TNA) was extracted using the High Pure viral nucleic acid large volume kit (Roche 05114403001) following manufacturer's instructions. For each experiment, up to 2.5 ml of the plasma/pellets mix (1.5:1) underwent a lysate preparation process, then purification and elution of 150 µl TNA using spin columns, including a High Pure Extender Assembly for large initial volume. Extrinsic controls, Phocine Herpesvirus (PhHV) and artificial construct containing the region targeted by the MS2 PCR assay were added to each sample during lysate preparation to evaluate extraction and amplification efficiency. For each batch of extractions, a blank (about 2.5 ml of nuclease-free water) was processed through the complete protocol, and later assayed to rule out contamination during the extraction and amplification processes. A positive target in the blank would invalidate positive results for that target in the same batch of TNA extractions. Testing of TNA on TAC was done either on the same day or the day following extraction.

Detection of targets using TAC RT-qPCR

Real-time reverse transcription PCR assays were performed using a custom TAC (Thermo Fisher, CA, USA) on a QuantStudio 7 Flex instrument (Life Technologies, USA) to detect 16 bacterial, four viral and one protozoal target (Figure 1)[42 43]. The choice of targets was based on previous studies on neonatal sepsis[28]. Organisms such as CoNS that have been previously shown to be clinically insignificant in our setting[44] were not included in the TAC panel. The uidA gene detects both *E. coli* and *Shigella* species, hence were included as a single target on the TAC cards. Primers and probes were adapted from published assays to detect acute febrile illness[42 45] and sepsis[43] optimized for the universal cycling conditions on the card. Positive controls were plasmids for DNA and *in vitro* transcripts for RNA. Cards were designed, quality-controlled, and validated at the University of Virginia who provided onsite training.

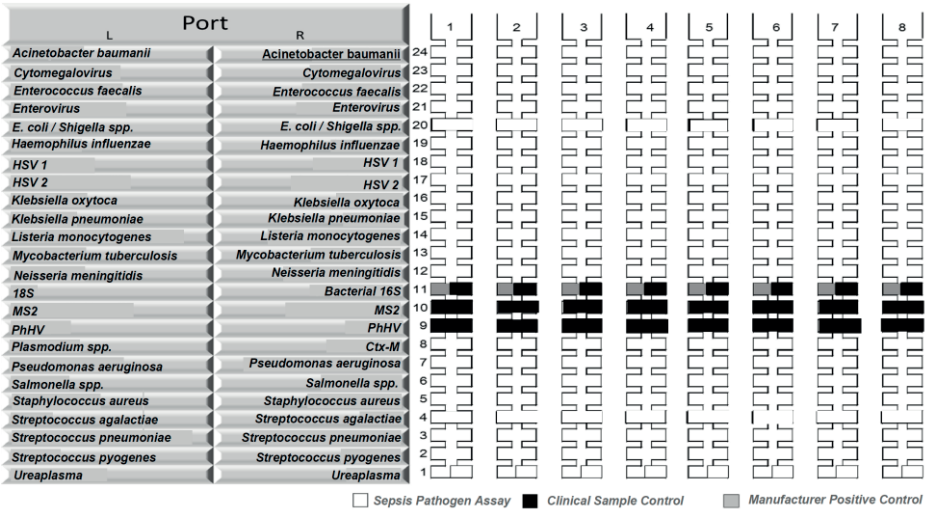

Figure 1. Organisms included in the whole blood TaqMan Array Card panel.

Pathogens interrogated with TaqMan Array Card (TAC) in whole blood. The TAC is a 384-well real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) based platform consisting of 8 (shown as no. 1 to 8 above) individual microfluidic channels that can be loaded with PCR reactions containing nucleic acid extract from a clinical specimen or control material. The TAC was customised to include 21 targets (16 bacterial, 4 viral and 1 protozoal organism, tested in duplicate) and two controls (MS2 bacteriophage and phocine Herpesvirus (PhHV)). The 21 targets are shown in alphabetical order as follows: *Acinetobacter baumanii*, Cytomegalovirus, *Enterococcus faecalis*, Enterovirus, *Escherichia coli*/Shigella spp., *Haemophilus influenzae*, Herpes Simplex Virus 1, Herpes Simplex Virus 2, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Neisseria meningitidis*, Plasmodium spp., *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and Ureaplasma spp.

For each experiment, 25 µL of TaqMan Fast Virus one-step master mix (4444434, Applied Biosystems, Thermo Fisher Scientific) was mixed with 75 µL of TNA extract or nuclease-free water (for no template control [NTC]) to make a 100 µL PCR reaction mix. Each 100 µL PCR reaction + sample mix was then transferred into the fill port of TAC after which the TAC was then centrifuged to ensure complete filling of the reaction wells, sealed and run. The reactions included a reverse transcription at 50°C for 10 minutes, initial denaturation at 95°C for 20 seconds, then 40 three-second cycles of 95°C, and 60°C for 30 seconds. Up to eight samples were tested per card, blinded to case-control status, with one NTC included in every 10 cards to check for reagent

contamination. Analysis utilized QuantStudio Real-Time PCR Software version 1.2 (Applied Biosystems, Thermo Fisher). Results were quality-checked by examining target amplification plots. Baseline adjustment for targets or reaction wells with irregular amplification was done when a false amplification curve was generated, or an inaccurate threshold cycle (Ct) value was yielded. Upon review of the reaction fluorescence curves for each target, we set the cut off threshold cycle (Ct) value for all targets at <40. Samples were deemed positive when any of the duplicate reactions yielded amplification curves that crossed the threshold as defined and controls were valid. We repeated *Ureaplasma* spp. testing using singleplex PCR on 261 samples which had parallel positive blanks on TAC and excluded TAC results from four samples for which repeat singleplex PCR was not possible due to depletion of TNA.

Statistical Analysis

Characteristics associated with case status were investigated using backward stepwise logistic regression retaining variables with P<0.1. We initially estimated the odds ratio for all cases (survived and died) versus controls. Then, since several organisms of potential public health relevance were not detected at all in controls and could not be meaningfully analysed in this way, we estimated the cumulative odds of pSBI across ordered groups of controls: cases-survived: cases-died using ordinal logistic regression which can accommodate zero values. We tested the proportional odds assumption to confirm that the relationship between each pair of outcome variable (controls, cases-survived, and cases-died) were similar prior to performing ordinal logistic regression. We estimated the attribution fraction (AF) among cases with “punafcc” in STATA v15 (StataCorp, TX, USA)[46].

RESULTS

Of 15,409 deliveries during the study period, 604 cases and 300 controls were selected (Figure 2). One case was subsequently excluded due to sample inhibition to amplification. Thus, 603 cases comprising 502 EONS survivors and 101 EONS deaths (58 [57%] and 74 [73%] of whom died within 24 and 48 hours after birth respectively) were included. Admissions on day 0, 1 and 2 of life among EONS cases were as follows: 256 (51%), 184 (37%) and 62 (12%) respectively in 502 survivors, compared with 93 (92%), 7 (6.9%) and 1 (1.0%) in 101 deaths (P<0.001).

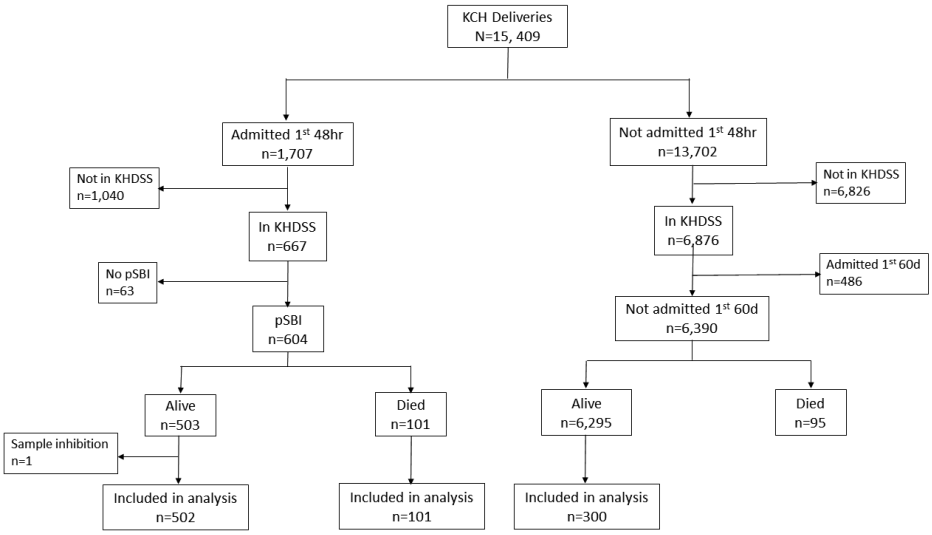

Figure 2. Study participant flow.

Selection of cases and controls from a cohort of 15,409 deliveries at Kilifi County Hospital (KCH) between March 2011 and March 2016. Cases were hospitalised within the first 48 hours of life, resident of the Kilifi Health Demographic Surveillance System (KHDSS) and resented with one or more of the WHO-defined criteria for possible serious bacterial infection (pSBI). Controls were resident of the KHDSS and not hospitalised within the first 60 days of life.

Compared with controls, pSBI cases were more likely to be born of mothers who were nulliparous (odds ratio [OR] 1.7, 95% confidence interval [CI] 1.2-2.3) or presented with drainage of liquor (OR 2.0, 95% CI 1.3-3.1), vaginal bleeding (OR 4.8, 95% CI 2.1-11) or oedema (OR 3.0, 95% CI 1.3-6.9) (Table 1). Admission with pSBI was not associated with maternal fever, prolonged rupture of membranes (PROM) or abnormal urinalysis at admission (prior to delivery).

Among newborns, assessment of appearance, pulse, grimace, activity, and respiration (APGAR) score <9 at 5 minutes (OR 15, 95% CI 6.2-35), resuscitation at birth (OR 3.6, 95% CI 1.8-7.3) and gestation of <32 weeks (OR 2.9, 95% CI 1.1-7.7) were associated with a pSBI case status (Table 2). Newborn mid-upper arm circumference (MUAC) (OR 0.77, 95% CI 0.64-0.94 per cm) and head circumference (OR 0.90, 95% CI 0.81-0.99 per cm) were also associated with pSBI, but birth weight was not associated with pSBI (OR 1.2, 95% CI 0.73-2.0) in this adjusted model.

Among 502 EONS survivors and 101 EONS deaths, 141 (28%) and 38 (38%) respectively tested positive for at least one TAC target, whilst 30 (6.0%) and 7 (6.9%) tested positive for multiple targets. The most frequent organisms detected were *K. oxytoca*, *E. coli/Shigella* spp., *P. aeruginosa,* and *S. pyogenes* (Table 3). Among 300 controls, 79 (26%) tested positive for at least one target, led by *K. oxytoca* and *P. aeruginosa*, and 11 (3.7%) were positive for multiple targets. *L. monocytogenes* and *M. tuberculosis* were not detected in either cases or controls. CMV was the commonest virus detected (19/603 (3.2%) cases versus 8/300 (2.7%) controls, p=0.7) and co-detection of CMV with a bacterial target was found in four cases and two controls.

Table 1. Maternal characteristics

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI)[a]	P value[a]	Multivariable Odds Ratio (95% CI)[b]	P value[b]
Age, years	26 (21-32)	26 (21-32)	1.0 (0.9 to 1.0)	0.593	-	-
Weight, kg	60 (53-70)	60 (54-67)	1.0 (0.9 to 1.0)	0.632	-	-
Height, cm	156 (151-160)	156 (151-160)	0.9 (0.9 to 1.0)	0.283	-	-
MUAC, cm	26 (24-28)	25 (24-28)	1.0 (0.9 to 1.1)	0.326	-	-
Marital status						
Married	543 (90)	264 (88)	1.0	-	-	-
Single	38 (6.3)	24 (8.0)	0.8 (0.5 to 1.3)	0.335	-	-
Divorced	2 (0.3)	2 (0.7)	0.5 (0.1 to 3.5)	0.472	-	-
Widowed	3 (0.5)	1 (0.3)	1.5 (0.2 to 14)	0.744	-	-
Missing	17 (2.8)	9 (3.0)	0.9 (0.4 to 2.1)	0.839	-	-
Education Level						
None	105 (17)	58 (19)	1.0	-	-	-
Primary	360 (60)	178 (59)	1.1 (0.8 to 1.6)	0.555	-	-
Secondary	86 (14)	36 (12)	1.3 (0.8 to 2.2)	0.281	-	-
Higher	34 (5.6)	12 (4.0)	1.6 (0.8 to 3.3)	0.230	-	-
Missing	18 (3.0)	16 (5.3)	0.6 (0.3 to 1.3)	0.211	-	-
Nulliparous						
No	383 (63)	207 (69)	1.0	-	1.0	-
Yes	185 (31)	65 (22)	1.5 (1.1 to 2.1)	0.010	1.7 (1.2 to 2.3)	0.004
Missing	35 (6.0)	28 (9.0)	0.7 (0.4 to 1.1)	0.143	0.7 (0.4 to 1.3)	0.304
Presenting complaints						
History of fever						
No	584 (97)	294 (98)	1.0	-	-	-
Yes	10 (1.7)	1 (0.3)	5.0 (0.6 to 40)	0.124	-	-
Missing	9 (1.5)	5 (1.7)	0.9 (0.3 to 2.7)	0.861	-	-

Table 1 Maternal characteristics (continued)

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI)[a]	P value[a]	Multivariable Odds Ratio (95% CI)[b]	P value[b]
Drainage of liquor						
No	489 (81)	266 (89)	1.0	-	1.0	-
Yes	109 (18)	30 (10)	2.0 (1.3 to 3.0)	0.002	2.0 (1.3 to 3.1)	0.002
Missing	5 (0.8)	4 (1.3)	0.7 (0.2 to 2.6)	0.568	0.0 (0.0 to 0.0)	0.975
Ruptured membranes						
No	401 (66)	210 (70)	1.0	-	-	-
Yes	172 (29)	77 (26)	1.2 (0.9 to 1.6)	0.331	-	-
Missing	30 (5.0)	13 (4.3)	1.2 (0.6 to 2.4)	0.581	-	-
PROM >18h						
No	471 (78)	240 (80)	1.0	-	-	-
Yes	27 (4.5)	9 (3.0)	1.5 (0.7 to 3.3)	0.280	-	-
Missing	105 (17)	51 (17)	1.0 (0.7 to 1.5)	0.799	-	-
Vaginal bleeding						
No	539 (89)	287 (96)	1.0	-	1.0	-
Yes	61 (10)	7 (2.3)	4.6 (2.1 to 10)	<0.001	4.8 (2.1 to 11)	<0.001
Missing	3 (1.0)	6 (2.0)	0.3 (0.1 to 1.1)	0.063	0.0 (0.0 to 0.0)	0.993
Dysuria						
No	577 (96)	287 (96)	1.0	-	-	-
Yes	21 (3.5)	8 (2.7)	1.3 (0.6 to 3.0)	0.527	-	-
Missing	5 (0.8)	5 (1.7)	0.5 (0.1 to 1.7)	0.273	-	-
Decreased foetal movements						
No	576 (96)	293 (98)	1.0	-	1.0	-
Yes	23 (3.8)	1 (0.3)	11.7 (1.6 to 87)	0.016	6.0 (0.8 to 47)	0.085
Missing	4 (0.7)	6 (2.0)	0.3 (0.1 to 1.2)	0.096	0.0 (0.0 to 0.0)	0.993

Table 1 Maternal characteristics (continued)

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI)[a]	P value[a]	Multivariable Odds Ratio (95% CI)[b]	P value[b]
Admission examination						
Emergency signs[c]						
No	500 (83)	271 (90)	1.0	-		
Yes	98 (16)	27 (9.0)	2.0 (1.3 to 3.1)	0.003	1.6 (1.0 to 2.5)	0.058
Missing	5 (0.8)	2 (0.7)	1.4 (0.3 to 7.0)	0.718	0.0 (0.0 to 0.0)	0.976
Temperature,˚C						
36-38	483 (80)	252 (84)	1.0	-	-	-
>38	7 (1.2)	0 (0.0)	1.0	-	-	-
<36	47 (7.8)	17 (5.7)	1.4 (0.8 to 2.6)	0.212	-	-
Missing	66 (11)	31 (10.3)	1.1 (0.7 to 1.7)	0.649	-	-
Oedema						
No	551 (91)	287 (96)	1.0	-	1.0	-
Yes	48 (8.0)	7 (2.3)	3.6 (1.6 to 8.0)	0.002	3.0 (1.3 to 6.9)	0.009
Missing	4 (0.7)	6 (2.0)	0.3 (0.1 to 1.2)	0.103	1.0	-
Positive nitrite and/or leucocytes (2+/3+)[d]						
No	417 (69)	214 (71)	1.0	-	-	-
Yes	108 (18)	46 (15)	1.2 (0.8 to 1.8)	0.339	-	-
Missing	78 (13)	40 (13)	1.0 (0.7 to 1.5)	0.997	-	-
Antibiotics in the last 4 weeks						
No	539 (89)	274 (91)	1.0	-	-	-
Yes	61 (10)	26 (8.7)	1.2 (0.7 to 1.9)	0.473	-	-
Missing	3 (1)	0 (0)	1.0	-	-	-

Table 1. Maternal characteristics (continued)

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratio (95% CI)[a]	P value[a]	Multivariable Odds Ratio (95% CI)[b]	P value[b]
Data are N (%) or median (IQR)						
Abbreviations: CI, confidence interval; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; PROM, prolonged rupture of membranes; °C, degree Celsius.						
[a]Univariable logistic model for all cases vs. controls [b]Multivariable logistic model for all cases vs. controls, including variables with P<0.1						
[c]Danger signs at triage suggesting need for emergency care (include airway not patent, respiratory rate >30 or <10 breaths/minute, systolic blood pressure >160 or <90 mmHg, diastolic blood pressure >90 mmHg, heart rate <40 or >120 beats/minute, unconscious or alert only to pain, other obstetric emergencies (including imminent delivery) requiring immediate intervention)						
[d]Urinalysis results at admission						

Table 2. Neonatal birth characteristics

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratios (95% CI)[a]	P value[a]	Multivariable Odds Ratios (95% CI)[b]	P value[b]
Weight, kg	2.7 (1.9-3.2)	3 (2.7-3.3)	0.4 (0.3 to 0.5)	<0.001	1.2 (0.7 to 2.0)	0.454
Length, cm	47.5 (43.0-49.5)	48.5 (47.0-50.0)	0.9 (0.8 to 0.9)	<0.001	1.0 (0.9 to 1.1)	0.814
MUAC, cm	10.0 (8.2-10.7)	10.5 (9.8-11.2)	0.6 (0.5 to 0.7)	<0.001	0.8 (0.6 to 0.9)	0.010
Head circumference, cm	33.3 (31.0-34.9)	34.0 (33.0-35.0)	0.8 (0.8 to 0.9)	<0.001	0.9 (0.8 to 0.9)	0.036
Sex						
Male	345 (57)	151 (50)	1.0	-	1.0	-
Female	258 (43)	149 (50)	0.8 (0.6 to 1.0)	0.051	0.8 (0.6 to 1.1)	0.177
Gestation, weeks						
≥37	373 (62)	247 (82)	1.0	-	1.0	-
≥32 to <37	122 (20)	44 (15)	1.8 (1.3 to 2.7)	0.002	1.2 (0.8 to 1.9)	0.457
<32	88 (15)	6 (2.0)	9.7 (4.2 to 23)	<0.001	2.9 (1.1 to 7.7)	0.035
Missing	20 (3.3)	3 (1.0)	4.4 (1.3 to 15)	0.017	3.0 (0.8 to 11)	0.096
Mode of delivery						
Vaginal	441 (73)	205 (68)	1.0	-	-	-
Caesarean section	162 (27)	91 (30)	0.8 (0.6 to 1.1)	0.225	-	-
Missing	0 (0.0)	4 (1.3)	1.0	-	-	-
Resuscitated at birth[c]						
No	415 (69)	287 (96)	1.0	-	1.0	-
Yes	186 (31)	11 (4.0)	11.7 (6.2 to 22)	<0.001	3.7 (1.8 to 7.3)	<0.001
Missing	2 (0.3)	2 (0.7)	0.7 (0.1 to 4.9)	0.713	1.0	-
APGAR Score at 5 minutes						
≥9	352 (58)	290 (97)	1.0	-	1.0	-
<9	229 (38)	6 (2.0)	31.4 (14 to 72)	<0.001	15 (6.2 to 35)	<0.001
Missing	22 (3.7)	4 (1.3)	4.5 (1.5 to 13)	0.006	8.8 (1.1 to 70)	0.039

Table 2. Neonatal birth characteristics (continued)

Characteristic	Cases (n=603)	Controls (n=300)	Univariable Odds Ratios (95% CI)[a]	P value[a]	Multivariable Odds Ratios (95% CI)[b]	P value[b]
Data are N (%) or median (IQR)						
Abbreviations: CI, confidence interval; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; APGAR, appearance, pulse, grimace, activity, and respiration.						
[a]Univariable logistic model for all cases vs. controls						
[b]Multivariable logistic model for all cases vs. controls, including variables with P<0.1						
[c]Resuscitation using bag mask ventilation with oxygen and/or cardiopulmonary resuscitation						

We observed four patterns of target detection by TAC (Figure 3):

i) Group 1: detected in a low proportion (<5.0%) in both cases (12/603 [2.0%]) and controls (6/300 [2.0%]), ((HSV 1, HSV 2, *S. aureus*, *Salmonella enterica*, *S. pneumoniae*, and *A. baumanii*);

ii) Group 2: detected in a low proportion (<5.0%) in cases (27/603 [4.5%]) but none in controls (0/300 [0.0%]), (*H. influenzae*, *N. meningitidis*, *E. faecalis*, Enterovirus, *Plasmodium* spp., and *S. agalactiae*);

iii) Group 3: detected in a high proportion (≥5.0%) in both cases (123/603 [20%]) and controls (70/300 [23%]), (CMV, *S. pyogenes*, *P. aeruginosa*, and *K. oxytoca*); and

iv) Group 4: detected in a high proportion (≥5%) in cases (47/603 [7.8%]) and low proportion (<5.0%) in controls (6/300 [2.0%]) (*K. pneumoniae*, *Ureaplasma* spp. and *E. coli/Shigella* spp.).

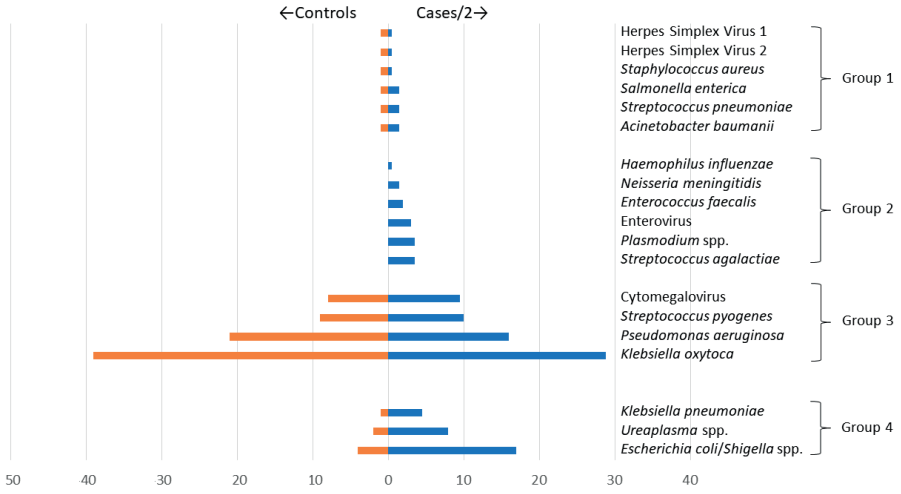

Figure 3. Patterns of detection of TaqMan Array Card Polymerase Chain Reaction targets.

Organisms included in the TAC were detected in 4 distinct groups: Group 1 (Herpes Simplex Virus 1, Herpes Simplex Virus 2, *Staphylococcus aureus*, *Salmonella enterica*, *Streptococcus pneumoniae*, and *Acinetobacter baumanii*); Group 2 (*Hemophilus influenzae*, *Neisseria meningitidis*, *Enterococcus faecalis*, Enterovirus, Plasmodium spp., and *Streptococcus agalactiae*); Group 3 (Cytomegalovirus, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca*); and Group 4 (*Klebsiella pneumoniae*, Ureaplasma spp. and *Escherichia coli*/Shigella spp.). Weighting of cases (represented as

cases/2) was done to allow for improved accuracy in assessing the distribution of organisms tested, comparing cases to controls, since cases (n=603) were ~twice more than controls (n=300). Weighting was done by calculating the number of eligible cases/controls divided by the number of enrolled cases/controls i.e., the inverse of the sampling fraction for cases/controls.

Upon examining cumulative odds, detection of any bacterial, viral or protozoal target was not associated with pSBI and death (OR 1.3, 95% CI 1.0-1.7) (Table 3). However, the high proportions of *K. oxytoca* and *P. aeruginosa* in both cases and controls suggested contamination or clinically insignificant traces of DNA in blood. Excluding these organisms, detection of any target was associated with pSBI and death (OR 2.1, 95% CI 1.4-2.8).

E. coli/Shigella spp. (P<0.001), *E. faecalis* (P=0.034), *S. agalactiae* (P=0.004), *Ureaplasma* spp. (P=0.010), Enterovirus (P=0.002), and *Plasmodium* spp. (P=0.004) were associated with pSBI and death (Table 3). *K. pneumoniae* (P=0.050) and *N. meningitidis* (P=0.054) had P values of borderline significance.

Overall, 6.6% (95% CI 0-14) of all pSBI cases were attributed to the bacterial, viral or protozoal targets. Excluding *K. oxytoca* and *P. aeruginosa* as likely contaminants, 9.4% (95% CI 5.1-13) of cases were attributed to the tested targets. Overall, 4.5% (95% CI 0-11) and 1.6% (95% CI 0-3.9) of pSBI cases were attributed to bacterial and viral targets respectively. The leading attributed pathogens were *E. coli/Shigella* spp. (AF 3.5%, 95% CI 1.7-5.3) and *Ureaplasma* spp. (AF 1.7, 95% CI 0.5-3.0). *E. faecalis*, *H. influenzae*, *K. pneumoniae*, *N. meningitidis*, *S. agalactiae*, *Salmonella enterica*, CMV, Enterovirus and *Plasmodium* spp. were each attributed to less than 1% of the pSBI cases

Table 3. TaqMan Results

	Controls (n=300)	Cases (n=603)	Cases survived (n=502)	Cases died (n=101)	Cases vs Controls odds ratio (95% CI)[a]	Cases (died) vs. Cases (survived) vs Controls cumulative odds ratio (95% CI)[b]	Attributable Fraction among cases (95% CI)[b]
Bacteria							
Acinetobacter baumanii	1 (0.3)	3 (0.5)	3 (0.6)	0 (0)	1.5 (0.2 to 14)	1.0 (0.3 to 3.6)	0
Escherichia coli/Shigella spp.	4 (1.3)	34 (5.6)	27 (5.4)	7 (6.9)	4.4 (1.6 to 13)	2.6 (1.6 to 4.4)	3.5 (1.7 to 5.3)
Enterococcus faecalis	0 (0)	4 (0.7)	3 (0.6)	1 (1.0)	-	4.0 (1.1 to 15)	0.5 (0.0 to 1.0)
Haemophilus influenzae	0 (0)	1 (0.2)	0 (0)	1 (1.0)	-	-	0.2 (0.0 to 0.5)
Klebsiella oxytoca	39 (13)	58 (9.6)	47 (9.4)	11 (11)	0.7 (0.5 to 1.1)	0.8 (0.5 to 1.2)	0
Klebsiella pneumoniae	1 (0.3)	9 (1.5)	7 (1.4)	2 (2.0)	4.5 (0.6 to 36)	2.7 (1.0 to 7.3)	0.9 (0.0 to 1.8)
Listeria monocytogenes	0 (0)	0 (0)	0 (0)	0 (0)	-	-	0
Mycobacterium tuberculosis	0 (0)	0 0)	0 (0)	0 (0)	-	-	0
Neisseria meningitidis	0 (0)	3 (0.5)	2 (0.4)	1 (1.0)	-	5.2 (1.0 to 28)	0.4 (0.0 to 0.9)
Pseudomonas aeruginosa	21 (7.0)	32 (5.3)	24 (4.8)	8 (7.9)	0.7 (0.4 to 1.3)	0.9 (0.5 to 1.6)	0
Streptococcus agalactiae	0 (0)	7 (1.2)	5 (1.0)	2 (2.0)	-	4.5 (1.6 to 13)	0.9 (0.2 to 1.6)
Staphylococcus aureus	1 (0.3)	1 (0.2)	1 (0.2)	0 (0)	0.5 (0.0 to 8.0)	0.4 (0.0 to 4.9)	0
Streptococcus pneumoniae	1 (0.3)	3 (0.5)	3 (0.6)	0 (0)	1.5 (0.2 to 14)	1.0 (0.3 to 3.6)	0
Streptococcus pyogenes	9 (3.0)	20 (3.3)	20 (4.0)	0 (0)	1.1 (0.5 to 2.5)	0.8 (0.5 to 1.4)	0
Salmonella spp.	1 (0.3)	3 (0.5)	2 (0.4)	1 (1.0)	1.5 (0.2 to 14)	2.0 (0.2 to 20)	0.2 (0.0 to 1.0)
Ureaplasma spp.	2 (0.7)	16 (2.7)	12 (2.4)	4 (4.0)	4.1 (0.9 to 18)	2.9 (1.3 to 6.4)	1.7 (0.5 to 3.0)
Any bacteria	72 (24)	156 (26)	123 (25)	33 (33)	1.1 (0.8 to 1.5)	1.2 (0.9 to 1.6)	4.5 (0.0 to 11)
Any bacteria excluding *K. oxytoca* and *P. aeruginosa*	20 (6.7)	89 (15)	70 (14)	19 (19)	2.4 (1.5 to 4.0)	2.1 (1.5 to 3.1)	7.9 (4.3 to 11)

Table 3. TaqMan Results (continued)

	Controls (n=300)	Cases (n=603)	Cases survived (n=502)	Cases died (n=101)	Cases vs Controls odds ratio (95% CI)[a]	Cases (died) vs. Cases (survived) vs Controls cumulative odds ratio (95% CI)[b]	Attributable Fraction among cases (95% CI)[b]
Viruses							
Cytomegalovirus	8 (2.7)	19 (3.2)	15 (3.0)	4 (4.0)	1.2 (0.5 to 2.7)	1.3 (0.6 to 2.7)	0.6 (0.0 to 2.7)
Enterovirus	0 (0)	6 (1.0)	3 (0.6)	3 (3.0)	-	9.1 (2.3 to 37)	0.9 (0.2 to 1.6)
Herpes Simplex Virus 1	1 (0.3)	1 (0.2)	1 (0.2)	0 (0)	0.5 (0.0 to 8.0)	0.4 (0.0 to 4.9)	0
Herpes Simplex Virus 2	1 (0.3)	1 (0.2)	1 (0.2)	0 (0)	0.5 (0.0 to 8.0)	0.4 (0.0 to 4.9)	0
Any viruses	10 3.3)	27 (4.5)	20 (4.0)	7 (6.9)	1.4 (0.6 to 2.8)	1.6 (0.8 to 3.2)	1.6 (0.0 to 3.9)
Protozoa							
Plasmodium spp.	0 (0)	7 (1.2)	6 (1.2)	1 (1.0)	-	2.9 (1.4 to 6.2)	0.8 (0.1 to 1.4)
Any bacteria, viruses and protozoa	79 (26)	179 (30)	141 (28)	38 (38)	1.2 (0.9 to 1.6)	1.3 (1.0 to 1.7)	6.6 (0.0 to 14)
Any bacteria (excluding *K. oxytoca* and *P. aeruginosa*), viruses and protozoa	30 (10)	115 (19)	91 (18)	24 (24)	2.1 (1.4 to 3.3)	2.0 (1.4 to 2.8)	9.4 (5.1 to 13)

[a]Ordinary logistic regression
[b]Ordinal logistic regression
[c]Attributable fraction is calculated from cumulative odds ratio

A total of 11 (1.8%) of 603 cases had presumed pathogens isolated from blood culture at admission and *S. aureus* (n=5) was the most common isolate (Table 4). The OR for a positive admission blood culture for death among admitted pSBI cases was 1.1 (95% CI 0.24-5.2) and 0.2% (95% CI 0-3.2) of pSBI were attributed to the pathogens identified through blood culture.

Table 4. Admission blood culture results among cases and corresponding TaqMan Array Card Polymerase Chain Reaction results

	Cases (n=603)	
Organisms	**Blood culture**	**TAC cord blood**
Presumed significant organisms		
Acinetobacter spp.	2	0
Klebsiella pneumoniae	1	0
Pantoea spp.*	1	0
Staphylococcus aureus	5	1 (*K. oxytoca* + *P. aeruginosa*)
Streptococcus Group B	1	1 (CMV)
Streptococcus Group G*	1	1 (*K. oxytoca*)
Total	11	3

Columns show number of neonates with either positive culture or TAC (TAC organisms are indicated within the brackets).
Abbreviations: CMV, cytomegalovirus.
*These organisms were not included on the TAC.

DISCUSSION

We used a novel approach to identify causes of EONS by investigating stored cord blood samples collected at birth with a custom TAC, spatially multiplexed PCR to interrogate the presence of multiple pathogens. This is the first study evaluating diagnostic performance using cord blood in an African setting. These samples were obtained at delivery prior to admission with signs of pSBI. Approximately 60% of 603 EONS cases and 92% of all deaths were admitted on day 0 of life. A total of 58 of 101 (57%) deaths occurred within the first 24 hours of life, after cord blood samples had been obtained. This underscores the importance of prompt diagnosis for targeted treatment and makes a cord blood approach potentially attractive in epidemiological studies, and possibly for managing 'at risk' neonates since cord blood could be collected, stored and tested at a later stage if the newborn develops signs of pSBI.

E. coli/Shigella spp. and *Ureaplasma* spp. had the highest causal attribution in our results, supporting the latter as an important pathogen in this setting. *Ureaplasma* spp. are associated with maternal colonization and adverse

pregnancy outcomes[47]. Appropriate treatment of high-risk neonates is needed given the emerging resistance of *Ureaplasma* spp. to macrolides. Two healthy controls and 16 pSBI cases, of whom 12 survived, tested positive for *Ureaplasma* spp. in our study. Although the clear association of *Ureaplasma* spp. with sepsis and mortality indicates pathogenicity, asymptomatic presentation or recovery in ill neonates without targeted antimicrobials has been reported and is not unusual[48]. Additionally, the culture-independent molecular method identified non-culturable organisms such as Enterovirus, which is shown to cause serious sepsis-like illness in neonates in other settings[49]. However, despite the use of sensitive molecular assays, 90% of pSBI cases still had unknown aetiology on cord blood analysis.

The overall causal attribution of 6.6% (95% CI 0-14) increased to 9.4% (95% CI 5.1-13) with exclusion of *K. oxytoca* and *P. aeruginosa* in our study. As expected, the attributable proportion was lower than in the Sepsis Aetiology in Neonates in South Africa study (SANISA [27%, 95% CI 23-32])[27] and the Aetiology of Neonatal Infection in South Asia study (ANISA [23%, 95% CI 19-26])[28] since much of the latter study's attribution went to detection of RSV in respiratory samples, which this study did not examine. Although SANISA (n=27) and ANISA (n=28) were large prospective studies and tested more targets by TAC than we did (n=21), they also failed to attribute aetiology to a large proportion of pSBI cases. There were also differences in pSBI case definitions (SANISA used a predefined set of clinical and laboratory criteria[27], while ANISA used WHO clinical criteria but excluded tachypnoea[28]) and differences in selection and sampling of controls (SANISA sampled healthy neonates at study hospital[27] while ANISA used an automated algorithm triggered at the first postnatal visit to select randomly registered controls[28]). Our pSBI definition was based on the WHO Young Infants Clinical Signs study which derived a decision rule (presence of ≥1 sign: history of difficulty feeding, history of convulsions, movement only when stimulated, respiratory rate of ≥60 breaths/min, severe chest indrawing, and a temperature of ≥37.5°C, or ≤35.5°C) predicting severe illness in neonates aged 0-6 days with 87% sensitivity and 74% specificity[39]. The performance of these signs in distinguishing neonates with sepsis from those without sepsis has not been adequately investigated. Current WHO[50] and Kenya national paediatric guidelines[51] for empiric antimicrobials in neonates suspected to have sepsis are based on this limited evidence. Neonates with sepsis often present with subtle and non-specific clinical signs that overlap with those seen in other non-infectious diagnoses[52]. Thus, our case definition may have resulted in the

inclusion of neonates who did not have true sepsis, contributing to low attribution rates. Development and use of a highly sensitive and specific consensus definition for neonatal sepsis is critically needed in clinical practice and research[6].

Bacterial organisms (25% bacterial compared to 4.1% viral targets detected) were predominant in our study, similar to SANISA[27] and ANISA[28] results from blood samples. Thirty percent of cord blood samples of pSBI cases in our study tested positive for at least one target by TAC, compared to blood samples in SANISA (37%)[27] and ANISA (12%)[28]. We identified multiple targets in 7% of pSBI cases compared to 11% cases in SANISA[27] and 1% in ANISA[28]. At least one target was positive in 28% of healthy controls in our study compared to 20% in SANISA[27]. Thus, background positivity of cord blood among healthy neonates in our study was greater than in SANISA. All cases and controls were first selected based on the presence or absence of pSBI. All 604 pSBI cases who resided in the KHDSS had cord blood samples available for testing and were included in this analysis. 300 controls were randomly selected from a subset of 6,295 neonates who were resident of the KHDSS, remained well during the first 60 days of life, and had cord blood samples available for testing. Therefore, we ensured that cases and controls had an equal chance of being selected in respective groups, with controls derived from similar circumstances to cases for optimal group comparison, unlike in ANISA where controls were recruited from the community. Although the cases had a lower gestation age and were generally smaller than the controls based on the anthropometric measurements, we believe that bias risk was minimal since we would expect sick neonates to present with known underlying risk factors of infection, such as prematurity.

K. oxytoca and *P. aeruginosa* were identified in large numbers in both cases and controls. This could be due to environmental contamination of laboratory materials or reagents, which has been widely reported for *K. oxytoca*[53], contamination of the specimen by gut flora or skin commensals post-delivery[54], as was reported in SANISA[27], or true subclinical detection of circulating non-viable genetic material, or low copies of organisms insufficient to cause disease. Overall causal attribution increased with exclusion of *K. oxytoca* and *P. aeruginosa*, suggesting non-significance of these bacteria in EONS. Cord blood sample contamination has been reported in studies evaluating the diagnostic use of cord blood cultures, by comparing results obtained to peripheral venous blood cultures[29 33 35]. Although cord blood provides a non-invasive alternative to peripheral blood sampling with better culture yields[30 55], the risk of

contamination cannot be ignored. Careful aseptic techniques and training of clinical staff are imperative to optimize sample collection and may improve the validity of results. Aseptic techniques were used during cord blood sample collection to minimise sample contamination, since identification of pathogens associated with adverse maternal and perinatal outcomes was planned[36], including a recently published study on the association of flavivirus exposure with congenital microcephaly[56]. In addition, cord blood analysis using PCR has mostly focused on vertically transmitted viruses[57-59], and more research on cord blood testing using molecular diagnostics is needed to better understand the clinical significance of detected organisms. Detection of organisms known to cause permanent neurodevelopmental sequelae in asymptomatic congenital infection such as CMV[60] (eight healthy controls in our study) may inform management. However, we did not follow up these infants for post-discharge outcomes in this retrospective analysis. Nonetheless, the PCR detections for *E. coli/Shigella* spp., *E. faecalis*, *K. pneumoniae*, *N. meningitidis*, *S. agalactiae*, *Ureaplasma* spp., and *Plasmodium* spp. had clear directional association across controls, surviving cases, and cases who died.

A limitation of this study was that we could not rigorously compare cord blood PCR to cord blood culture since we did not have paired specimens. However, studies such as ANISA[28] and SANISA[27] which performed blood TAC PCR and culture in parallel reported discordance of results between the two tests. In our study, blood culture was performed on later specimens at ward admission. Although blood culture is the gold standard test for sepsis, culture-negative neonatal sepsis is common[19], and this is evident in the low positivity rate among the pSBI cases in our study. In addition, the tests differed in the volumes of blood used for processing (0.1 ml equivalent per PCR reaction versus ~2ml for culture[61]) and timing of testing (immediately for culture, stored for ~5 years for TAC). Low burden of infection at the limit of detection, different sampling timepoints, and decreased *S. agalactiae* sampling sensitivity due to antisepsis measures associated with caesarean section delivery[14], may have contributed to failure to detect *S. agalactiae* by cord blood TAC, in a pSBI case from whom *S. agalactiae* was isolated from admission blood culture five hours after delivery. In addition, some of the pathogens were fastidious/unculturable and were only detected by TAC. Low aetiological attribution by culture among pSBI cases underscores the need for better diagnostics as bacteraemia was associated with an increased likelihood of case fatality. Sensitivity analysis of neonates excluded in our study despite having available cord blood samples,

some of which were of low volumes would have been useful in checking for selection bias in our case-control selection, and in comparing our PCR results.

Maternal variables at delivery can aid prompt initiation of antimicrobials. Intrapartum fever (temperature ≥38°C), chorioamnionitis, pre-labour rupture of membranes ≥18 hours, preterm pre-labour rupture of membranes, PROM ≥18 hours, maternal GBS colonization or bacteriuria, multiparity, and poor intrapartum and postpartum infection control practices have previously been shown to predispose neonates to infection[5 62 63]. We lacked complete data on intrapartum antibiotic use and were unable to assess its impact on pathogen identification. In addition to an immunological immaturity[64], prematurity, low birth weight, complicated or instrument-assisted delivery, and low APGAR scores, contribute to an increased risk of admission with EONS. Although not the primary aim of our study, we observed that being identified as very preterm (<32 weeks) as well as head circumference and MUAC, which are associated with maturity[65], were associated with EONS. However, low birth weight was not associated with EONS in our study.

Although TAC provided epidemiological data on potential causes of EONS in our setting, including the role of nonculturable organisms such as *Ureaplasma* spp. and Enterovirus, 90% of pSBI cases lacked epidemiological attribution. The presence of presumed contaminants in both cases and controls was only discernible on a population basis rather than from an individual's results. Thus, despite allowing for customization of a panel of pathogen targets, requirement of small blood volumes, and rapid pathogen detection, TAC in its current form may have a limited role in individual diagnosis in clinical practice, particularly in settings like ours where associated costs of setting up and using this platform will be prohibitive. Further research using this technology alongside highly specific diagnostic methods is needed to better understand the aetiology, distribution and determinants of disease. In addition, our study was limited by use of archived samples and retrospective analysis of data. Future prospective studies using specific definitions of EONS alongside paired cord blood and peripheral blood cultures are needed to better understand the performance of TAC in detection of pathogens associated with EONS.

In conclusion, we were able to identify organisms associated with subsequent EONS and death using cord blood at birth and an identically sampled comparator group of healthy neonates in sub-Saharan Africa. Further prospective research on the clinical utility of cord blood in our setting is needed

alongside development and use of rapid and specific point-of-care diagnostics, that will guide prompt management in seriously ill neonates. Robust evidence of the causes of EONS is vital, given the potential for prevention and targeted treatment strategies such as maternal immunization and intrapartum antibiotic prophylaxis[66], including oral azithromycin for reduction of bacterial carriage and risk of EONS[67]. Coverage for *Ureaplasma* spp. in at-risk neonates should be considered when updating antimicrobial guidelines given the strength of combined data from three studies (ours, SANISA and ANISA) and the potential adverse outcomes associated with this organism[68].

Data availability

Underlying data

Harvard Dataverse:Replication Data for: Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR, https://doi.org/10.7910/DVN/FXKGRB (Obiero *et al.*, 2021)[69]

This project contains the following underlying data:

- Maternal variables-1.tab
- Neonatal variables-1.tab
- PCR Ct values-1.tab

Extended data

Harvard Dataverse:Replication Data for: Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR, https://doi.org/10.7910/DVN/FXKGRB (Obiero *et al.*, 2021)[69]

This project contains the following extended data:

- CObiero_Detection of pathogens in cord blood_Codebook.pdf
- CObiero_Detection of pathogens in cord blood_readme.txt
- Detection of pathogens at birth_Extended data.pdf

Competing interests

No competing interests were disclosed

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REFERENCES

1. Hug L, Alexander M, You D, et al. National, regional, and global levels and trends in neonatal mortality between 1990 and 2017, with scenario-based projections to 2030: a systematic analysis. *The Lancet Global health* 2019;7(6):e710-e20. doi: 10.1016/S2214-109X(19)30163-9 [published Online First: 2019/05/18]
2. Baqui AH, Mitra DK, Begum N, et al. Neonatal mortality within 24 hours of birth in six low- and lower-middle-income countries. *Bulletin of the World Health Organization* 2016;94(10):752-58B. doi: 10.2471/BLT.15.160945 [published Online First: 08/30]
3. Alliance for M, Newborn Health Improvement mortality study g. Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study. *The Lancet Global health* 2018;6(12):e1297-e308. doi: 10.1016/S2214-109X(18)30385-1
4. Seale AC, Blencowe H, Zaidi A, et al. Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. *Pediatric research* 2013;74 Suppl 1:73-85. doi: 10.1038/pr.2013.207 [published Online First: 2013/12/25]
5. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. *Pediatr Clin North Am* 2013;60(2):367-89. doi: 10.1016/j.pcl.2012.12.003 [published Online First: 01/17]
6. McGovern M, Giannoni E, Kuester H, et al. Challenges in developing a consensus definition of neonatal sepsis. *Pediatric research* 2020;88(1):14-26. doi: 10.1038/s41390-020-0785-x [published Online First: 2020/03/04]
7. Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN surveillance network. *Archives of disease in childhood Fetal and neonatal edition* 2011;96(1):F9-F14. doi: 10.1136/adc.2009.178798 [published Online First: 2010/09/30]
8. Prakesh Shah, E. W. Y., Chan, P. & Members of the Annual Report Review Committee. The Canadian Neonatal Network Annual Report 2016. http://www.canadianneonatalnetwork.org/Portal/LinkClick.aspx?fileticket=PJSDwNECsMI%3d&tabid=39 (2016).
9. Stoll BJ, Gordon T, Korones SB, et al. Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *The Journal of pediatrics* 1996;129(1):72-80.
10. Centre for Disease Control and Prevention. ABCs Report: group B streptococcus, 2017. https://www.cdc.gov/abcs/reports-findings/survreports/gbs17.html (2017).
11. Zaidi AK, Huskins WC, Thaver D, et al. Hospital-acquired neonatal infections in developing countries. *Lancet* 2005;365(9465):1175-88. doi: 10.1016/S0140-6736(05)71881-X [published Online First: 2005/03/30]
12. Zaidi AK, Thaver D, Ali SA, et al. Pathogens associated with sepsis in newborns and young infants in developing countries. *The Pediatric infectious disease journal* 2009;28(1 Suppl):S10-8. doi: 10.1097/INF.0b013e3181958769 [published Online First: 2009/01/10]
13. Seale AC, Bianchi-Jassir F, Russell NJ, et al. Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017;65(suppl_2):S200-S19. doi: 10.1093/cid/cix664 [published Online First: 2017/11/09]
14. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya. *Nat*

Microbiol 2016;1(7):16067. doi: 10.1038/nmicrobiol.2016.67 [published Online First: 2016/08/31]
15. Quan V, Verani JR, Cohen C, et al. Invasive Group B Streptococcal Disease in South Africa: Importance of Surveillance Methodology. *PLoS One* 2016;11(4):e0152524. doi: 10.1371/journal.pone.0152524 [published Online First: 2016/04/08]
16. Harris JB, Holmes AP. Neonatal Herpes Simplex Viral Infections and Acyclovir: An Update. *The journal of pediatric pharmacology and therapeutics : JPPT : the official journal of PPAG* 2017;22(2):88-93. doi: 10.5863/1551-6776-22.2.88 [published Online First: 2017/05/05]
17. Yadav SS, Narula G, Narayan S, et al. Cytomegalovirus infection in six neonates. *Indian pediatrics* 2010;47(2):174-5. [published Online First: 2009/05/12]
18. Cantey JB, Baird SD. Ending the Culture of Culture-Negative Sepsis in the Neonatal ICU. *Pediatrics* 2017;140(4) doi: 10.1542/peds.2017-0044 [published Online First: 2017/09/21]
19. Klingenberg C, Kornelisse RF, Buonocore G, et al. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Frontiers in pediatrics* 2018;6:285. doi: 10.3389/fped.2018.00285
20. Schelonka RL, Chai MK, Yoder BA, et al. Volume of blood required to detect common neonatal pathogens. *The Journal of pediatrics* 1996;129(2):275-8. [published Online First: 1996/08/01]
21. Viel-Theriault I, Fell DB, Grynspan D, et al. The transplacental passage of commonly used intrapartum antibiotics and its impact on the newborn management: A narrative review. *Early human development* 2019;135:6-10. doi: 10.1016/j.earlhumdev.2019.05.020 [published Online First: 2019/06/10]
22. Pammi M, Flores A, Versalovic J, et al. Molecular assays for the diagnosis of sepsis in neonates. *The Cochrane database of systematic reviews* 2017;2:CD011926. doi: 10.1002/14651858.CD011926.pub2 [published Online First: 2017/02/27]
23. Investigators of the Delhi Neonatal Infection Study c. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. *The Lancet Global health* 2016;4(10):e752-60. doi: 10.1016/S2214-109X(16)30148-6 [published Online First: 2016/09/17]
24. Seedat F, Stinton C, Patterson J, et al. Adverse events in women and children who have received intrapartum antibiotic prophylaxis treatment: a systematic review. *BMC Pregnancy Childbirth* 2017;17(1):247. doi: 10.1186/s12884-017-1432-3 [published Online First: 2017/07/28]
25. Águeda S, Leitão A, Rocha G, et al. Viral Infections in a Neonatal Intensive Care Unit. *Pediat Therapeut* 2013;3(154):2161-0665.1000154.
26. Diaz MH, Waller JL, Napoliello RA, et al. Optimization of Multiple Pathogen Detection Using the TaqMan Array Card: Application for a Population-Based Study of Neonatal Infection. *PLoS One* 2013;8(6):e66183. doi: 10.1371/journal.pone.0066183 [published Online First: 2013/06/28]
27. Velaphi SC, Westercamp M, Moleleki M, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. *PLoS One* 2019;14(4):e0214077. doi: 10.1371/journal.pone.0214077 [published Online First: 2019/04/11]
28. Saha SK, Schrag SJ, El Arifeen S, et al. Causes and incidence of community-acquired serious infections among young children in south Asia (ANISA): an observational cohort study. *Lancet* 2018;392(10142):145-59. doi: 10.1016/S0140-6736(18)31127-9

29. Polin JI, Knox I, Baumgart S, et al. Use of umbilical cord blood culture for detection of neonatal bacteremia. *Obstetrics and gynecology* 1981;57(2):233-7. [published Online First: 1981/02/01]
30. Newberry DM. Comparison of Placental and Neonatal Admission Complete Blood Cell Count and Blood Cultures. *Adv Neonatal Care* 2018;18(3):215-22. doi: 10.1097/ANC.0000000000000482 [published Online First: 2018/03/27]
31. Mithal LB, Palac HL, Yogev R, et al. Cord Blood Acute Phase Reactants Predict Early Onset Neonatal Sepsis in Preterm Infants. *PLoS One* 2017;12(1):e0168677. doi: 10.1371/journal.pone.0168677 [published Online First: 2017/01/04]
32. Su H, Chang SS, Han CM, et al. Inflammatory markers in cord blood or maternal serum for early detection of neonatal sepsis-a systemic review and meta-analysis. *Journal of perinatology : official journal of the California Perinatal Association* 2014;34(4):268-74. doi: 10.1038/jp.2013.186 [published Online First: 2014/01/25]
33. Herson VC, Block C, McLaughlin JC, et al. Placental blood sampling: an aid to the diagnosis of neonatal sepsis. *Journal of perinatology : official journal of the California Perinatal Association* 1998;18(2):135-7. [published Online First: 1998/05/30]
34. Mithal LB, Malczynski M, Qi C, et al. Umbilical Cord Blood Diagnostics for Early Onset Sepsis in Premature Infants: Detection of Bacterial DNA and Systemic Inflammatory Response. *bioRxiv* 2017:200337. doi: 10.1101/200337
35. Beeram MR, Loughran C, Cipriani C, et al. Utilization of umbilical cord blood for the evaluation of group B streptococcal sepsis screening. *Clin Pediatr (Phila)* 2012;51(5):447-53. doi: 10.1177/0009922811431882 [published Online First: 2011/12/27]
36. Seale AC, Barsosio HC, Koech AC, et al. Embedding surveillance into clinical care to detect serious adverse events in pregnancy. *Vaccine* 2015;33(47):6466-8. doi: 10.1016/j.vaccine.2015.07.086 [published Online First: 2015/08/10]
37. Lang'at E, Mwanri L, Temmerman M. Effects of implementing free maternity service policy in Kenya: an interrupted time series analysis. *BMC Health Serv Res* 2019;19(1):645. doi: 10.1186/s12913-019-4462-x [published Online First: 2019/09/08]
38. Scott JA, Bauni E, Moisi JC, et al. Profile: The Kilifi Health and Demographic Surveillance System (KHDSS). *Int J Epidemiol* 2012;41(3):650-7. doi: 10.1093/ije/dys062 [published Online First: 2012/05/01]
39. Young Infants Clinical Signs Study G. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *Lancet* 2008;371(9607):135-42. doi: 10.1016/S0140-6736(08)60106-3 [published Online First: 2008/01/15]
40. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *The New England journal of medicine* 2005;352(1):39-47. doi: 10.1056/NEJMoa040275 [published Online First: 2005/01/07]
41. Obiero CW, Mturi N, Mwarumba S, et al. Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital. *Archives of disease in childhood* 2020 doi: 10.1136/archdischild-2020-318913 [published Online First: 2020/08/21]
42. Liu J, Ochieng C, Wiersma S, et al. Development of a TaqMan Array Card for Acute-Febrile-Illness Outbreak Investigation and Surveillance of Emerging Pathogens, Including Ebola Virus. *Journal of clinical microbiology* 2016;54(1):49-58. doi: 10.1128/JCM.02257-15 [published Online First: 12/30]
43. Moore CC, Jacob ST, Banura P, et al. Etiology of Sepsis in Uganda Using a Quantitative Polymerase Chain Reaction-based TaqMan Array Card. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of*

America 2019;68(2):266-72. doi: 10.1093/cid/ciy472 [published Online First: 06/04]
44. Seale AC, Obiero CW, Jones KD, et al. Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya? *The Pediatric infectious disease journal* 2017;36(11):1073-78. doi: 10.1097/INF.0000000000001699 [published Online First: 2017/07/22]
45. Abade A, Eidex RB, Maro A, et al. Use of TaqMan Array Cards to Screen Outbreak Specimens for Causes of Febrile Illness in Tanzania. *Am J Trop Med Hyg* 2018;98(6):1640-42. doi: 10.4269/ajtmh.18-0071 [published Online First: 04/02]
46. Newson R. PUNAFCC: Stata module to compute population attributable fractions for case-control and survival studies. 2017
47. Sprong KE, Mabenge M, Wright CA, et al. Ureaplasma species and preterm birth: current perspectives. *Crit Rev Microbiol* 2020;46(2):169-81. doi: 10.1080/1040841X.2020.1736986 [published Online First: 2020/03/07]
48. Viscardi RM, Terrin ML, Magder LS, et al. Randomised trial of azithromycin to eradicate Ureaplasma in preterm infants. *Archives of disease in childhood Fetal and neonatal edition* 2020;105(6):615-22. doi: 10.1136/archdischild-2019-318122 [published Online First: 2020/03/15]
49. Chuang YY, Huang YC. Enteroviral infection in neonates. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2019;52(6):851-57. doi: 10.1016/j.jmii.2019.08.018 [published Online First: 2019/10/15]
50. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed: World Health Organization, 2013:412.
51. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
52. Hermansen CL, Mahajan A. Newborn Respiratory Distress. *Am Fam Physician* 2015;92(11):994-1002. [published Online First: 2016/01/14]
53. Lowe C, Willey B, O'Shaughnessy A, et al. Outbreak of extended-spectrum beta-lactamase-producing Klebsiella oxytoca infections associated with contaminated handwashing sinks(1). *Emerg Infect Dis* 2012;18(8):1242-7. doi: 10.3201/eid1808.111268 [published Online First: 2012/07/31]
54. Underwood MA, Sohn K. The Microbiota of the Extremely Preterm Infant. *Clinics in perinatology* 2017;44(2):407-27. doi: 10.1016/j.clp.2017.01.005 [published Online First: 2017/05/10]
55. Meena R, Meena KK, Athwani V, et al. Umbilical Cord Blood Culture in Diagnosis of Early Onset Neonatal Sepsis. *Indian journal of pediatrics* 2020;87(10):793-97. doi: 10.1007/s12098-020-03345-5 [published Online First: 2020/05/30]
56. Barsosio HC, Gitonga JN, Karanja HK, et al. Congenital microcephaly unrelated to flavivirus exposure in coastal Kenya. *Wellcome Open Res* 2019;4:179. doi: 10.12688/wellcomeopenres.15568.1 [published Online First: 2020/03/17]
57. Hou GQ, Chen SS, Lee CP. Pathogens in maternal blood and fetal cord blood using Q-PCR assay. *Taiwan J Obstet Gynecol* 2006;45(2):114-9. doi: 10.1016/S1028-4559(09)60207-2 [published Online First: 2007/01/02]
58. Theiler RN, Caliendo AM, Pargman S, et al. Umbilical cord blood screening for cytomegalovirus DNA by quantitative PCR. *J Clin Virol* 2006;37(4):313-6. doi: 10.1016/j.jcv.2006.08.019 [published Online First: 2006/10/13]
59. Avila EC, Finger-Jardim F, Goncalves CV, et al. High Incidence of Herpes Simplex Virus-1 in Cord Blood and Placenta Infection of Women in Southern Brazil. *Rev Bras Ginecol Obstet* 2020;42(1):5-11. doi: 10.1055/s-0039-1700794 [published Online First: 2020/02/29]
60. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol* 2007;17(5):355-63. doi: 10.1002/rmv.544 [published Online First: 2007/06/02]

61. Huber S, Hetzer B, Crazzolara R, et al. The correct blood volume for paediatric blood cultures: a conundrum? *Clin Microbiol Infect* 2020;26(2):168-73. doi: 10.1016/j.cmi.2019.10.006 [published Online First: 2019/10/28]
62. Schuchat A, Zywicki SS, Dinsmoor MJ, et al. Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. *Pediatrics* 2000;105(1 Pt 1):21-6. [published Online First: 2000/01/05]
63. Chan GJ, Lee AC, Baqui AH, et al. Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. *PLoS Med* 2013;10(8):e1001502. doi: 10.1371/journal.pmed.1001502 [published Online First: 2013/08/27]
64. Fleer A, Gerards LJ, Verhoef J. Host defence to bacterial infection in the neonate. *The Journal of hospital infection* 1988;11 Suppl A:320-7. [published Online First: 1988/02/01]
65. Thawani R, Dewan P, Faridi MM, et al. Estimation of gestational age, using neonatal anthropometry: a cross-sectional study in India. *Journal of health, population, and nutrition* 2013;31(4):523-30. doi: 10.3329/jhpn.v31i4.20051 [published Online First: 2014/03/07]
66. Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. *The New England journal of medicine* 1986;314(26):1665-9. doi: 10.1056/NEJM198606263142603 [published Online First: 1986/06/26]
67. Roca A, Oluwalana C, Bojang A, 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(6):565 e1-9. doi: 10.1016/j.cmi.2016.03.005 [published Online First: 2016/03/31]
68. Viscardi RM. Ureaplasma species: role in neonatal morbidities and outcomes. *Archives of disease in childhood Fetal and neonatal edition* 2014;99(1):F87-92. doi: 10.1136/archdischild-2012-303351 [published Online First: 2013/08/21]
69. Obiero CW, Gumbi W, Mwakio S, et al. Replication Data for: Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR. V2 ed: Harvard Dataverse, 2021.

Supplementary Tables

Supplementary Table 1. Annual distribution of KCH deliveries included in this analysis

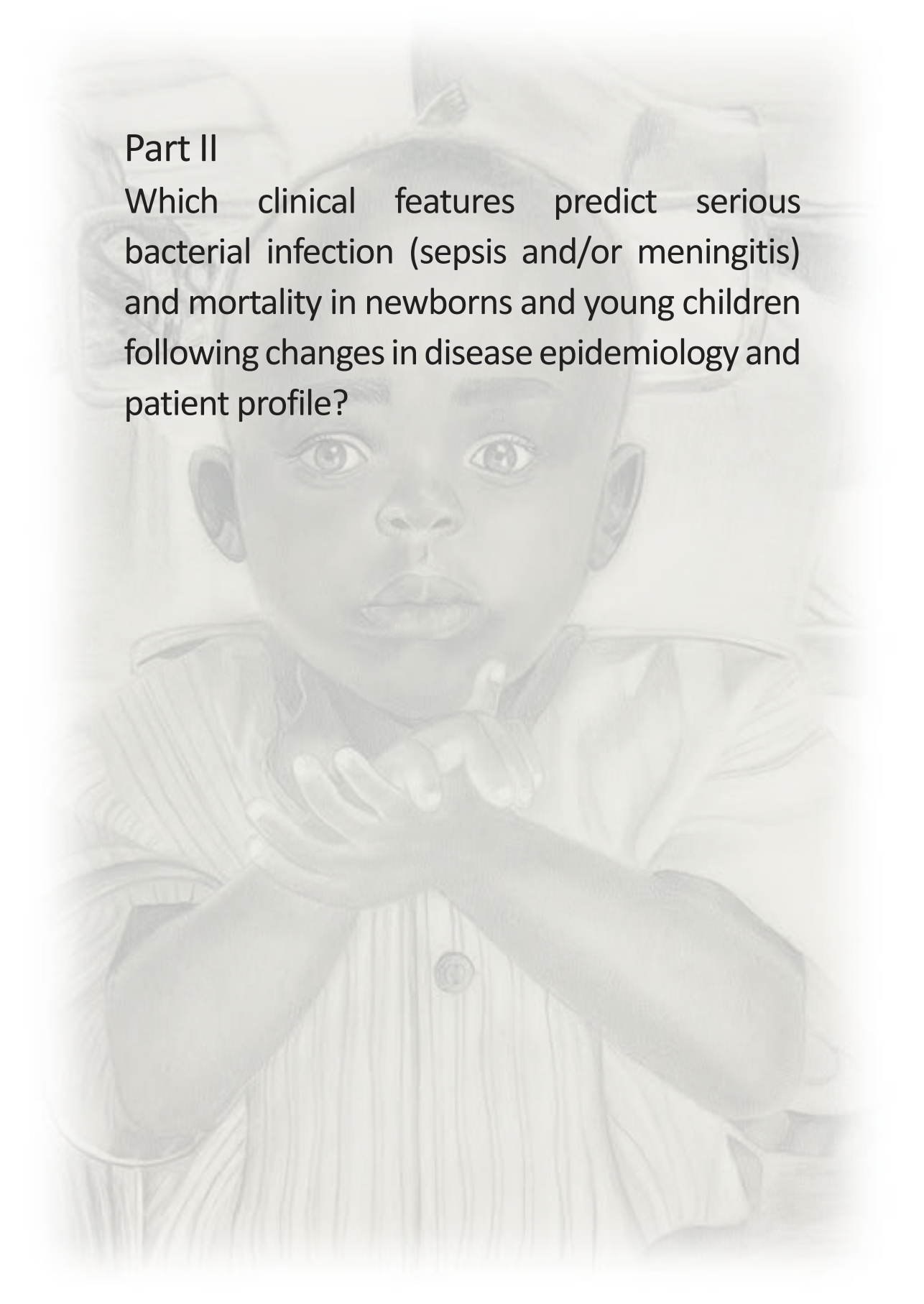
Year	All (n=903)	Controls (n=300)	Cases survived (n=502)	Cases died (n=101)
2011	115 (13)	47 (16)	68 (11)	12 (12)
2012	173 (19)	59 (20)	114 (19)	19 (19)
2013	146 (16)	51 (17)	95 (16)	14 (14)
2014	191(21)	53 (18)	138 (23)	24 (24)
2015	186 (21)	59 (20)	127 (21)	26 (26)
2016	92 (10)	31 (10)	61 (10)	6 (5.9)

Data are n (%)
Study period was March 2011 to March 2016

Supplementary Table 2. Proportional odds (parallel regression) assumption

	Controls (n=300)	Cases survived (n=502)	Cases died (n=101)	P value*
Bacteria				
Acinetobacter baumanii	1 (0.3)	3 (0.6)	0 (0)	-
Escherichia coli/Shigella spp.	4 (1.3)	27 (5.4)	7 (6.9)	0.1319
Enterococcus faecalis	0 (0)	3 (0.6)	1 (1.0)	0.2667
Haemophilus influenzae	0 (0)	0 (0)	1 (1.0)	-
Klebsiella oxytoca	39 (13)	47 (9.4)	11 (11)	0.3190
Klebsiella pneumoniae	1 (0.3)	7 (1.4)	2 (2.0)	0.4633
Listeria monocytogenes	0 (0)	0 (0)	0 (0)	-
Mycobacterium tuberculosis	0 (0)	0 (0)	0 (0)	-
Neisseria meningitidis	0 (0)	2 (0.4)	1 (1.0)	0.4100
Pseudomonas aeruginosa	21 (7.0)	24 (4.8)	8 (7.9)	0.1281
Streptococcus agalactiae	0 (0)	5 (1.0)	2 (2.0)	0.1702
Staphylococcus aureus	1 (0.3)	1 (0.2)	0 (0)	-
Streptococcus pneumoniae	1 (0.3)	3 (0.6)	0 (0)	-
Streptococcus pyogenes	9 (3.0)	20 (4.0)	0 (0)	-
Salmonella enterica	1 (0.3)	2 (0.4)	1 (1.0)	0.6859
Ureaplasma spp.	2 (0.7)	12 (2.4)	4 (4.0)	0.4883
Any bacteria	72 (24)	123 (25)	33 (33)	0.2024
Any bacteria excluding *K. oxytoca* and *P. aeruginosa*	20 (6.7)	70 (14)	19 (19)	0.3946
Viruses				
Cytomegalovirus	8 (2.7)	15 (3.0)	4 (4.0)	0.7901
Enterovirus	0 (0)	3 (0.6)	3 (3.0)	0.3918
Herpes Simplex Virus 1	1 (0.3)	1 (0.2)	0 (0)	-
Herpes Simplex Virus 2	1 (0.3)	1 (0.2)	0 (0)	-
Any viruses	10 3.3)	20 (4.0)	7 (6.9)	0.4934
Protozoa				
Plasmodium spp.	0 (0)	6 (1.2)	1 (1.0)	0.0684
Any bacteria, viruses and protozoa	79 (26)	141 (28)	38 (38)	0.2039
Any bacteria (excluding *K. oxytoca* and *P. aeruginosa*), viruses and protozoa	30 (10)	91 (18)	24 (24)	0.5136

*Proportional odds (parallel regression) assumption tested using *omodel* or *oparallel* commands on Stata software. An insignificant likelihood ratio chi-square value suggests that the proportional odds assumption is met. A significant likelihood ratio chi-square value provides evidence that the proportional odds assumption has been violated. Absent p values are due to perfect prediction hence full models not estimated.



Part II

Which clinical features predict serious bacterial infection (sepsis and/or meningitis) and mortality in newborns and young children following changes in disease epidemiology and patient profile?

Chapter 4

Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital

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ABSTRACT

Background Detection of meningitis is essential to optimize the duration and choice of antimicrobial agents to limit mortality and sequelae. In developing countries most health facilities lack laboratory capacity and rely on clinical features to empirically treat meningitis.

Objective We conducted a diagnostic validation study to investigate the performance of clinical features (fever, convulsions, irritability, bulging fontanel and temperature ≥39^{o}C) and WHO-recommended signs (drowsiness, lethargy, unconsciousness, convulsions, bulging fontanel, irritability, or a high-pitched cry) in discriminating meningitis in young infants.

Design Retrospective cohort study.

Setting Kilifi County Hospital.

Patients Infants aged <60 days hospitalised between 2012-2016.

Main outcome measure Definite meningitis defined as positive cerebrospinal fluid (CSF) culture, microscopy or antigen test, or leukocytes ≥50/µL.

Results Of 4,809 infants aged <60 days included, 81(1.7%) had definite meningitis. WHO-recommended signs had sensitivity of 58% (95%CI: 47-69) and specificity of 57% (95%CI: 56-59) for definite meningitis. Addition of history of fever improved sensitivity to 89% (95% CI: 80-95) but reduced specificity to 26% (95%CI: 25-27). Presence of ≥1 of five previously identified signs had sensitivity of 79% (95%CI: 69-87) and specificity of 51% (95%CI: 50-53).

Conclusions

Despite a lower prevalence of definite meningitis, the performance of previously identified signs at admission in predicting meningitis was unchanged. Presence of history of fever improves the sensitivity of WHO-recommended signs but loses specificity. Careful evaluation, repeated assessment and capacity for lumbar puncture and CSF microscopy to exclude meningitis in most young infants with potential signs are essential to management in this age group.

INTRODUCTION

Meningitis is a life-threatening disease associated with significant mortality and disabling neuropsychological sequelae.[1-4] Disease burden is highest in developing countries where about a quarter of children who survive vaccine-preventable meningitis develop post-discharge complications.[5 6] Prompt recognition and treatment with appropriate antimicrobial coverage and cerebrospinal fluid (CSF) penetration for an adequate duration is critical to optimize outcomes.

CSF culture is the gold standard diagnostic test for meningitis, however, it has limited sensitivity[7], is compromised by prior antibiotic exposure[8] and is frequently unavailable or unreliable in resource-limited hospitals. Changes in CSF cytological and biochemical parameters have diagnostic utility but rarely CSF may be normal in the presence of meningitis.[9 10] Most health facilities in developing countries lack CSF diagnostic capacity and so management decisions are based on clinical presentation only.

Young infants typically present to hospital with subtle symptoms and signs,[1 11] making it challenging for clinicians to decide when to perform a lumbar puncture (LP) (if laboratory facilities exist) or continue empiric antibiotics. The World Health Organization (WHO) advises suspecting meningitis if an infant: i) is drowsy, lethargic or unconscious; ii) has convulsions; iii) has a bulging fontanel; iv) is irritable, or; v) has a high-pitched cry.[12] These guidelines are based on limited evidence collected prior to widespread use of conjugate vaccines,[13 14] including a study that reported clinical signs not specific to meningitis.[14]

Between 2001 and 2007, we conducted a study of clinical features associated with meningitis among infants aged <60 days at Kilifi County Hospital (KCH) and found history of fever, convulsions, irritability, bulging fontanel or temperature ≥39^{o}C to be useful indicators of meningitis.[15] These results, including the WHO recommendation, were incorporated into Kenyan national guidelines.[16] However, since the introduction of conjugate *Haemophilus influenzae* type b (Hib) vaccine in 2001, an 89% reduction in Hib meningitis in Kenyan children has been observed.[17] Similarly, 10-valent pneumococcal conjugate vaccine (PCV-10) was introduced in 2011 and was associated with significant reduction in nasopharyngeal carriage of vaccine serotypes[18], and incidence and mortality from pneumococcal meningitis.[19-21] Additionally, the introduction of a voucher scheme and free maternity care in 2013 in Kenya has resulted in a greatly

increased number of hospital deliveries and admissions directly to paediatric care.[22] Although intrapartum antibiotic prophylaxis is recommended in the presence of risk factors for infection such as prolonged rapture of membranes ≥18 hours, maternal screening for Group B Streptococci (GBS) is not included in the Kenya national guidelines,[23] despite high incidence of early-onset neonatal infection secondary to GBS.[24]

Thus, changes in both epidemiology and patient profile, may have altered the associations between clinical features and meningitis, hence clinical decision rules derived from earlier studies may no longer be optimal. We therefore performed a revalidation study of clinical features at admission to hospital in infants aged <60 days, examining those identified in the previous study at our centre and those recommended by the WHO.

METHODS

Location and Participants

KCH is a government hospital located on the Kenyan coast serving a mostly rural population. Routine vaccination with Hib and PCV-10 vaccines are provided free of charge at government health facilities as a three-dose primary series without a booster dose at 6, 10, and 14 weeks of age. All infants <60 days old hospitalized at KCH between 1st January 2012 and 31st December 2016 were included in this retrospective cohort study.

Procedures

All infants were systematically assessed by trained clinicians at admission and standardized demographic and clinical data were prospectively collected and entered on a surveillance database in real time. Laboratory investigations done at admission on all infants included hemogram, blood slide for malaria parasites, and blood culture. Infants presenting with signs suggestive of meningitis underwent LP and were started on broad-spectrum antibiotics according to WHO[25] and Kenyan national guidelines.[26] LP was deferred in infants with cardiorespiratory compromise or signs of raised intracranial pressure.[27] Infants were assessed daily by clinicians and an LP performed once stable if LP had been delayed at admission, or if an infant developed new clinical features suggestive of meningitis during hospitalization. Collection of surveillance data (KEMRI SSC 1433) and this retrospective analysis (KEMRI SSC 3001) were

reviewed and approved by the Kenya Medical Research Institute Scientific Steering Committee.

Laboratory Analysis

CSF examination included leukocyte count, Gram and/or Indian ink staining and latex antigen agglutination tests (Wellcogen™ Bacterial Antigen kit for *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, and CrAg Lateral Flow Assay kit Ref CR2003 for *Cryptococcus neoformans*). All CSF and blood samples were cultured as previously described and pathogens identified using standard methods, including antimicrobial susceptibility testing.[15 28] Known commensals including coagulase-negative Staphylococci were considered non-significant. CSF protein and glucose and concurrent blood glucose were measured on an Instrument Laboratory Aries analyzer (Werfen, Germany).

Sample processing and analysis was performed at the KEMRI Centre for Geographic Medicine (Coast) laboratory which is externally monitored for quality assurance by the United Kingdom (UK) External Quality Assessment Service and accredited in Good Clinical Laboratory Practice by Qualogy, UK.

Definitions

For this analysis, we defined definite meningitis according to the criteria used in our previous study:[15] 1) positive CSF culture for a known pathogen; or 2) organisms observed on CSF Gram stain microscopy; or 3) positive CSF antigen test; or 4) CSF leukocytes ≥50 cells/µL. Possible meningitis was defined in infants without definite meningitis as: CSF leukocytes ≥20/µL in infants age 0-28 days, and CSF leukocytes ≥10/µL in infants age 28-59 days. Infants not meeting either criterion were defined as no meningitis. Possible meningitis and the narrow microbiological criteria for definite meningitis (positive CSF culture, antigen test or microscopy, or CSF leukocytes ≥50/µL plus positive blood culture) were used for sensitivity analysis.

Statistical analysis

We extracted data from the surveillance database. Infants who died before an LP had been performed were then excluded as we could not ascertain their meningitis status. We analysed data from all infants and then separately examined those 0-6 days and 7-59 days because of potential differences in aetiology and clinical presentation.[15]

We calculated the prevalence of meningitis and tabulated the frequency distribution of CSF findings, including pathogens identified and the highest CSF criterion for definite meningitis attained in the order of the four criteria given above.

We examined the performance of the previously identified clinical features (history of fever, convulsions, irritability, bulging fontanel and temperature ≥39^{o}C)[15] and the WHO-recommended signs[25] by calculating their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under receiver operating characteristic (ROC) curve for definite meningitis versus no meningitis.

We calculated the number of LPs needed to identify one case of definite meningitis using each combination of features as the inverse of the risk difference obtained by subtracting the risk of meningitis in the group with indicator(s) of interest from risk of meningitis in the group without indicator(s) of interest. As a sensitivity analysis we then repeated these analyses for possible or definite meningitis versus no meningitis.

Proportions were compared using the chi-squared test or Fishers exact test, while continuous variables were compared using Wilcoxon rank-sum test. We performed analyses using Stata version 15 (Stata Corp, USA).

RESULTS

During the study period, 5,591 infants were admitted, of which 4,196 (75%) were aged 0-6 days. Three thousand two hundred and fifty-three (58%) infants were born at KCH, including 2,747 (65%) of those aged 0-6 days of whom 2,476 (90%) were hospitalized within the first 72 hours of life. Overall, 853/5,591 (15%) infants died during hospitalization and 640/853 (75%) deaths occurred during the first day of life. Seven hundred and eighty-two (92%) of 853 deaths occurred before an LP had been performed and were excluded from this analysis (Figure 1). Thus, 4,809 infants of which 3,508 (73%) were aged 0-6 days were included in the analysis.

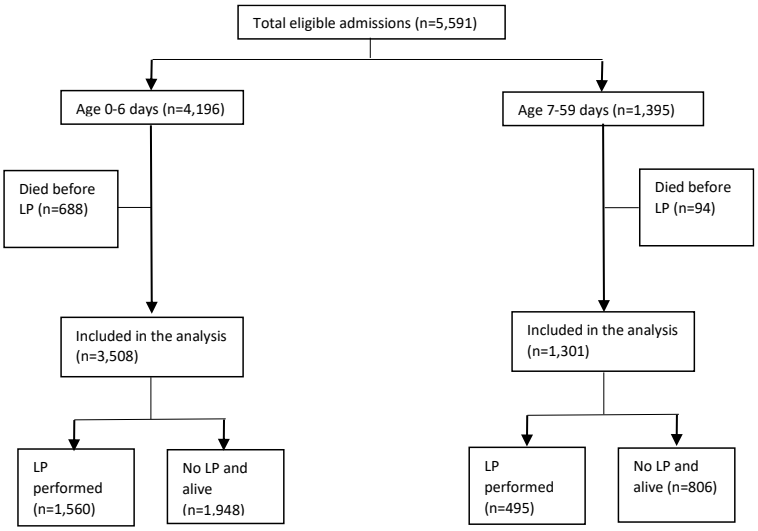

Figure 1. Flow Chart of Study Participants

Eighty-one (1.7%) infants had definite meningitis; 39/3,508 (1.1%) aged 0-6 days and 42/1,301 (3.2%) aged 7-59 days (*P*<0.001). Eighteen (22%) infants had positive CSF culture (Table 1) of which 8 had a positive blood culture and 6 grew similar organisms [4 GBS, 1 Group A Streptococcus (GAS) and 1 *Escherichia coli*] in blood and CSF. GBS was the commonest CSF isolate followed by *E. coli* and *Klebsiella pneumoniae*. Two infants had Hib and one had pneumococcal meningitis; none had positive CSF or blood cultures. Twenty-seven (33%) of the 81 definite meningitis cases had visibly turbid CSF. Three of 11 infants with positive blood culture had CSF leukocytes ≥50/µL. Seven (8.6%) infants with definite meningitis died during admission compared with 64 (1.4%) in the non-meningitis group, *P*<0.001. Table S1 shows meningitis cases and number of LPs during our study period versus our previous analysis.

Table 1. Diagnostic Criteria for Meningitis and Bacterial Species Detected

Diagnostic Criteria	Age 0-6d (3,508/4,809, 73%)	Age 7-59d (1,301/4,809, 27%)	Total Positives	Highest criteria for Meningitis
CSF Culture				
Gram positive				
Group B Streptococci	0	7	7[a]	7
Group A Streptococci	0	1	1[b]	1
Gram negative				
Escherichia coli	2	3	5[c]	5
Klebsiella pneumonia	1	1	2[d]	2
Klebsiella oxytoca	0	1	1	1
Enterobacter aerogenes	0	1	1	1
Citrobacter species	1	0	1[e]	1
Total	**4**	**14**	**18**	**18**
Latex antigen test				
Streptococcus pneumoniae	0	1	1[f]	1
Haemophilus influenzae	1	1	2[g]	2
Cryptococcus neoformans	1	0	1	1
Total positive antigen test	**2**	**2**	**4**	**4**
Gram-stain				
Gram-positive cocci	0	6	6	0
Gram-negative rods	5	1	6	2
Total	**5**	**7**	**12[h]**	**2**
Indian Ink	1	0	1	1
CSF WCC ≥50 cells/µl	33	37	70	56
Total				**81**

[a] 6/7 had WCC ≥50 cells/µl and 5/7 had positive Gram stain.
[b] Had WCC ≥50 cells/µl and positive Gram stain.
[c] 1/5 Had Streptococcus species isolated as well; 3/5 had WCC≥50 cells/µl; 2/5 had positive Gram stain.
[d] 1/2 had WC C≥50 cells/µl; 1 had positive Gram stain.
[e] Had WCC ≥50 cells/µl and positive Gram stain.
[f] Had WCC ≥50 cells/µl.
[g] 1/2 Had WCC ≥50 cells/µl.
[h] 10/12 had positive CSF culture; 9/10 had WCC ≥50 cells/µl.

Clinical features at admission

History of fever, bulging fontanel, axillary temperature ≥39^{o}C, and irritability were associated with definite meningitis among infants aged 0-6 days (Table 2). Bulging fontanel, convulsions and irritability were associated with definite meningitis in older infants. A bulging fontanel, stiff neck and inability to breastfeed were each observed in only 5% of meningitis cases.

Performance of clinical features in all infants

Previously identified signs

Sixty-four (2.7%) of 2,377 infants presenting with one or more of: history of fever, irritability, axillary temperature ≥39^{o}C, convulsions or bulging fontanel, had definite meningitis compared with 17/2,432 (0.7%) infants lacking these features (*P*<0.001): sensitivity 79% (95%CI: 69-87), specificity 51% (95%CI: 50-53), PPV 2.7% (95%CI: 2.1-3.4), NPV 99% (95%CI: 99-100). Fifty infants (95%CI: 37-79) presenting with one or more of these clinical features would need to undergo an LP for each case of meningitis to be identified (Table 3).

WHO-recommended signs or a history of fever

Forty-seven (2.3%) of 2,072 infants presenting with one or more of WHO-recommended signs had definite meningitis compared with 34/2,737 (1%) infants lacking these signs (*P*=0.006): sensitivity 58% (95%CI: 47-69), specificity 57% (95%CI: 56-59), PPV 2.3% (95%CI: 1.7-3), and NPV 99% (95%CI: 98-99). Ninety-eight infants (95%CI: 56-381) presenting with one or more of WHO-recommended signs would need to undergo an LP for each meningitis case to be identified (Table 3). Addition of history of fever to these WHO signs resulted in sensitivity 89% (95%CI: 80-95), specificity 26% (95%CI: 25-27), PPV 2% (95%CI: 1.6-2.5), and NPV 99% (95%CI: 99-100).

Table 2. Clinical history and examination findings among neonates and young infants

Characteristic		Age 0-6 days			Age 7-59 days		
		No Meningitis (n=3,469)	Meningitis (n=39)	P value[a]	No Meningitis (n=1,259)	Meningitis (n=42)	P value[a]
Bulging fontanel	No	3,419 (99)	37 (95)	<0.001	1,233 (98)	33 (79)	<0.001
	Yes	15 (0.4)	2 (5.1)		11 (0.9)	9 (21)	
	Missing	35 (1.0)	0 (0)		15 (1.2)	0 (0)	
Convulsions	No	3,269 (94)	34 (87)	0.063	1,162 (92)	31 (74)	<0.001
	Yes	168 (4.8)	5 (13)		82 (6.5)	11 (26)	
	Missing	32 (0.9)	0 (0)		15 (1.2)	0 (0)	
Axillary temperature ^{o}C	<36	1,061 (31)	4 (10)	0.021	77 (6.1)	1 (2.4)	0.269
	36-38.9	2,202 (63)	30 (77)		1,092 (87)	35 (83)	
	≥39	199 (5.7)	5 (13)		89 (7.1)	6 (14)	
	Missing	7 (0.2)	0 (0)		1 (0.1)	0 (0)	
Agitation/Irritability	No	3,375 (97)	33 (85)	<0.001	1,198 (95)	36 (86)	0.002
	Yes	59 (1.7)	6 (15)		46 (3.7)	6 (14)	
	Missing	35 (1.0)	0 (0)		15 (1.2)	0 (0)	
History of fever [b]	No	2,086 (60)	14 (36)	0.006	470 (37)	9 (21)	0.074
	Yes	1,350 (39)	25 (64)		774 (61)	33 (79)	
	Missing	33 (1.0)	0 (0)		15 (1.1)	0 (0)	
Drowsy, lethargic or unconscious	No	2,626 (76)	27 (69)	0.466	1,048 (83)	31 (74)	0.148
	Yes	808 (23)	12 (31)		196 (16)	11 (26)	
	Missing	35 (1.0)	0 (0)		15 (1.2)	0 (0)	
Abnormal cry	No	2,489 (72)	33 (85)	0.093	846 (67)	31 (74)	0.587
	Yes	945 (27)	5 (13)		118 (9.4)	4 (9.5)	
	Missing	35 (1.0)	1 (2.6)		295 (23)	7 (!7)	

Data are n (%)

Group percentages may not add to 100% due to rounding off

[a] Univariate comparison of characteristics; [b] Elevated tactile temperature as reported by the parent or guardian

Table 3. Performance of indicators of meningitis among all infants 0-59 days old

Indicators	Number with indicator	Number with meningitis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95%CI)	N.N. LP (95% CI)
Previously identified							
Bulging fontanel	37	11	13.6 (7.0-23.0)	99.4 (99.2-99.6)	29.7 (15.9-47.0)	98.5 (98.1-98.8)	4 (2-7)
Convulsions or any of the above	292	24	29.6 (20.0-40.8)	94.3 (93.6-95.0)	8.2 (5.3-12.0)	98.7 (98.4-99.0)	14 (10-26)
Axillary temp ≥39^{o}C or any of the above	555	32	39.5 (28.8-51.0)	88.9 (88.0-89.8)	5.8 (4.0-8.0)	98.8 (98.5-99.1)	22 (15-38)
Agitation/irritability or any of the above	640	37	45.7 (34.6-57.1)	87.2 (86.3-88.2)	5.8 (4.1-7.9)	98.9 (98.6-99.2)	21(15-35)
History of fever or any of the above	2,377	64	79.0 (68.5-87.3)	51.1 (49.6-52.5)	2.7 (2.1-3.4)	99.3 (98.9-99.6)	50 (37-79)
WHO recommended							
One or more of the WHO suggested signs	2,072	47	58.0 (46.5-68.9)	57.2 (55.7-58.6)	2.3 (1.7-3.0)	98.8 (98.3-99.1)	98 (56-381)
One or more of the WHO suggested signs or history of fever	3,566	72	88.9 (80.0-94.8)	26.1(24.9-27.4)	2.0 (1.6-2.5)	99.3 (98.6-99.7)	77 (51-157)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; N.N. LP, number needed to lumbar puncture to identify one case of definite meningitis

Clinical features in infants 0-6 days old compared to infants 7-59 days old

Previously identified signs were less sensitive but more specific in detecting meningitis in infants in the first week of life than among infants 7-59 days old (Tables S2 and S3). WHO-recommended signs had similar sensitivity and specificity in both age groups. History of fever markedly improved the sensitivity of WHO-recommended signs but resulted in low specificity in both age groups.

The overall area under the ROC curve for previously identified signs was 0.72 (95%CI: 0.66-0.78) and there was no evidence that this differed between infants age 0-6 days and age 7-59 days (p=0.19) (Figure 2).

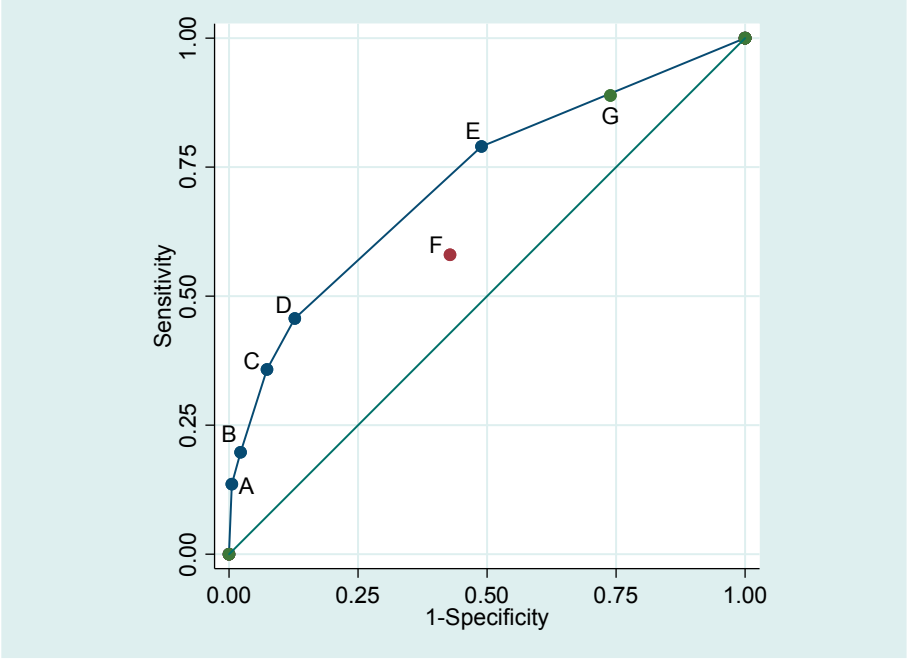

Figure 2. Area under ROC curve for previously identified signs and WHO-recommended signs of meningitis

Figure showing the area under ROC curve for previously identified signs and WHO-recommended signs or history of fever in infants aged 0-59 days. A. Bulging fontanel; B. Convulsions; C. Axillary temperature ≥39ºC; D. Agitation/irritability; E. History of fever; F. WHO-recommended signs; G. WHO-recommended signs or history of fever

Sensitivity analysis

One hundred and twenty-six infants had possible or definite meningitis. Fewer LPs were needed to identify a single case of possible or definite meningitis compared to a cut-off ≥50/μL in all infants (Table S4). The overall area under the ROC curve for previously identified signs was 0.69 (95%CI: 0.64-0.74) and there was no evidence that this differed between infants aged 0-6 days and age 7-59 days (*P*=0.17).

For the 28 (0.6%) infants with definite meningitis based on microbiological criteria, point estimates for sensitivity and specificity for previously identified signs, and WHO-recommended signs or a history of fever respectively were similar to the main analysis and more LPs were needed to identify a single case of meningitis (Table S5).

DISCUSSION

This study aimed to determine if clinical features at admission to hospital that were found to discriminate young infants with meningitis before widespread use of conjugate vaccines in developing countries are still applicable for decision making. Overall, 1.7% infants included in our study had definite meningitis, lower than 4.2% in 2006-2007 and 4.1% in 2001-2005 previously at our centre using the same definition and inclusion criteria.[15] The number of infants hospitalized at KCH has increased, predominantly due to increased admissions on the first day of life related to uptake of free maternity care.[15 22] Meningitis cases decreased with time despite an increase in the number of LPs performed. Comparing the present study (2012-2016) to our previous study (1994-1998)[29], there were 1 versus 24 cases of *S. pneumoniae* and 2 versus 11 cases of *H. influenzae* meningitis demonstrating an effect of herd immunity from conjugate vaccination on caseload.

In addition to limited CSF diagnostic capacity, ancillary tests that may be helpful in stratifying infants at risk of meningitis such as peripheral blood leukopaenia,[30] absolute neutrophilia,[31] and biomarkers e.g. procalcitonin[32] are usually unavailable and have not been validated in our setting. This further underscores the need to optimise clinical guidelines to identify infants needing urgent treatment.

Whilst the presence of one or more of the previously identified signs missed less cases than WHO-recommended signs alone (21% versus 42%), history of fever

improved the performance of WHO signs. Our results were similar to those reported by our previous study[15] suggesting that although meningitis is now less common, performance of these signs, including those recommended by WHO has not significantly changed over time. Of importance, none of these signs were highly specific in discriminating meningitis at admission, hence careful clinical review and LP are essential, especially in consideration of duration of antibiotics once started.

Capacity for CSF analysis is often unavailable in resource-limited settings[33] and more LPs are now needed to identify a single meningitis case than previously, especially with the narrowest meningitis case definition.[15] There are limited data on the 'acceptable' number of LPs needed to diagnose a single case of meningitis in young infants, with most studies focusing on the clinical utility of LPs in older children with seizures.[34]

Typically, about 50% meningitis deaths occur within the first 24 hours of admission and post-mortem LP may be useful for surveillance and studies of aetiology,[29] but undertaking LP promptly is vital for diagnosis. The low specificity of clinical signs leads to overdiagnosis. However, given the high risk of morbidity and mortality associated with bacterial meningitis and the contribution to development of antimicrobial resistance of overtreatment, any reluctance to perform LPs, even where full laboratory support is unavailable, needs to be addressed as failing to do so will miss 10-40% cases and/or overtreat the vast majority of infants with indicator signs (Table S4). Meningitis is now less common, and more difficult to exclude purely on clinical grounds and clinicians should maintain a low threshold for doing LPs, such as in all infants with fever in addition to more specific signs, especially in 0-6-day olds.

Thirty seven percent of meningitis cases in our study had turbid CSF, similar to 28% cases in 2001-2005 (P=0.18). CSF leukocyte count may fail to discriminate infants with culture-proven meningitis from those without,[10] but together with visual turbidity would have identified 71 (88%) of definite cases and 118 (94%) of possible cases. Sensitivity analysis done in our study with a lower cut-off (≥20/μL), which has been shown to provide sufficient diagnostic precision for culture-proven meningitis[7] did not alter our results. Support to establish basic CSF cell counting and Gram stain in resource-limited settings to optimize antimicrobial treatment is essential to providing inpatient paediatric services.

Limitations

We lacked data on pre-hospital antibiotic exposure which has been shown to lower CSF culture yield.[8 35] Potential misclassification of infants with negative CSF findings or asymptomatic infants may have diminished the validity of clinical features studied. Infants who died prior to an LP were excluded from this analysis, however, we did not aim to estimate the overall burden of meningitis, rather to address the challenges faced in clinical practice among infants in whom a decision to admit to hospital had been made. Delays in presentation to hospital[36] and rapid disease progression may have led to early mortality before LP. This may have a pathogen-specific impact on our findings as, for example, GBS mortality commonly occurs in the first few hours of life.[24]

Conclusions

Meningitis is an uncommon but important diagnosis in young infants. Despite declining incidence, clinical features of meningitis do not perform less well now than in the pre-conjugate vaccine era. However, clinicians and policymakers should be aware of the number of LPs or empirical treatments needed for each case of definite meningitis identified. The clinical signs currently recommended by WHO to guide decisions to perform an LP and initiate antibiotics poorly discriminate infants with meningitis, particularly in neonates age <1 week. History of fever is an important indicator and clinicians should not rely on 'classical' signs such as neck stiffness or bulging fontanel only. Even the best performing clinical decision rule fails to identify all cases when applied at admission and has poor specificity, hence it is important that all young infants hospitalized with serious illness undergo an LP.

What is already known on this topic

Meningitis is associated with significant mortality and long-term neurological impairment, particularly in developing countries where disease burden is highest and diagnostic resources are constrained.

We previously independently identified simple predictors of meningitis (fever, convulsions, irritability, bulging fontanel or temperature ≥39^{o}C) in young infants at our centre.

Current management guidelines are based on limited evidence obtained prior to use of conjugate vaccines and may not be optimal given changes in disease epidemiology.

What this study adds

This study investigated the performance of previously identified clinical features and WHO-recommended signs in discriminating meningitis in young infants hospitalised at a rural hospital.

Meningitis is less common than previously found but performance of clinical features in discriminating meningitis has not changed since the introduction of conjugate vaccines.

Low specificity of clinical features means that the capacity for basic cerebrospinal fluid analysis is essential to avoid unnecessary treatment.

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Authors' contributions

CWO, MN, CRN, MBH, and JAB contributed to the conception and design of the study. CWO, NM and JAB contributed to inpatient care and data collection. SM was responsible for laboratory analysis. CWO, NM, SM, MN, CRN, MBH, and JAB contributed to the analysis and interpretation of the data. CWO, MBH, and JAB contributed to the drafting of the article. The views expressed in this manuscript are those of the authors and not necessarily those of the KEMRI, or the Wellcome Trust. All authors read and approved the final manuscript.

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Competing interests

None declared.

Ethical approval

Collection of surveillance data included in this analysis was reviewed and approved by the Kenya Medical Research Institute Scientific Steering Committee (KEMRI SSC 1433). This retrospective analysis was reviewed and approved by the KEMRI SSC (KEMRI SSC 3001).

REFERENCES

1. Mann K, Jackson MA. Meningitis. *Pediatrics in review* 2008;29(12):417-29; quiz 30. doi: 10.1542/pir.29-12-417 [published Online First: 2008/12/03]
2. Khowaja AR, Mohiuddin S, Cohen AL, et al. Mortality and neurodevelopmental outcomes of acute bacterial meningitis in children aged <5 years in Pakistan. *The Journal of pediatrics* 2013;163(1 Suppl):S86-S91 e1. doi: 10.1016/j.jpeds.2013.03.035 [published Online First: 2013/06/21]
3. Christie D, Rashid H, El-Bashir H, et al. Impact of meningitis on intelligence and development: A systematic review and meta-analysis. *PLoS One* 2017;12(8):e0175024. doi: 10.1371/journal.pone.0175024 [published Online First: 2017/08/25]
4. Hudson LD, Viner RM, Christie D. Long-term sequelae of childhood bacterial meningitis. *Current infectious disease reports* 2013;15(3):236-41. doi: 10.1007/s11908-013-0332-6 [published Online First: 2013/03/30]
5. Ramakrishnan M, Ulland AJ, Steinhardt LC, et al. Sequelae due to bacterial meningitis among African children: a systematic literature review. *BMC medicine* 2009;7:47. doi: 10.1186/1741-7015-7-47 [published Online First: 2009/09/16]
6. Edmond K, Clark A, Korczak VS, et al. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *The Lancet Infectious diseases* 2010;10(5):317-28. doi: 10.1016/S1473-3099(10)70048-7 [published Online First: 2010/04/27]
7. Manning L, Laman M, Mare T, et al. Accuracy of cerebrospinal leucocyte count, protein and culture for the diagnosis of acute bacterial meningitis: a comparative study using Bayesian latent class analysis. *Tropical medicine & international health : TM & IH* 2014;19(12):1520-4. doi: 10.1111/tmi.12400 [published Online First: 2014/10/16]
8. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 2001;108(5):1169-74. [published Online First: 2001/11/06]
9. Polk DB, Steele RW. Bacterial meningitis presenting with normal cerebrospinal fluid. *The Pediatric infectious disease journal* 1987;6(11):1040-2. [published Online First: 1987/11/01]
10. Garges HP, Moody MA, Cotten CM, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics* 2006;117(4):1094-100. doi: 10.1542/peds.2005-1132 [published Online First: 2006/04/06]
11. Ku LC, Boggess KA, Cohen-Wolkowiez M. Bacterial meningitis in infants. *Clinics in perinatology* 2015;42(1):29-45, vii-viii. doi: 10.1016/j.clp.2014.10.004 [published Online First: 2015/02/14]
12. Group WTYIS. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *The Lancet* 2008;371(9607):135-42.
13. Clinical prediction of serious bacterial infections in young infants in developing countries. The WHO Young Infants Study Group. *The Pediatric infectious disease journal* 1999;18(10 Suppl):S23-31. [published Online First: 1999/10/26]
14. Young Infants Clinical Signs Study G. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *Lancet* 2008;371(9607):135-42. doi: 10.1016/S0140-6736(08)60106-3 [published Online First: 2008/01/15]
15. Mwaniki MK, Talbert AW, Njuguna P, et al. Clinical indicators of bacterial meningitis among neonates and young infants in rural Kenya. *BMC infectious diseases* 2011;11:301. doi: 10.1186/1471-2334-11-301 [published Online First: 2011/11/03]

16. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
17. Cowgill KD, Ndiritu M, Nyiro J, et al. Effectiveness of Haemophilus influenzae type b Conjugate vaccine introduction into routine childhood immunization in Kenya. *Jama* 2006;296(6):671-8. doi: 10.1001/jama.296.6.671 [published Online First: 2006/08/10]
18. Hammitt LL, Akech DO, Morpeth SC, et al. Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae and non-typeable Haemophilus influenzae in Kilifi, Kenya: findings from cross-sectional carriage studies. *The Lancet Global health* 2014;2(7):e397-405. doi: 10.1016/S2214-109X(14)70224-4
19. Gessner BD, Adegbola RA. The impact of vaccines on pneumonia: key lessons from Haemophilus influenzae type b conjugate vaccines. *Vaccine* 2008;26 Suppl 2:B3-8. doi: 10.1016/j.vaccine.2008.04.013 [published Online First: 2008/09/20]
20. Collaborators GBDCM. Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;388(10053):1725-74. doi: 10.1016/S0140-6736(16)31575-6 [published Online First: 2016/10/14]
21. 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. *The Lancet Global health* 2018;6(7):e744-e57. doi: 10.1016/S2214-109X(18)30247-X [published Online First: 2018/06/16]
22. Wamalwa EW. Implementation challenges of free maternity services policy in Kenya: the health workers' perspective. *Pan Afr Med J* 2015;22(1):375. doi: 10.11604/pamj.2015.22.375.6708
23. Okola VO. Effectiveness of a Group B Streptococcus (Gbs) Protocol on Gbs Screening and Intrapartum Antibiotic Prophylaxis at Kenyatta National Hospital. University of Nairobi, 2017.
24. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya. *Nat Microbiol* 2016;1(7):16067. doi: 10.1038/nmicrobiol.2016.67 [published Online First: 2016/08/31]
25. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed, 2013:412.
26. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, . February 2016.
27. Srinivasan L, Harris MC, Shah SS. Lumbar puncture in the neonate: challenges in decision making and interpretation. *Seminars in perinatology* 2012;36(6):445-53. doi: 10.1053/j.semperi.2012.06.007 [published Online First: 2012/11/28]
28. Berkley JA, Mwangi I, Mellington F, et al. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM : monthly journal of the Association of Physicians* 1999;92(3):151-7. [published Online First: 1999/05/18]
29. Mwangi I, Berkley J, Lowe B, et al. Acute bacterial meningitis in children admitted to a rural Kenyan hospital: increasing antibiotic resistance and outcome. *The Pediatric infectious disease journal* 2002;21(11):1042-8. doi: 10.1097/00006454-200211000-00013 [published Online First: 2002/11/21]
30. Bonsu BK, Harper MB. A low peripheral blood white blood cell count in infants younger than 90 days increases the odds of acute bacterial meningitis relative to bacteremia. *Acad Emerg Med* 2004;11(12):1297-301. doi: 10.1197/j.aem.2004.06.012 [published Online First: 2004/12/04]
31. Nigrovic LE, Malley R, Kuppermann N. Meta-analysis of bacterial meningitis score validation studies. *Archives of disease in childhood* 2012;97(9):799-805. doi: 10.1136/archdischild-2012-301798 [published Online First: 2012/07/06]

32. Kuppermann N, Dayan PS, Levine DA, et al. A Clinical Prediction Rule to Identify Febrile Infants 60 Days and Younger at Low Risk for Serious Bacterial Infections. *JAMA pediatrics* 2019;173(4):342-51. doi: 10.1001/jamapediatrics.2018.5501 [published Online First: 2019/02/19]
33. English M, Gathara D, Mwinga S, et al. Adoption of recommended practices and basic technologies in a low-income setting. *Archives of disease in childhood* 2014;99(5):452-6. doi: 10.1136/archdischild-2013-305561 [published Online First: 2014/02/01]
34. Najaf-Zadeh A, Dubos F, Hue V, et al. Risk of bacterial meningitis in young children with a first seizure in the context of fever: a systematic review and meta-analysis. *PLoS One* 2013;8(1):e55270. doi: 10.1371/journal.pone.0055270
35. Saez-Llorens X, McCracken GH, Jr. Bacterial meningitis in children. *Lancet* 2003;361(9375):2139-48. doi: 10.1016/S0140-6736(03)13693-8 [published Online First: 2003/06/27]
36. Molyneux E, Walsh A, Phiri A, et al. Acute bacterial meningitis in children admitted to the Queen Elizabeth Central Hospital, Blantyre, Malawi in 1996-97. *Tropical medicine & international health : TM & IH* 1998;3(8):610-8. [published Online First: 1998/09/15]

Supplementary Tables

Supplementary Table 1. Comparison of annual admissions, lumbar punctures and meningitis cases during our study period and our previous analysis

Study period	2012-2016	2006-2007	2001-2005
Admissions/year	1,118	839	690
Lumbar punctures/year	411	517	210
Meningitis cases/year	16	32	20

Data are N

Supplementary Table 2. Performance of indicators of meningitis among neonates 0-6 days old

Indicators	Number with indicator	Number with meningitis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	NNLP (95% CI)
Bulging fontanel	17	2	5.1 (0.6-17.3)	99.6 (99.3-99.8)	11.8 (1.5-36.4)	98.9 (98.5-99.2)	9 (4 - -22)
Convulsions or any of the above	184	7	17.9 (7.5-33.5)	94.9 (94.1-95.6)	3.8 (1.5-7.7)	99.0 (98.6-99.3)	35 (18-1,734)
Axillary temp ≥39^{o}C or any of the above	365	11	28.2 (15.0-44.9)	89.8 (88.7-90.8)	3.0 (1.5 (5.3)	99.1 (98.7-99.4)	47 (26-296)
Agitation/irritability or any of the above	414	15	38.5 (23.4-55.4)	88.5 (87.4-89.5)	3.6 (2.0-5.9)	99.2 (98.8-99.5)	35 (21-98)
History of fever or any of the above	1,509	26	66.7 (49.8-80.9)	57.2 (55.6-58.9)	1.7 (1.1-2.5)	99.3 (98.9-99.7)	93 (55-305)
One or more of the WHO suggested signs	1,598	22	56.4 (39.6-72.2)	54.6 (52.9-72.2)	1.4 (0.9-2.1)	99.1 (98.6-99.5)	206 (84- -448)
One or more of the WHO suggested signs or history of fever	2,573	31	79.5 (63.5-90.7)	26.7 (25.3-28.2)	1.2 (0.8-1.7)	99.1 (98.3-99.6)	286 (93- -266)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NNLP, number needed to lumbar puncture

Supplementary Table 3. Performance of indicators of meningitis among neonates 7-59 days old

Indicators	Number with indicator	Number with meningitis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	NNLP (95% CI)
Bulging fontanel	20	9	21.4 (10.3-36.8)	99.1 (98.4-99.6)	45.0 (23.1-68.5)	97.4 (96.4-98.2)	2 (2-5)
Convulsions or any of the above	108	17	40.5 (25.6-56.7)	92.8 (91.2-94.1)	15.7 (9.5-24.0)	97.9 (96.9-98.6)	7 (5-15)
Axillary temp ≥39^{o}C or any of the above	190	21	50.0 (34.2-65.8)	86.6 (84.6-88.4)	11.1 (7.0-16.4)	98.1 (97.1-98.8)	11 (7-22)
Agitation/irritability or any of the above	226	22	52.4 (36.4-68.0)	83.8 (81.6-85.8)	9.7 (6.2-14.4)	98.1 (97.1-98.9)	13 (9-26)
History of fever or any of the above	868	38	90.5 (77.4-97.3)	34.1(31.5-36.8)	4.4 (3.1-6.0)	99.1 (97.7-99.7)	29 (20-55)
One or more of the WHO suggested signs	474	25	59.5 (43.3-74.4)	64.3 (61.6-67.0)	5.3 (3.4-7.7)	97.9 (96.7-98.8)	31 (18-101)
One or more of the WHO suggested signs or history of fever	993	41	97.6 (87.4-99.9)	24.4 (22.0-26.9)	4.1 (3.0-5.6)	99.7 (98.2-100)	26 (19-41)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NNLP, number needed to lumbar puncture

Supplementary Table 4. Performance of indicators of meningitis among all infants 0-59 days old, including possible meningitis[a]

Indicators	Number with indicator	Number with meningitis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95%CI)	NNLP (95% CI)
Bulging fontanel	37	14	11.1 (6.2-17.9)	99.5 (99.3-99.7)	37.8 (22.5-55.2)	97.6 (97.2-98.0)	3 (2-5)
Convulsions or any of the above	292	36	28.6 (20.9-37.3)	94.5 (93.8-95.2)	12.3 (8.8-16.7)	98.0 (97.6-98.4)	10 (7-15)
Axillary temp ≥39^{o}C or any of the above	555	46	36.5 (28.1-45.6)	89.1 (88.2-90.0)	8.3 (6.1-10.9)	98.1 (97.7-98.5)	16 (11-25)
Agitation/irritability or any of the above	640	52	41.3 (32.6-50.4)	87.4 (86.5-88.4)	8.1 (6.1-10.5)	98.2 (97.8-98.6)	16 (12-24)
History of fever or any of the above	2,377	94	74.6 (66.1-81.9)	51.2 (49.8-52.7)	4.0 (3.2-4.8)	98.7 (98.1-99.1)	38 (28-58)
One or more of the WHO suggested signs	2,072	77	61.1 (52.0-69.7)	57.4 (56.0-58.8)	3.7 (2.9-4.6)	98.2 (97.6-98.7)	52 (35-103)
One or more of the WHO suggested signs or history of fever	3,566	113	89.7 (83.0-94.4)	26.3 (25.0-27.6)	3.2 (2.6-3.8)	99.0 (98.2-99.4)	47 (34-76)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NNLP, number needed to lumbar puncture

[a] Possible meningitis defined in infants without definite meningitis as CSF WBC ≥20/µL and <50/µL in infants age 0-28 days, or CSF WBC ≥10/µL and <50/µL in infants age 29-59 days.

Supplementary Table 5. Performance of indicators of meningitis among all infants 0-59 days old using microbiologically confirmed definite meningitis[a]

Indicators	Number with indicator	Number with meningitis	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95%CI)	NNLP (95% CI)
Previously identified							
Bulging fontanel	37	6	21.4 (8.3-41.0)	99.3 (99.1-99.6)	16.2 (6.2-32.0)	99.5 (99.3-99.7)	6 (4-26)
Convulsions or any of the above	292	8	28.6 (13.2-48.7)	94.1 (93.4-94.7)	2.7 (1.2-5.3)	99.6 (99.3-99.7)	44 (24-241)
Axillary temp ≥39^{o}C or any of the above	555	14	50.0 (30.6-69.4)	88.7 (87.8-89.6)	2.5 (1.4-4.2)	99.7 (99.4-99.8)	46 (28-114)
Agitation/irritability or any of the above	640	15	53.6 (33.9-72.5)	86.9 (85.9-87.9)	2.3 (1.3-3.8)	99.7 (99.5-99.8)	49 (31-118)
History of fever or any of the above	2,377	24	85.7 (67.3-96.0)	50.8 (49.4-52.2)	1.0 (0.6-1.5)	99.8 (99.6-100.0)	118 (78-243)
WHO recommended							
One or more of the WHO suggested signs	2,072	15	53.6 (33.9-72.5)	57.0 (55.6-58.4)	0.7 (0.4-1.2)	99.5 (99.2-99.7)	402 (144-506)
One or more of the WHO suggested signs or history of fever	3,566	24	85.7 (67.3-96.0)	25.9(24.7-27.2)	0.7 (0.4-1.0)	99.7 (99.2-99.9)	285 (131-1,601)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; NNLP, number needed to lumbar puncture to identify one case of definite meningitis

[a]Includes positive CSF culture, positive latex agglutination test, positive microscopy, or CSF leukocyte count ≥50 cells/μL plus positive blood culture

Chapter 5

Clinical features of bacterial meningitis among hospitalised children in Kenya

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ABSTRACT

Background Diagnosing bacterial meningitis is essential to optimise the type and duration of antimicrobial therapy to limit mortality and sequelae. In sub-Saharan Africa, many public hospitals lack laboratory capacity, relying on clinical features to empirically treat or not treat meningitis. We investigated whether clinical features of bacterial meningitis identified prior to the introduction of conjugate vaccines still discriminate meningitis in children aged ≥60 days.

Methods We conducted a retrospective cohort study to validate seven clinical features identified in 2002 (*KCH-2002*): bulging fontanel, neck stiffness, cyanosis, seizures outside the febrile convulsion age range, focal seizures, impaired consciousness, or fever without malaria parasitaemia; and Integrated Management of Childhood Illness (IMCI) signs: neck stiffness, lethargy, impaired consciousness or seizures, and assessed at admission in discriminating bacterial meningitis after the introduction of conjugate vaccines. Children aged ≥60 days hospitalised between 2012 and 2016 at Kilifi County Hospital were included in this analysis. Meningitis was defined as positive cerebrospinal fluid (CSF) culture, organism observed on CSF microscopy, positive CSF antigen test, leukocytes ≥50/µL, or CSF to blood glucose ratio <0.1.

Results Among 12,837 admissions, 98 (0.8%) had meningitis. The presence of *KCH-2002* signs had a sensitivity of 86% (95%CI 77-92) and specificity of 38% (95%CI 37-38). Exclusion of 'fever without malaria parasitaemia' reduced sensitivity to 58% (95%CI 48-68) and increased specificity to 80% (95%CI 79-80). IMCI signs had a sensitivity of 80% (95%CI 70-87) and specificity of 62% (95%CI 61-63).

Conclusions A lower prevalence of bacterial meningitis and less typical signs than in 2002 meant the lower performance of *KCH-2002* signs. Clinicians and policymakers should be aware of the number of lumbar punctures (LPs) or empirical treatments needed for each case of meningitis. Establishing basic capacity for CSF analysis is essential to exclude bacterial meningitis in children with potential signs.

BACKGROUND

Childhood bacterial meningitis is associated with significant mortality and neurocognitive sequelae.[1 2] The disease burden is highest in low- and middle-income countries (LMICs) where a quarter of children who survive vaccine-preventable meningitis develop post-discharge complications.[2 3] Prompt recognition and antimicrobial treatment with cerebrospinal fluid (CSF) penetration for an adequate duration are critical.

CSF culture is the gold standard for bacterial meningitis but has limited sensitivity[4] as it may be compromised by prior administration of antimicrobials[5] and is usually unavailable or unreliable in public hospitals in sub-Saharan Africa. Public hospitals also often lack adequate CSF microscopy capacity, and lumbar puncture (LP) may be commonly ordered but not done.[6 7] Thus, antimicrobial management decisions are often based on clinical features only.

The World Health Organization (WHO) advises suspecting bacterial meningitis if one or more of the following are present: convulsions, inability to drink, irritability, lethargy, impaired consciousness, a bulging fontanel, or neck stiffness.[8] However, this recommendation is based on limited evidence collected prior to the introduction of *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* conjugate vaccines targeting the leading causes of bacterial meningitis.

In the Gambia ~20 years ago, a set of Integrated Management of Childhood Illness (IMCI) signs (lethargy, impaired consciousness, convulsions, or a stiff neck)[9] had 98% sensitivity and 72% specificity in predicting bacterial meningitis.[10] Concurrently, among children aged ≥60 days at Kilifi County Hospital (KCH), Kenya, a bulging fontanel, neck stiffness, cyanosis, seizures outside the febrile convulsions age range, focal seizures, and impaired consciousness were identified as indicators of bacterial meningitis (*KCH-2002*).[11] These findings were incorporated into Kenyan national paediatric guidelines.[12]

Hib and 10-valent pneumococcal conjugate vaccines at 6, 10, and 14 weeks of age without booster were introduced in Kenya in 2001 and 2011, respectively, resulting in a markedly reduced incidence and mortality from bacterial meningitis.[13-17] Since the early 2000's severe malaria, which may mimic bacterial meningitis [18], has declined, with changes in age and disease profile reported at several centres in Africa.[19-21]

Changes in epidemiology, patient profile and differential diagnoses may have altered associations between clinical features and bacterial meningitis. We therefore performed a revalidation study of the *KCH-2002* and IMCI signs among children aged ≥60 days.

METHODS

Location and participants

KCH is a public hospital serving a mostly rural population. Paediatric care is supported by the KEMRI/Wellcome Trust Research Programme. Children aged 60 days to 13 years hospitalised at KCH between January 1, 2012, and December 31, 2016, were included in this analysis.

Procedures

All children admitted were systematically assessed using standardised demographic and clinical proforma by trained clinicians at admission, and data were entered on a database in real-time. All admissions had a complete blood count, malaria slide, and blood culture. LP was performed at admission if suggestive signs were present, or if a child developed new clinical features of meningitis according to the WHO[8] and Kenyan guidelines[12] detected through daily clinical reviews until discharge. LP was deferred in children with cardiorespiratory compromise or suspicion of raised intracranial pressure.[22] Children with suspected meningitis were treated empirically with penicillin plus chloramphenicol or ceftriaxone (as per national and WHO guidelines[8 12]) while awaiting LP results. Once available, treatment was modified based on culture and susceptibility profile as needed. Data collection (SSC1433) and this analysis (SSC3001) were approved by the KEMRI Scientific and Ethics Review Unit.

Laboratory analysis

CSF examination included leukocyte and red blood cell (RBC) count using the Neubauer counting chamber method, and if leukocyte count >10 cells/µl, differential leukocyte count, Gram and Indian ink staining, latex antigen agglutination tests (Wellcogen™ Bacterial Antigen kit for *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, and CrAg Lateral Flow Assay kit Ref CR2003 for *Cryptococcus neoformans*) were done. CSF and blood samples were cultured, and pathogens identified using standard methods as previously described.[11 18] Coagulase-negative S*taphylococci* were considered non-significant.[23] CSF protein, glucose, and concurrent blood glucose were measured on an ILab Aries

analyser (Werfen, Germany). External quality assurance was by the United Kingdom External Quality Assessment Service, and Good Clinical Laboratory Practice was accredited by Qualogy, UK.[11]

Definitions

For this analysis, we used the *KCH-2002*[11] definition of bacterial meningitis:(i) positive CSF culture for a known pathogen, (ii) positive CSF antigen test, (iii) an organism observed on CSF microscopy (Gram stain or Indian Ink), (iv) CSF leucocyte count ≥50 cells/μL, or (v) CSF to blood glucose ratio <0.1. We also defined possible meningitis as CSF leucocyte count >10-49 cells/μL in the absence of the above criteria.

Statistical analysis

For the primary analysis, children who underwent LP not meeting meningitis criteria or without an LP were classified as not having meningitis, as was assumed in *KCH-2002*. We initially excluded children with possible meningitis[11], and calculated the highest criterion for meningitis in the order given above.

We examined the performance of *KCH-2002*[11] and IMCI signs (neck stiffness, lethargy, impaired consciousness, or seizures)[9] at admission by calculating their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for meningitis diagnosed by LP either at admission or at any time during hospitalisation versus no meningitis, defined as negative CSF analysis or no clinical suspicion of meningitis until discharge from hospital. We calculated the number of LPs needed to identify one case of meningitis as the inverse of the risk difference obtained by subtracting the prevalence of meningitis in each group from that in the group without the indicators of interest. As sensitivity analyses, we (i) included possible meningitis cases, (ii) excluded those who died before LP, and (iii) used a narrow microbiological definition of meningitis (positive CSF culture for a known pathogen, positive CSF antigen test, an organism observed on CSF microscopy (Gram stain or Indian Ink), or CSF leucocyte count >10 cells/μL plus a positive blood culture).

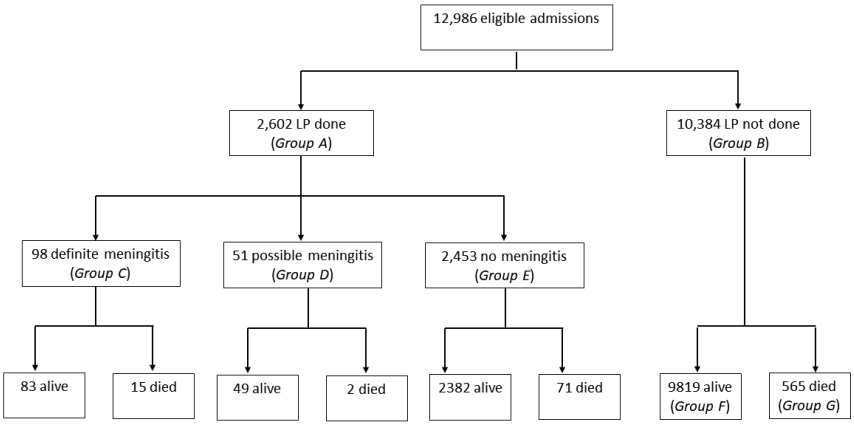
Proportions were compared using the chi-squared test or Fisher's exact test. Continuous variables were compared using Wilcoxon rank-sum test. All analyses used Stata version 15 (Stata Corp, USA).

RESULTS

There were 12,986 admissions aged 60 days to 13 years: 2,975 (23%) <1 year, 6,248 (48%) 1-4 years, and 3,763 (29%) ≥5 years old; 463 (3.6%) were HIV antibody positive. Two thousand six hundred and two (20%) children had an LP, of which 409 (16%) were aged <1 year. LPs were more commonly done among children aged 1-5 years [1,484/6,248 (24%)] than in children aged >5 years [709/3,763 (19%)] or <1 year [409/2,975 (14%)], *P<0.001*. A positive malaria smear was present in 1,189 (46%) children who had an LP. Of 10,384 children who did not have an LP, 565 died before an LP (193 (34%) <1 year, 230 (41%) 1-5 years and 142 (25%) ≥5 years) while 9,819 survived (2,373 (24%) <1 year, 4,534 (46%) 1-5 years and, 2,912 (30%) ≥5 years) (*P<0.001*). Median [interquartile range (IQR)] age of 565 children who died before an LP was 21 months (8.0-60) compared to 20 months (9.5-59) in 88 children who died after an LP (*P=0.874*).

Meningitis cases

Ninety-eight children had meningitis (Figure 1, Group C): 0.8% of 12,986 admissions and 3.8% of 2,602 children with an LP. Fifty-one (0.4%) children had possible meningitis (Group D) and were excluded from the primary analysis. Median (IQR) ages of children with meningitis, possible meningitis, or no meningitis were 25 (7.4-77), 40 (11-83) and 29 (12-67) months respectively *(P=0.167)*. Fifteen (15%) meningitis cases died during hospitalisation; 2.3% (15/653) of all inpatient deaths.



Abbreviation: LP, lumbar puncture

Figure 1. Flow chart of study participants

Leading CSF pathogens were *S. pneumoniae* (16 culture-positive and 4 antigen-positive) and *H. influenzae* (5 culture-positive and 3 antigen-positive) (Table 1). Fifty (51%) meningitis cases had CSF leukocyte count ≥50/µl only. One hundred and twenty (4.8%) of 2,521 children had differential leukocyte count done of which 118 (98%) had polymorphonuclear cell predominance (≥60%) and 77 had meningitis. Five (2.0%) of 249 children with CSF RBC count ≥500 cells/µL had positive CSF cultures while 4 (1.6%) children missed leukocyte counting due to grossly blood-stained CSF. Thirty-three (34%) children with meningitis had positive blood culture; 23 matched CSF isolates (13 *S. pneumoniae*, 4 *H. influenzae*, 2 *Salmonella* spp., and 1 each of *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *C. neoformans*). Forty-one (42%) meningitis cases had turbid CSF.

Admission clinical features

Two thousand three hundred and thirty (79%), 3,762 (60%) and 2,007 (54%) children aged <1, 1-5 and ≥5 years respectively presented with *KCH-2002* signs (*P<0.001*), while 899 (30%), 2,661 (43%) and 1,391 (37%) had IMCI signs (*P<0.001*). Bulging fontanel, neck stiffness, impaired consciousness, seizures outside the febrile convulsion age range, focal seizures, history of fever and axillary temperature ≥39^{o}C were more common among children with meningitis than without, and malaria was less common (Table 2). Of 8,099 children with *KCH-2002* signs, 485 (6.0%) died before LP (277 (57%) within 24 hours of admission). Of 4,951 children with IMCI signs, 359 (7.3%) died before LP (240 (67%) within 24 hours of admission).

Table 1. Diagnostic criteria for meningitis and organisms detected

Diagnostic criteria	Age <1year (n=33)	Age 1-4years (n=35)	Age ≥5years (n=30)	Total positives	Highest criteria for meningitis[a]
CSF culture					
Gram positive					
Streptococcus pneumoniae	5	5	6	16[b]	16
Haemophilus influenzae	3	2	0	5[c]	5
Staphylococcus aureus	1	0	0	1[d]	1
Gram negative					
Escherichia coli	0	1	0	1	1
Klebsiella pneumonia	1	1	0	2[e]	2
Non-typhoidal *Salmonella* sp.	2	0	0	2[f]	2
Pseudomonas aeruginosa	1	0	0	1[g]	1
Proteus mirabilis	1	0	0	1[h]	1
Cryptococcus neoformans	0	0	2	2[i]	2
Total	**14**	**9**	**8**	**31**	**31**
Latex antigen test					
Streptococcus pneumoniae	7	5	6	18	4[j]
Haemophilus influenzae	2	3	0	5	3[k]
Cryptococcus neoformans	0	0	2	2	1
Total	**9**	**8**	**8**	**25**	**8**
Gram-stain					
Gram-positive cocci	8	8	5	21	4
Gram-negative rods	4	3	0	7	0
Total	**12**	**11**	**5**	**28**	**4**
Indian ink	**0**	**0**	**1**	**1**	**0**
CSF WCC ≥50 cells/µl	29	29	27	85	52[l]
CSF/blood glucose ratio <0.1	**7**	**5**	**3**	**15**	**3**
Total					**98**

[a]Hierarchical order of criteria for meningitis:(i) positive CSF culture for a known pathogen, (ii) positive CSF antigen test, (iii) an organism observed on CSF microscopy (Gram stain or Indian Ink), (iv) CSF leucocyte count ≥50 cells/µL, and (v) CSF to blood glucose ratio <0.1

[b]14/16 had WCC ≥50 cells/µl, 14/16 had positive *S. pneumoniae* antigen test, 14/16 had positive Gram stain, and 5/16 had CSF/blood glucose ratio <0.1

[c]5/5 had WCC ≥50 cells/µl, 2/5 had positive *H. influenzae* antigen test, 3/5 had positive Gram stain, 3/5 had CSF/blood glucose ratio <0.1

[d]Had WCC ≥50 cells/µl and positive Gram stain

[e]1/2 had WCC ≥50 cells/µl and positive Gram stain

[f]1/2 had WCC ≥50 cells/µl and positive Gram stain. 1/2 had CSF/blood glucose ratio <0.1

[g]Had WCC ≥50 cells/µl and positive Gram stain

[h]Had WCC ≥50 cells/µl, positive Gram stain and CSF/blood glucose ratio <0.1

[i]1/2 had WCC ≥50 cells/µl, positive Indian Ink stain and positive Cryptococcal antigen test

[j]2/4 had WCC ≥50 cells/µl and positive Gram stain

[k]3/3 had WCC ≥50 cells/µl, 2/3 had positive Gram stain, and 1/3 had CSF/blood glucose <0.1

[l]2/52 had CSF/Blood glucose ratio <0.1

Table 2. Clinical features at admission

	No meningitis (n=12,837)	Meningitis (n=98)	P value[a]
Bulging fontanelle[b]			
Absent	4,490 (98)	37 (82)	<0.001
Present	51 (1.1)	8 (18)	
Missing	29 (0.6)	0 (0)	
Neck stiffness			
Absent	12,652 (99)	79 (81)	<0.001
Present	96 (0.8)	19 (19)	
Missing	89 (0.7)	0 (0)	
Cyanosis			
Absent	12,702 (99)	98 (100)	0.594
Present	46 (0.3)	0 (0)	
Missing	89 (0.7)	0 (0)	
History of seizures within febrile convulsion age range[c]			
No seizures	9,990 (78)	48 (49)	<0.001
Seizures within febrile convulsion age range	2,187 (17)	34 (35)	
Seizures outside febrile convulsion age range	573 (4.5)	16 (16)	
Missing	87 (0.7)	0 (0)	
Type of convulsion at any age			
No seizures	9,990 (78)	48 (49)	<0.001
Unknown type	81 (0.6)	2 (2.0)	
Generalised[d]	2,354 (18)	40 (41)	
Focal[e]	325 (2.5)	8 (8.2)	
Missing	87 (0.7)	0 (0)	
Conscious level			
Normal	9,338 (73)	46 (47)	<0.001
Lethargic	1,444 (11)	16 (16)	
Agitated	159 (1.2)	2 (2.0)	
Impaired consciousness[f]	1,807 (14)	34 (35)	
Missing	89 (0.7)	0 (0)	
History of fever			
Absent	4,070 (32)	10 (10)	<0.001
Present	8,681 (68)	88 (90)	
Missing	86 (0.7)	0 (0)	
Axillary temperature, ^{o}C			
<36	717 (5.6)	4 (4.1)	0.032
36-38.9	10,080 (79)	68 (69)	
≥39	2,016 (16)	26 (27)	
Missing	24 (0.2)	0 (0)	
Malaria			
Negative	10,346 (81)	84 (86)	0.201
Positive	2,491 (19)	14 (14)	

[a]Compares children with definite meningitis (Group B, n=98) to children with no meningitis [Group A (n=10,384) + Group D (n=2,453)]. Excludes children with possible meningitis (Group C, n=51)
[b]Cut-off age for assessment of fontanel closure was 18 months. Analysis of bulging fontanel was limited to 4,615 children aged ≤18 months (45 with meningitis and 4,570 without meningitis) hence column totals are less than those of other variables on the table; [c]Febrile convulsions occurring in children age between 6 months and 6 years; [d]Involving both sides of the body with associated loss of consciousness
[e]Focal, involving one side of the body, and may or may not become generalised
[f]Blantyre Coma Score (BCS) <4 up to 9 months and <5 at ≥9 months (Eyes: 1=watches/follows, 0=none; Cry: 2=normal, 1=moan/weak, 0=none; Motor: 2=localises stimulus, 1=withdraws, 0=other/none)

Performance of clinical features

KCH-2002

One or more *KCH-2002* signs were present in 8,099 children, of whom 84 (1.0%) had meningitis compared with 14/4,836 (0.3%) without *KCH-2002* signs: sensitivity 86% (95%CI 77-92), specificity 38% (95%CI 37-38), PPV 1.0% (95%CI 0.8-1.3), and NPV 100% (95%CI 99-100). One hundred and thirty-four children (95%CI 99-208) presenting with ≥1 *KCH-2002* signs would need to undergo an LP for each case of meningitis identified (Table 3).

IMCI

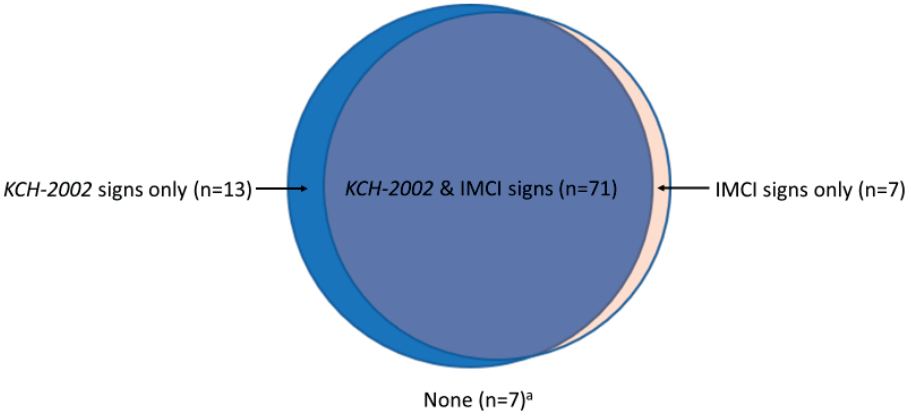
One or more IMCI signs were present in 4,951 children, of whom 78 (1.6%) had meningitis compared with 20/7,984 (0.3%) without IMCI signs: sensitivity 80% (95%CI 70-87), specificity 62% (95%CI 61-63), PPV 1.6% (95%CI 1.3-2.0), and NPV 100% (99%CI 99-100). Seventy-six children (95%CI: 59-104) presenting with ≥1 IMCI signs would need to undergo an LP for each case of meningitis identified (Table 3).

Admission versus later LP

Thirty-three (34%) meningitis cases had their LP after admission, of which 6/33 (18%) and 8/33 (24%) were not identified by *KCH-2002* signs and IMCI signs, respectively, at admission. Seven (7.1%) meningitis cases were not identified by either *KCH-2002* signs or IMCI signs at admission (Figure 2).

Sensitivity analysis

Excluding 565 who died before an LP (Group G) and including 51 cases with possible meningitis (Group D) as 'meningitis' gave similar results for *KCH-2002* and IMCI signs (Supplementary Table 2). Fifty children with microbiologically confirmed meningitis fulfilled criteria as follows: 31 positive CSF cultures only (of which 23 had positive blood culture), 7 positive antigen tests only (of which 2 had positive blood culture), 5 positive microscopies (of which 2 had CSF leukocyte count >10), and 7 CSF leukocyte counts >10 cells/µL plus positive blood culture. For microbiologically confirmed meningitis, *KCH*-2002 signs had a sensitivity of 90% (95%CI 78-97) and specificity of 39% (95%CI 38-39). IMCI signs had a sensitivity 76% (95%CI 62-87) and specificity 63% (95%CI 62-64).



Abbreviations: KCH-2002, previously identified signs at Kilifi County Hospital; IMCI, Integrated Management of Childhood Illness.
[a] History of fever with positive malaria smear (n=1), history of diarrhoea (n=2), history of vomiting (n=2), oedema (n=1), palmar pallor (n=1), severe acute malnutrition (n=2), died (n=2)

Figure 2. Clinical features of meningitis in 98 children with definite meningitis

DISCUSSION

Misdiagnosis of bacterial meningitis based on the clinical signs only may result in overtreatment with prolonged courses of antimicrobials, or undertreatment of missed cases,[24] both contributing to mortality and selection of resistant organisms.

We studied a large cohort of hospitalised children to validate clinical features of bacterial meningitis. Using the same definitions and inclusion criteria as in 2002, we observed a reduction in the prevalence of bacterial meningitis among paediatric admissions at our centre from 2% in 2001-2002[11] to 0.8% in 2012-2016. There was also a decline in annual paediatric admissions and number of LPs done. However, we observed an increase in the prevalence of *KCH-2002* signs (55% in 2001-2002 vs 63% in 2012-2016, *P<0.001*) and a decrease in the prevalence of IMCI signs (42% in 2001-2002 vs 38% in 2012-2016, *P<0.001*) [11]. Although *S. pneumoniae* and *H. influenzae* remained the leading causes of bacterial meningitis, cases arising from these organisms declined over time (57 vs 20 pneumococcal, and 66 vs 8 *H. influenzae* cases, comparing 1994-1998[25] to 2012-2016). These changes may be attributed to conjugate vaccination and herd immunity in older children. Our study excluded infants aged <60 days who typically have bacterial meningitis due to different pathogens,[17] different clinical presentation[26] and alternative diagnoses such as birth asphyxia,[27] and associated higher risk of neurological disability and mortality.[17]

Table 3. Comparison of potential screening criteria at admission for definite meningitis (excluding children with possible meningitis only (Group C, n=51))

Screening criteria	No. with criteria	No. with meningitis	Sensitivity % (95% CI)	Specificity % (95%CI)	PPV % (95% CI)	NPV % (95% CI)	NNLP (95% CI)
Bulging fontanel[a] or neck stiffness	161	23	23.5 (15.5-33.1)	98.9 (98.7-99.1)	14.3 (9.3-20.7)	99.4 (99.3-99.5)	7 (5-12)
Cyanosis or any of the above	207	23	23.5 (15.5-33.1)	98.6 (98.3-98.8)	11.1 (7.2-16.2)	99.4 (99.3-99.5)	10 (7-16)
Seizures outside 6 months to 6 years or any of the above	771	33	33.7 (24.4-43.9)	94.3 (93.8-94.6)	4.3 (3.0-6.0)	99.5 (99.3-99.6)	27 (19-43)
Focal seizures or any of the above	1,023	39	39.8 (30.0-50.2)	92.3 (91.9-92.8)	3.8 (2.7-5.2)	99.5 (99.4-99.6)	30 (22-47)
Impaired consciousness or any of the above	2,659	57	58.2 (47.8-68.1)	79.7 (79.0-80.4)	2.1 (1.6-2.8)	99.6 (99.5-99.7)	57 (43-85)
Fever without malaria parasitemia or any of the above	8,099	84	85.7 (77.2-92.0)	37.6 (36.7-38.4)	1.0 (0.8-1.3)	99.7 (99.5-99.8)	134 (99-208)
IMCI referral criteria: neck stiffness, lethargy, impaired consciousness, or seizures	4,951	78	79.6 (70.3-87.1)	62.0 (61.2-62.9)	1.6 (1.3-2.0)	99.7 (99.6-99.8)	76 (59-104)

Abbreviations: CI, confidence interval; PPV, positive predictive values; NPV, negative predictive value; NNLP, number needed to lumbar puncture; IMCI, integrated management of childhood infection

'Or any of the above' refers to the presence of ≥1 of the signs indicated in the preceding rows. This means that children represented on each row had the sign indicated on a particular row +/- any of the preceding signs. For example, 161 children had either bulging fontanel or neck stiffness (13 had both). 1,023 children had ≥1 focal seizures, seizures outside the febrile convulsion age range, cyanosis, bulging fontanel, or neck stiffness (252 had focal seizures only while 771 had focal seizures and ≥1 of seizures outside the febrile convulsion age range, cyanosis, bulging fontanel and neck stiffness [e.g., 76 had focal seizures which occurred outside the febrile convulsion age range; 1 had focal seizures which occurred outside the febrile convulsion age range and cyanosis])

[a]Bulging fontanel was only deemed present if the age was ≤18 months

Clinical guidelines for limited-resource settings should comprise straightforward features, easily identifiable by clinicians.[28] Overall, we found that the clinical signs at admission had lower sensitivity and PPV in discriminating children with bacterial meningitis than in 2002.[11] *KCH-2002* and IMCI signs did not statistically significantly differ in the proportions of meningitis cases missed (14% vs 20%, *P=0.258*), although numbers were limited for this comparison. Results did not appear to be altered by the exclusion of children who died before LP or using a narrower microbiological case definition.

History of fever was common with (90%) or without meningitis (68%) and nearly half of the LPs were done in children with malaria since signs overlap. The previous *KCH-2002* analysis found that exclusion of fever without malaria parasitaemia from the screening rule had lower sensitivity but higher specificity (sensitivity 79%, specificity 80%, PPV 8.0%) than when it was included (sensitivity 97%, specificity 44%, PPV 3.5%).[11] The present analysis also shows that although the specificity of *KCH-2002* signs excluding fever without malaria parasitaemia has not changed, sensitivity was again markedly reduced (to 58% from 86%). Malaria parasitaemia has been shown to augment predictive models for bacterial meningitis;[11 29] however, the significant morbidity and mortality associated with meningitis means a screening rule with higher sensitivity may be favourable despite lower specificity.

Although conjugate vaccination has resulted in a reduction in bacterial meningitis cases, antimicrobial resistance to penicillin[30] and chloramphenicol[31 32] is reported. Ceftriaxone as a first-line treatment for bacterial meningitis has been associated with lower resistance rates, and reduction in mortality and neurological complications compared to chloramphenicol.[32 33] Thus, clinical decision rules with optimal performance in predicting bacterial meningitis contribute to antimicrobial stewardship by guiding initiation of treatment and minimising selection of resistant microorganisms.

Limitations

An inescapable limitation is that a selective group of children underwent an LP based on clinical suspicion at admission or later during admission. It is possible that a number of bacterial meningitis cases may have been missed due to apparent recovery and discharge. However, we believe that the higher than usual clinical staffing, training oversight, and availability of laboratory resources due to the presence of the research programme helped limit the chances of missed meningitis cases. Although performing LPs in all children is diagnostically

optimal and would provide an understanding of the true prevalence of meningitis, this is not possible due to the risks involved and would not be ethically justified.[22] Our dataset may not be perfect, but it addresses research gaps in similar settings. Of 2,602 LPs done, 1,026 (39%) were performed after admission; 33/98 (34%) meningitis cases were diagnosed after admission, underscoring the importance of daily clinical reviews following standard guidelines. Our assumption of true negatives in children who did not develop signs suggestive of meningitis during hospitalisation and were discharged home alive is valid. The highest proportion of children having an LP was in those aged 1-5 years. *KCH-2002* signs were most frequent among children aged <1 year, fewer LPs done in this age group may be attributed to early deaths or more LPs being deferred due to contraindications since most deaths occurred in young infants (7.4%, 4.3%, and 4.4% deaths in children aged <1, 1-5, and ≥5 years, respectively, *P<0.001*). However, age bias in LPs may have affected our findings. Importantly, our aim was to inform clinical guidelines for empiric treatment and indications for LP rather than describe the epidemiology of meningitis for which post-mortem LPs would have been necessary.

Molecular tests for bacterial and viral causes were not routinely done, potentially missing true bacterial meningitis cases and falsely including viral meningitis cases. Although differential leukocyte count was done in some CSF samples, it was not included in our standard definition of meningitis. Polymorphonuclear cell predominance can occur in both bacterial and aseptic meningitis.[34] We lacked data on pre-hospital antibiotic exposure which may be common and has been shown to alter CSF leukocyte count and biochemical profile and impede detection of bacterial pathogens.[5 35] Diagnostic delay may decrease survival[36] and increase neurological sequelae in Hib meningitis,[37] and may be more of a problem in settings without advanced diagnostic resources such as CSF polymerase chain reaction (PCR).[38]

Low LP rates reported in settings like ours have raised concerns regarding missing meningitis cases.[6 7] Knowing that a large number of LPs is needed in order to diagnose each case of bacterial meningitis is important in this regard. The *KCH-2002* or IMCI signs at admission suggest an LP may be needed in ~40 to 60% of children presenting to the hospital with these signs to achieve >80% sensitivity. There are no studies evaluating the additional discriminatory value of a structured repeated evaluation of signs that develop later during admission, or of biomarkers in this context. Although traumatic LPs are common and may complicate CSF leukocyte interpretation, adjustment of CSF leukocyte count has

been shown to lack additional value in predicting meningitis.[39] In our study, only 5 children with CSF RBC ≥500 cells/μL met our laboratory meningitis criteria. Our results provided important guidance for performing LPs in LMICs settings where there is a paucity of comprehensive data on this important question.

Conclusions

Bacterial meningitis is an uncommon but important diagnosis in children. Declining incidence is welcome but identifying children with meningitis has become more difficult. Clinicians and policymakers should be aware of the number of LPs or empirical treatments needed for each case of bacterial meningitis to be identified, and this may vary with malaria endemicity. The IMCI criteria offer a balance between the more specific *KCH-2002* signs (impaired consciousness or any one of: bulging fontanel, neck stiffness, cyanosis, seizures outside 6 months to 6 years, or focal seizures) and non-malarial fever. While the IMCI criteria will continue to be used, the number of LPs needed to identify a single case of bacterial meningitis has increased 3-fold from 24 to 76. Clinicians should continue to have a high index of suspicion while assessing children during daily reviews. Support to establish accurate CSF cell counting, Gram stain, and glucose measurement as a minimum in resource-poor settings to optimise antimicrobial treatment is essential to providing effective inpatient paediatric services.

List of abbreviations

LMICs: low- and middle-income countries; CSF: cerebrospinal fluid; LP: lumbar puncture; WHO: World Health Organization; Hib: Haemophilus influenzae type b; IMCI: Integrated Management of Childhood Illness; KCH: Kilifi County Hospital; PPV: positive predictive value; NPV: negative predictive value.

Ethics approval and consent to participate

The collection of surveillance data included in this analysis was reviewed and approved by the Kenya Medical Research Institute Scientific Steering Committee (KEMRI SSC 1433). This retrospective analysis was reviewed and approved by the KEMRI SSC (KEMRI SSC 3001).

Availability of data and materials

The dataset used and analysed during the current study is available from the KWTRP Data Governance Committee (DGC) on reasonable request (dgc@kemri-

wellcome.org), ensuring the protection of the privacy, rights and interests of research participants and primary researchers, and upholding transparency and accountability. KWTRP is the custodian of the data used in this analysis and the KWTRP DGC oversees the internal data repository.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CWO, MN, CRN, MBH, and JAB contributed to the conception and design of the study. CWO, NM, and JAB contributed to inpatient care and data collection. SM was responsible for laboratory analysis. CWO, NM, SM, MN, CRN, MBH, and JAB contributed to the analysis and interpretation of the data. CWO, MBH, and JAB contributed to the drafting of the article. The views expressed in this manuscript are those of the authors and not necessarily those of the KEMRI, or the Wellcome Trust. The authors read and approved the final manuscript.

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REFERENCES

1. Mann K, Jackson MA. Meningitis. *Pediatrics in Review* 2008;29(12):417-30. doi: 10.1542/pir.29-12-417 [published Online First: 2008/12/03]
2. Edmond K, Clark A, Korczak VS, et al. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *The Lancet Infectious Diseases* 2010;10(5):317-28. doi: 10.1016/s1473-3099(10)70048-7 [published Online First: 2010/04/27]
3. Ramakrishnan M, Ulland AJ, Steinhardt LC, et al. Sequelae due to bacterial meningitis among African children: a systematic literature review. *BMC medicine* 2009;7:47. doi: 10.1186/1741-7015-7-47 [published Online First: 2009/09/16]
4. Manning L, Laman M, Mare T, et al. Accuracy of cerebrospinal leucocyte count, protein and culture for the diagnosis of acute bacterial meningitis: a comparative study using Bayesian latent class analysis. *Tropical medicine & international health : TM & IH* 2014;19(12):1520-4. doi: 10.1111/tmi.12400 [published Online First: 2014/10/16]
5. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 2001;108(5):1169-74. [published Online First: 2001/11/06]
6. Ayieko P, Ogero M, Makone B, et al. Characteristics of admissions and variations in the use of basic investigations, treatments and outcomes in Kenyan hospitals within a new Clinical Information Network. *Archives of disease in childhood* 2016;101(3):223-9. doi: 10.1136/archdischild-2015-309269 [published Online First: 2015/12/15]
7. Herbert G, Ndiritu M, Idro R, et al. Analysis of the indications for routine lumbar puncture and results of cerebrospinal fluid examination in children admitted to the paediatric wards of two hospitals in East Africa. *Tanzania Journal of Health Research* 2006;8(1):7-10. doi: 10.4314/thrb.v8i1.14263 [published Online First: 2006/10/25]
8. WHO. Pocket book of hospital care for children. Guidelines for the management of common childhood illnesses. Second edition ed: WHO Press, 2013.
9. WHO IMCI. Integrated Management of Childhood Illness (IMCI) Chart Booklet. Distance Learn Course. 2014;(March):1–76.
10. Weber MW, Herman J, Jaffar S, et al. Clinical predictors of bacterial meningitis in infants and young children in The Gambia. *Trop Med Int Health* 2002;7(9):722-31. doi: 10.1046/j.1365-3156.2002.00926.x [published Online First: 2002/09/13]
11. Berkley JA, Versteeg AC, Mwangi I, et al. Indicators of Acute Bacterial Meningitis in Children at a Rural Kenyan District Hospital. *Pediatrics* 2004;114(6):e713-e19. doi: 10.1542/peds.2004-0007 [published Online First: 2004/12/03]
12. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
13. Mwenda JM, Soda E, Weldegebriel G, et al. Pediatric Bacterial Meningitis Surveillance in the World Health Organization African Region Using the Invasive Bacterial Vaccine-Preventable Disease Surveillance Network, 2011–2016. *Clinical Infectious Diseases* 2019;69(Supplement_2):S49-S57. doi: 10.1093/cid/ciz472 [published Online First: 2019/09/11]
14. Cowgill KD, Ndiritu M, Nyiro J, et al. Effectiveness of Haemophilus influenzae Type b Conjugate Vaccine Introduction Into Routine Childhood Immunization in Kenya. *Jama* 2006;296(6):671-8. doi: 10.1001/jama.296.6.671 [published Online First: 2006/08/10]
15. Gessner BD, Adegbola RA. The impact of vaccines on pneumonia: Key lessons from Haemophilus influenzae type b conjugate vaccines. *Vaccine* 2008;26:B3-B8. doi: 10.1016/j.vaccine.2008.04.013 [published Online First: 2008/09/20]

16. 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. *The Lancet Global health* 2018;6(7):e744-e57. doi: 10.1016/S2214-109X(18)30247-X [published Online First: 2018/06/16]
17. Zunt JR, Kassebaum NJ, Blake N, et al. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology* 2018;17(12):1061-82. doi: 10.1016/s1474-4422(18)30387-9
18. Berkley JA, Mwangi I, Mellington F, et al. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM : monthly journal of the Association of Physicians* 1999;92(3):151-7. [published Online First: 1999/05/18]
19. Mogeni P, Williams TN, Fegan G, et al. Age, Spatial, and Temporal Variations in Hospital Admissions with Malaria in Kilifi County, Kenya: A 25-Year Longitudinal Observational Study. *PLoS Med* 2016;13(6):e1002047. doi: 10.1371/journal.pmed.1002047 [published Online First: 2016/06/29]
20. Nkumama IN, O'Meara WP, Osier FHA. Changes in Malaria Epidemiology in Africa and New Challenges for Elimination. *Trends Parasitol* 2017;33(2):128-40. doi: 10.1016/j.pt.2016.11.006 [published Online First: 2016/12/13]
21. Njuguna P, Maitland K, Nyaguara A, et al. Observational study: 27 years of severe malaria surveillance in Kilifi, Kenya. *BMC medicine* 2019;17(1):124. doi: 10.1186/s12916-019-1359-9 [published Online First: 2019/07/10]
22. Schulga P, Grattan R, Napier C, et al. How to use... lumbar puncture in children. *Arch Dis Child Educ Pract Ed* 2015;100(5):264-71. doi: 10.1136/archdischild-2014-307600 [published Online First: 2015/06/25]
23. Chun S, Kang C-I, Kim Y-J, et al. Clinical Significance of Isolates Known to Be Blood Culture Contaminants in Pediatric Patients. *Medicina* 2019;55(10) doi: 10.3390/medicina55100696 [published Online First: 2019/10/20]
24. Aipit J, Laman M, Hwaiwhanje I, et al. Accuracy of initial clinical diagnosis of acute bacterial meningitis in children from a malaria-endemic area of Papua New Guinea. *Trans R Soc Trop Med Hyg* 2014;108(7):444-8. doi: 10.1093/trstmh/tru067 [published Online First: 2014/05/06]
25. Mwangi I, Berkley J, Lowe B, et al. Acute bacterial meningitis in children admitted to a rural Kenyan hospital: increasing antibiotic resistance and outcome. *The Pediatric infectious disease journal* 2002;21(11):1042-8. doi: 10.1097/00006454-200211000-00013 [published Online First: 2002/11/21]
26. Obiero CW, Mturi N, Mwarumba S, et al. Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital. *Archives of disease in childhood* 2020 doi: 10.1136/archdischild-2020-318913 [published Online First: 2020/08/21]
27. Abdul-Mumin A, Cotache-Condor C, Owusu SA, et al. Timing and causes of neonatal mortality in Tamale Teaching Hospital, Ghana: A retrospective study. *PLoS One* 2021;16(1):e0245065. doi: 10.1371/journal.pone.0245065 [published Online First: 2021/01/14]
28. Curtis S, Stobart K, Vandermeer B, et al. Clinical Features Suggestive of Meningitis in Children: A Systematic Review of Prospective Data. *Pediatrics* 2010;126(5):952-60. doi: 10.1542/peds.2010-0277 [published Online First: 2010/10/27]
29. Laman M, Manning L, Greenhill AR, et al. Predictors of acute bacterial meningitis in children from a malaria-endemic area of Papua New Guinea. *Am J Trop Med Hyg* 2012;86(2):240-5. doi: 10.4269/ajtmh.2012.11-0312 [published Online First: 2012/02/04]

30. Nhantumbo AA, Gudo ES, Caierao J, et al. Serotype distribution and antimicrobial resistance of Streptococcus pneumoniae in children with acute bacterial meningitis in Mozambique: implications for a national immunization strategy. *BMC Microbiol* 2016;16(1):134. doi: 10.1186/s12866-016-0747-y [published Online First: 2016/07/01]
31. Sanneh B, Okoi C, Grey-Johnson M, et al. Declining Trends of Pneumococcal Meningitis in Gambian Children After the Introduction of Pneumococcal Conjugate Vaccines. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2019;69(Suppl 2):S126-S32. doi: 10.1093/cid/ciz505 [published Online First: 2019/09/11]
32. Manning L, Laman M, Greenhill AR, et al. Increasing chloramphenicol resistance in Streptococcus pneumoniae isolates from Papua New Guinean children with acute bacterial meningitis. *Antimicrob Agents Chemother* 2011;55(9):4454-6. doi: 10.1128/AAC.00526-11 [published Online First: 2011/06/29]
33. Duke T, Michael A, Mokela D, et al. Chloramphenicol or ceftriaxone, or both, as treatment for meningitis in developing countries? *Archives of disease in childhood* 2003;88(6):536-9. doi: 10.1136/adc.88.6.536 [published Online First: 2003/05/27]
34. Negrini B, Kelleher KJ, Wald ER. Cerebrospinal fluid findings in aseptic versus bacterial meningitis. *Pediatrics* 2000;105(2):316-9. doi: 10.1542/peds.105.2.316 [published Online First: 2000/02/02]
35. Nigrovic LE, Malley R, Macias CG, et al. Effect of Antibiotic Pretreatment on Cerebrospinal Fluid Profiles of Children With Bacterial Meningitis. *Pediatrics* 2008;122(4):726-30. doi: 10.1542/peds.2007-3275 [published Online First: 2008/10/03]
36. Tadesse BT, Foster BA, Shibeshi MS, et al. Empiric Treatment of Acute Meningitis Syndrome in a Resource-Limited Setting: Clinical Outcomes and Predictors of Survival or Death. *Ethiop J Health Sci* 2017;27(6):581-88. doi: 10.4314/ejhs.v27i6.3 [published Online First: 2018/03/01]
37. Kaplan SL, O'Brian Smith E, Wills C, et al. Association between preadmission oral antibiotic therapy and cerebrospinal fluid findings and sequelae caused by Haemophilus influenzae type b meningitis. *The Pediatric infectious disease journal* 1986;5(6):626-32. doi: 10.1097/00006454-198611000-00005 [published Online First: 1986/11/01]
38. Khumalo J, Nicol M, Hardie D, et al. Diagnostic accuracy of two multiplex real-time polymerase chain reaction assays for the diagnosis of meningitis in children in a resource-limited setting. *PLoS One* 2017;12(3):e0173948. doi: 10.1371/journal.pone.0173948 [published Online First: 2017/03/28]
39. Bonsu BK, Harper MB. Corrections for Leukocytes and Percent of Neutrophils Do Not Match Observations in Blood-Contaminated Cerebrospinal Fluid and Have No Value Over Uncorrected Cells for Diagnosis. *The Pediatric infectious disease journal* 2006;25(1)

Supplementary Tables

Supplementary Table 1. Comparison of annual admissions, lumbar punctures and meningitis cases during our study period and our previous analysis

Study period	2012-2016	2001-2002
Admissions/year	2,597	4,616
Lumbar punctures/year	520	999
Meningitis cases/year	20	91

Data are N

Supplementary Table 2. Sensitivity analysis of potential screening criteria at admission for meningitis

Screening Criteria	Meningitis (n=98) vs no meningitis (n= 12,272)[a]	Meningitis (plus possible meningitis) (n=149) vs no meningitis (n=12,837)[b]	Meningitis (plus possible meningitis) (n=149) vs no meningitis (n=12,272)[c]	Microbiologically confirmed meningitis[d] (n=50) vs no meningitis (n=12,371)[c]
Bulging fontanel[e] or neck stiffness				
No. with criteria	147	166	152	152
No. with meningitis	23	28	28	14
Sensitivity (95% CI)	23.5 (15.5-33.1)	18.8 (12.9-26.0)	18.8 (12.9-26.0)	28.0 (16.2-42.5)
Specificity (95% CI)	99.0 (98.8-99.2)	98.9 (98.7-99.1)	99.0 (98.8-99.2)	98.9 (98.7-99.1)
PPV (95% CI)	15.6 (10.2-22.5)	16.9 (11.5-23.4)	18.4 (12.6-25.5)	9.2 (5.1-15.0)
NPV (95% CI)	99.4 (99.2-99.5)	99.1 (98.9-99.2)	99.0 (98.8-99.2)	99.7 (99.6-99.8)
NNLP (95% CI)	7 (5-11)	6 (5-10)	6 (4-9)	11 (7-23)
Cyanosis or any of the above				
No. with criteria	178	212	183	183
No. with meningitis	23	28	28	14
Sensitivity (95% CI)	23.5 (15.5-33.1)	18.8 (12.9-26.0)	18.8 (12.9-26.0)	28.0 (16.2-42.5)
Specificity (95% CI)	98.7 (98.5-98.9)	98.6 (98.3-98.8)	98.7 (98.5-98.9)	98.6 (98.4-98.8)
PPV (95% CI)	12.9 (8.4-18.8)	13.2 (9.0-18.5)	15.3 (10.4-21.3)	7.7 (4.3-12.5)
NPV (95% CI)	99.4 (99.2-99.5)	99.1 (98.9-99.2)	99.0 (98.8-99.2)	99.7 (99.6-99.8)
NNLP (95% CI)	8 (6-14)	8 (6-13)	7 (5-11)	14 (9-29)
Seizures outside 6 mo to 6 y or any of the above				
No. with criteria	715	787	731	731
No. with meningitis	33	49	49	21
Sensitivity (95% CI)	33.7 (24.4-43.9)	32.9 (25.4-41.0)	32.9 (25.4-41.0)	42.0 (28.2-56.8)
Specificity (95% CI)	94.4 (94.0-94.8)	94.3 (93.8-94.6)	94.4 (94.0-94.8)	94.3 (93.8-94.7)
PPV (95% CI)	4.6 (3.2-6.4)	6.2 (4.6-8.2)	6.7 (5.0-8.8)	2.9 (1.8-4.4)
NPV (95% CI)	99.4 (99.3-99.6)	99.2 (99.0-99.3)	99.1 (99.0-99.3)	99.8 (99.6-99.8)
NNLP (95% CI)	25 (18-40)	19 (14-27)	17 (13-25)	38 (26-71)

Supplementary Table 2. Sensitivity analysis of potential screening criteria at admission for meningitis (continued)

Screening Criteria	Meningitis (n=98) vs no meningitis (n= 12,272)[a]	Meningitis (plus possible meningitis) (n=149) vs no meningitis (n=12,837)[b]	Meningitis (plus possible meningitis) (n=149) vs no meningitis (n=12,272)[c]	Microbiologically confirmed meningitis[d] (n=50) vs no meningitis (n=12,371)[c]
Focal seizures or any of the above				
No. with criteria	954	1,043	974	974
No. with meningitis	39	59	59	23
Sensitivity (95% CI)	39.8 (30.0-50.2)	39.6 (31.7-47.9)	39.6 (31.7-47.9)	46.0 (31.8-60.7)
Specificity (95% CI)	92.5 (92.1-93.0)	92.3 (91.9-92.8)	92.5 (92.1-93.0)	92.3 (91.8-92.8)
PPV (95% CI)	4.1 (2.9-5.6)	5.7 (4.3-7.2)	6.1 (4.6-7.7)	2.4 (1.5-3.5)
NPV (95% CI)	99.5 (99.3-99.6)	99.2 (99.1-99.4)	99.2 (99.0-99.4)	99.8 (99.7-99.8)
NNLP (95% CI)	28 (21-43)	20 (16-29)	19 (15-27)	47 (32-86)
Impaired consciousness or any of the above				
No. with criteria	2,354	2,690	2,385	2,385
No. with meningitis	57	88	88	31
Sensitivity (95% CI)	58.2 (47.8-68.1)	59.1 (50.7-67.0)	59.1 (50.7-67.0)	62.0 (47.2-75.3)
Specificity (95% CI)	81.3 (80.6-82.0)	79.7 (79.0-80.4)	81.3 (80.6-82.0)	81.0 (80.3-81.7)
PPV (95% CI)	2.4 (1.8-3.1)	3.3 (2.6-4.0)	3.7 (3.0-4.5)	1.3 (0.9-1.8)
NPV (95% CI)	99.6 (99.4-99.7)	99.4 (99.2-99.5)	99.4 (99.2-99.5)	99.8 (99.7-99.9)
NNLP (95% CI)	50 (38-73)	37 (30-50)	32 (26-43)	90 (64-154)
Fever without malaria parasitaemia or any of the above				
No. with criteria	7,614	8,142	7,657	7,657
No. with meningitis	84	127	127	45
Sensitivity (95% CI)	85.7 (77.2-92.0)	85.2 (78.5-90.5)	85.2 (78.5-90.5)	90.0 (78.2-96.7)
Specificity (95% CI)	38.6 (37.8-39.5)	37.6 (36.7-38.4)	38.6 (37.8-39.5)	38.5 (37.6-39.3)
PPV (95% CI)	1.1 (0.9-1.4)	1.6 (1.3-1.9)	1.7 (1.4-2.0)	0.6 (0.4-0.8)
NPV (95% CI)	99.7 (99.5-99.8)	99.5 (99.3-99.7)	99.5 (99.3-99.7)	99.9 (99.8-100.0)
NNLP (95% CI)	124 (92-189)	90 (70-129)	84 (65-117)	207 (148-347)

Supplementary Table 2. Sensitivity analysis of potential screening criteria at admission for meningitis (continued)

Screening Criteria	Meningitis (n=98) vs no meningitis (n= 12,272)[a]	Meningitis (plus possible meningitis) (n=149) vs no meningitis (n=12,837)[b]	Meningitis (plus possible meningitis) (n=149) vs no meningitis (n=12,272)[c]	Microbiologically confirmed meningitis[d] (n=50) vs no meningitis (n=12,371)[c]
IMCI referral criteria: neck stiffness, lethargy, impaired consciousness, or seizures				
No. with criteria	4,592	4,994	4,635	4,635
No. with meningitis	78	121	121	38
Sensitivity (95% CI)	79.6 (70.3-87.1)	81.2 (74.0-87.1)	81.2 (74.0-87.1)	76.0 (61.8-86.9)
Specificity (95% CI)	63.2 (62.4-64.1)	62.0 (61.2-62.9)	63.2 (62.4-64.1)	62.8 (62.0-63.7)
PPV (95% CI)	1.7 (1.3-2.1)	2.4 (2.0-2.9)	2.6 (2.2-3.1)	0.8 (0.6-1.1)
NPV (95% CI)	99.7 (99.6-99.8)	99.6 (99.5-99.8)	99.6 (99.5-99.8)	99.8 (99.7-99.9)
NNLP (95% CI)	69 (55-95)	48 (40-61)	44 (37-56)	150 (106-255)

Abbreviations: CI, confidence interval; PPV, positive predictive values; NPV, negative predictive value; NNLP, number needed to lumbar puncture; IMCI, integrated management of childhood infection.

'Or any of the above' refers to presence of ≥1 of the signs indicated in the preceding rows. This means that children represented on each row had the sign indicated on a particular row +/- any of the preceding signs.

[a]Excludes children with possible meningitis (Group D, n=51) and children who died before an LP (Group G, n=565)

[b]Includes children who died before an LP (Group G, n=565)

[c]Excludes children who died before an LP (Group G, n=565)

[d]Positive CSF culture, antigen test, microscopy, or CSF leukocytes >10/ µL plus positive blood culture

[e]Bulging fontanel was only deemed present if the age was ≤18 months

Chapter 6

Validation of clinical predictors of mortality among hospitalised young infants with suspicion of serious bacterial infection

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Manuscript in preparation

ABSTRACT

Background Serious bacterial infection (SBI) is associated with significant mortality in young infants in low- and middle-income countries (LMICs). However, diagnostic capacity and clinical decision tools to guide empiric antibiotics are limited. We aimed to validate a new neonatal severity score (NSS) in predicting mortality among young infants hospitalised with SBI.

Methods We performed a retrospective cohort study of infants aged <60 days hospitalised at Kilifi County Hospital (KCH), February 2017 - January 2020. NSS was derived from the infants with suspected SBI meeting the NeoOBS study inclusion criteria (NSIC). We estimated the performance of NSS by area under the receiver operating characteristic curve (AUC) and investigated potential screening rules for mortality risk.

Results 621/3746 (17%) infants died, of which 63 (10%) had SBI. Among all 3746 admissions and 2804 infants fulfilling NSIC, the NSS AUC for mortality was 0.82 (95% CI 0.80-0.84) and 0.80 (95% CI 0.78-0.82) respectively. NSIC had sensitivity 89% (95%CI 84-93%), specificity 21% (95%CI 19-22%), PPV 5.2% (95%CI 4.4-6.1%), and NPV 98% (95%CI 96-99%) for confirmed SBI. 522/1708 (31%) infants with NSS ≥4 died compared with 99/2038 (4.9%) for NSS <4 (*P<0.001*); sensitivity 84% (95%CI 81-87), specificity 62% (95%CI 60-64), PPV 31% (95%CI 28-33), and NPV 95% (95%CI 94-96).

Conclusion NSIC can support clinicians to identify infants likely to have SBI in the absence of confirmatory cultures. NSS is prognostic for mortality risk in young infants, may guide prioritization of resources and antibiotic use and can inform selection of high-risk infants for clinical trials.

INTRODUCTION

Serious bacterial infection (SBI) causes significant mortality, morbidity and long-term neurodevelopmental sequelae among young infants in low- and middle-income countries (LMICs).[1-4] *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli*, *Streptococcus pneumoniae*, and *Salmonella* spp. cause the majority of culture positive bacteraemia and sepsis,[5 6] while *S. pneumoniae*, *S. aureus*, and Group B *Streptococcus* are predominant causes of meningitis.[5] Although blood and cerebrospinal fluid (CSF) cultures remain the gold standard tests for SBI, they are often not available in public health facilities in LMICs.[7] Where available, inadequate sample volumes and pre-treatment with antibiotics contribute to low sensitivity.[8] Thus, empiric antibiotic use is based on clinical guidelines and judgement, usually in the absence of confirmatory cultures or biomarker tests, and influenced by the perceived risk of mortality associated with failure to treat. However, this may also result in unnecessary treatment.

The World Health Organization (WHO) recommends ampicillin/penicillin plus gentamicin as first-line antibiotics in all infants presenting with signs of possible SBI (pSBI).[9 10] These guidelines are based on limited evidence recommending hospitalization in infants presenting with signs of pSBI[11] or severe illness[12] in first-level or referral hospitals in LMICs, and have not been recently updated despite changes in disease epidemiology and increasing antimicrobial resistance (AMR).[13] Consequently, hospitals commonly employ other antibiotic combinations, sometimes informed by local susceptibility patterns or literature.

Most studies of clinical predictors of severe illness and mortality in LMICs, including SBI, have not evaluated their utility against confirmatory cultures as 'gold standard' diagnostic references,[12 14] limiting their value in identifying infants who may benefit from prompt initiation of antibiotics. In addition, there are limited data to guide clinicians on when to stop antibiotics in infants suspected to have SBI.[14] Clinical signs may be useful in identifying and stratifying infants with presumed infection at risk of mortality. Existing mortality prediction scores developed and validated in high-income countries with different disease and patient profiles, and better resource capacity than LMICs frequently include laboratory parameters not routinely available in LMICs, and may not be applicable in these settings.[15-20] It is likely that clinical decision making will benefit from scores tailored to their specific setting.

The Neonatal Antimicrobial (NeoAMR) Global Neonatal Sepsis Observational Study (NeoOBS [ClinicalTrial.gov ID. NCT03721302]) is a multisite prospective

observational study that aimed to help generate robust evidence for managing sepsis in settings with high AMR rates. Hospitalized infants aged <60 days were enrolled in a clinical sepsis cohort (CSC) based on the NeoOBS study inclusion criteria (NSIC) comprising clinical and laboratory criteria (derived from the European Medicines Agency (EMA)[21 22] and WHO pSBI[12] criteria). NeoOBS data were used to develop and internally validate a neonatal severity score (NSS) to predict 28-day mortality among infants meeting the NSIC.

At the Kenyan NeoOBS study site, infants not fulfilling NSIC were not enrolled in NeoOBS, but comprehensive data at admission on all infants hospitalised were collected as part of routine surveillance.[2] We therefore conducted a broader analysis including all admissions at Kilifi County Hospital (KCH) aged <60 days to validate the prognostic utility of the NSS for inhospital death among i) infants who met NSIC but were not enrolled in NeoOBS, and ii) all young infants admitted. We also examined how the NSIC performed in discriminating confirmed SBI.

METHODS

Location and participants

KCH is a public hospital located on the Kenyan coast serving a mostly rural population. Paediatric care is supported by the KEMRI/Wellcome Trust Research Programme. The NeoOBS study was conducted between February 1, 2019, and January 31, 2020. For this analysis, data from all infants aged <60 days and hospitalised at KCH between February 1, 2017, and January 31, 2020, collected for a long-term disease surveillance study were included in this retrospective analysis (including infants enrolled in NeoOBS).

Procedures

All young infants admitted to KCH are routinely systematically assessed using standardised demographic and clinical proforma by trained clinicians at admission. Data are prospectively entered on a database in real-time. All admissions have a complete blood count (CBC), malaria slide and blood culture. Blood gas analysis is done in seriously ill infants requiring high dependency unit (HDU) care. Lumbar puncture (LP) is performed at admission or later in infants with signs suggestive of meningitis according to WHO[9] and Kenya paediatric guidelines.[23] A repeat blood culture is done in case of clinical deterioration as part of a septic screen. Blood and CSF cultures are processed as previously

described.[2 24] Infants with suspected SBI are treated empirically with ampicillin/penicillin plus gentamicin as first-line antibiotics.[9 23] Surveillance data collection including consent for use of data for future research (SSC1433), NeoOBS (SERU 3758) and this analysis (SERU 3853) were approved by the KEMRI Scientific and Ethical Review Unit.

Definitions

The NSIC and NSS are shown in Supplementary Table 1 and 2 respectively. C-reactive protein and immature-to-total polymorph ratio were not routine admission tests at our site and were not included in our NSIC assessment. Confirmed SBI was defined as the presence of bacteraemia and/or bacterial meningitis. Blood and CSF culture isolates were classified as presumed pathogens (known true pathogens), likely pathogenic (not common isolates but observed to cause significant infection in young infants[25-28]) or likely non-pathogenic (potentially contaminants e.g. Coagulase-negative Staphylococci are considered clinically non-significant in our setting[29 30]). Bacteraemia was defined as the growth of presumed pathogens or likely pathogenic bacteria on blood culture. Bacterial meningitis was defined as: i) isolation of presumed pathogens or likely-pathogenic bacteria from CSF; or ii) bacteraemia plus CSF pleocytosis (≥20 cells /μL for infants 0-28 days of age and ≥10 cells/μL for infants 29-59 days of age); or iii) positive CSF latex agglutination test.

Statistical analysis

We used data from February 2017 to January 2019 and compared baseline characteristics of survivors and non-survivors hospitalised during these 3 years. To assess the predictive accuracy for mortality of the NSS, we calculated the area under the receiver operating characteristic curve (AUC) for: i) all infants who met NSIC but were not enrolled NeoOBS study, and ii) all infants hospitalised during the 3 years. All 10 NSS factors (Supplementary Table 2) that predicted mortality in the NeoObs study were available in our surveillance dataset. However, 67% of infants were missing birth weight, while only 0.1% infants missed admission weight. We performed the primary analysis using admission weight and undertook a sensitivity analysis for birth weight using multiple imputation by chained equations (MICE) for all missing values. We then validated screening rules incorporating different NSS score values by calculating their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Since the NSS was derived from a cohort of infants who met NSIC and participated in the NeoOBS study, we determined the performance of the NSIC for SBI by calculating their sensitivity, specificity, PPV and NPV among all admissions and by age categories (≤48h, >48h-7d, and >7d-59d). This analysis included all admissions in the study period, and we compared baseline characteristics of infants hospitalised before NeoOBS (February 2017-January 2019) to all infants hospitalised during NeoOBS (February 2019-January 2020) to assess potential differences. In addition, we calculated the proportion of infants meeting NSIC and compared prevalence of SBI among infants who were eligible and ineligible for the NeoOBS study. We used logistic regression to determine the association of NSIC with mortality.

Categorical variables were presented as proportions and compared using the chi-squared or Fishers exact test as appropriate. Continuous variables were summarised using median and interquartile range (IQR) and compared using Wilcoxon rank-sum test. *P <0.05* was threshold for statistical significance. All analyses used Stata version 15 (Stata Corp, USA).

RESULTS

Patients

Three thousand, seven hundred and forty-six infants were hospitalised between February 1, 2017, and January 31, 2020. Median (IQR) age, weight and length were 1 (0-5) day, 2.7 (1.8-3.2) kg, and 48 (43-50) cm (Table 1). Sixty-eight percent of all infants were hospitalised within the first 48 hours of life and half of all infants were born at KCH. Six hundred and twenty-one (17%) infants died during hospitalization. Non-survivors were younger and smaller than survivors (Table 1). Gram-negative bacteria predominated blood and CSF culture isolates (Supplementary Table 3). Of 23 infants with bacterial meningitis, 12 had bacteraemia and CSF pleocytosis, 6 had similar CSF and blood culture isolates, and 4 (2 with positive blood and CSF cultures each) had positive CSF *H. influenzae type b* and *S. pneumoniae* antigen tests. Overall, 175/3746 (4.7%) infants had SBI of which 152 (87%) had bacteraemia only, 10 (5.7%) had bacterial meningitis only, and 13 (7.4%) had bacteraemia and bacterial meningitis. Ninety-nine (57%) infants with SBI were aged ≤48 hours compared to 27 (15%) aged >48 hours-7days and 49 (28%) aged >7d-59d (*p=0.004*). Sixty-three (10%) of 621 non-survivors had SBI compared to 112/3125 (3.6%) survivors, p<*0.001*.

Table 1. Baseline characteristics of all infants at admission

Infant characteristics	All (n=3746)	Alive (n=3125)	Died (n=621)	P value
Age, days	1 (0-5)	1 (0-6)	0 (0-1)	<0.001
Age categories				
≤48 h	2542 (68)	2035 (65)	507 (82)	<0.001
>48 h – 7 d	397 (11)	355 (11)	42 (6.8)	
>7 d – 59 d	807 (22)	735 (24)	72 (12)	
Weight, kg	2.7 (1.8-3.2)	2.8 (2.0-3.3)	2.1 (1.2-2.9)	<0.001
Length, cm	48 (43-50)	48 (44-45)	45 (37-49)	<0.001
MUAC, cm	9.5 (8.0-11)	9.6 (8.3-11)	8.5 (7.0-10)	<0.001
Head circumference, cm	34 (31-36)	35 (32-36)	32 (27-35)	<0.001
Male	2159 (58)	1805 (58)	354 (57)	0.322
Born at KCH	1861 (50)	1626 (52)	235 (38)	<0.001
Serious bacterial infection	175 (4.7)	112 (3.6)	63 (10)	<0.001
Bacteraemia only	152 (4.1)	94 (3.0)	58 (9.3)	<0.001
Meningitis only	10 (0.3)	8 (0.3)	2 (0.3)	0.675
Bacteraemia and meningitis	13 (0.4)	10 (0.3)	3 (0.5)	0.463
Met NSIC	2994 (80)	2378 (76)	616 (99)	<0.001
Had ≥1 NSS factors	3694 (99)	3078 (99)	616 (99)	0.174

Data are median (IQR), n (%)
Abbreviations: h, hour; d, day; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; KCH, Kilifi County Hospital.

Performance of NeoOBS study inclusion criteria (NSIC)

Baseline characteristics among 2284/3746 (61%) infants hospitalised before the NeoOBS study period appeared similar to those among 1462/3746 (39%) infants hospitalised during the NeoObs study period (Supplementary Table 4). The distribution of individual NSIC signs is shown in Supplementary Table 5. Eighty percent (2994/3746) infants fulfilled NSIC; 156/175 (89%) and 2838/3571 (79%) among those with and without confirmed SBI respectively, *p=0.002* (Table 2). Overall, NSIC had sensitivity 89% (95%CI 84-93%), specificity 21% (95%CI 19-22%), PPV 5.2% (95%CI 4.4-6.1%), and NPV 98% (95%CI 96-99%) for confirmed SBI. Performance of NSIC by age is shown on Table 2 and Supplementary Table 6.

Of 2994 infants who fulfilled NSIC, 616 (21%) died compared to 5/752 (0.7%) of infants not meeting NSIC, *p<0.001*. NSIC was associated with increased odds of mortality both in univariable (OR 39; 95%CI 16-94, *p<0.001*) and multivariable

logistic regression (adjusted OR 32; 95%CI 13-77, *p<0.001*) (Supplementary Table 7).

Table 2. Performance of the NeoOBS study inclusion criteria

NSIC	SBI[a]	No SBI	P value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Overall (n=3746)	n=175	n=3571					
No	19 (11)	733 (21)	0.002	89 (84-93)	21 (19-22)	5.2 (4.4-6.1)	98 (96-99)
Yes	156 (89)	2838 (79)					
≤48 hr (n=2542)	n=99	n=2443					
No	10 (10)	420 (17)	0.065	90 (82-95)	17 (16-19)	4.2 (3.4-5.2)	98 (96-99)
Yes	89 (90)	2023 (83)					
>48h-7d (n=397)	n=27	n=370					
No	4 (15)	141 (38)	0.021	85 (66-96)	38 (33-43)	9.1 (5.9-13)	97 (93-99)
Yes	23 (85)	229 (62)					
>7d-59d (n=807)	n=49	n=758					
No	5 (10)	172 (23)	0.048	90 (78-97)	23 (20-26)	7.0 (5.1-9.3)	97 (94-99)
Yes	44 (90)	586 (77)					

Abbreviations: SBI, serious bacterial infection; CI, confidence interval.

Validation of NeoSEP severity score (NSS)

Table 3 shows proportions of infants with factors included in the NSS, including those with missing values. In the primary analysis, using admission weight as part of NSS, the AUC for mortality in the complete case analysis was 0.79 (95% CI 0.77-0.82) among 2804 infants who fulfilled NSIC excluding the 197 enrolled in NeoOBS, and 0.82 (95% CI 0.80-0.84) among all 3746 admissions. When missing NSS values were imputed, the AUC for mortality was 0.80 (95% CI 0.78-0.82) among 2804 infants who fulfilled NSIC, excluding the 197 enrolled in NeoOBS, and 0.82 (95% CI 0.80-0.84) among all 3746 admissions. Performance at different NSS cutoffs is shown on Table 4 and Figure 1. Six hundred and sixteen (17%) of 3694 infants with NSS ≥1 died compared with 5 (9.6%) of 52

infants with NSS <1; sensitivity 99% (95%CI 98-100), specificity 1.5% (95%CI 1.1-2.0), PPV 17% (95%CI 16-18), and NPV 90% (95%CI 79-97). Five hundred and twenty-two (31%) of 1708 infants with NSS ≥4 died compared with 99 (4.9%) of 2038 infants with NSS <4; sensitivity 84% (95%CI 81-87), specificity 62% (95%CI 60-64), PPV 31% (95%CI 28-33), and NPV 95% (95%CI 94-96).

Table 3. Distribution of the NeoSEP severity score factors at admission

Factor	Score value if present	Maximum possible score value per factor[a]	Infants with factor n (%)	Missing values n (%)
Time in hospital: ≤10 days	1	1	2936 (78)	45 (1.2)
Gestational age: <37 weeks	1	1	1198 (32)	234 (6.3)
Birth weight:		2		2507 (67)
• >2 kg	0		912 (24)	
• 1-2 kg	1		292 (7.8)	
• <1 kg	2		35 (0.9)	
Congenital anomalies	2	2	196 (5.2)	105 (2.8)
Temperature:		2		4 (0.1)
• <35.5ºC	1		842 (22)	
• 35.5-37.9ºC	0		2079 (56)	
• 38-38.9ºC	1		617 (16)	
• ≥39ºC	2		204 (5.4)	
Maximum respiratory support:		3		4 (0.1)
• None	0		2,633 (70)	
• Oxygen supplementation	2		1,109 (30)	
• CPAP, BiPAP, HFNC	3		0 (0)	
• Invasive ventilation	3		0 (0)	
Abdominal distension	1	1	81 (2.2)	40 (1.1)
Difficulty in feeding	1	1	253 (6.8)	40 (1.1)
Evidence of shock[b]	1	1	428 (11)	40 (1.1)
Lethargy/no or reduced movement:		2		40 (1.1)
• Lethargy only	1		49 (1.3)	
• No movement or movement only on stimulation +/- lethargy	2		1442 (39)	

Abbreviations: Kg, kilogram; ºC, degree Celsius; CPAP, continuous positive airway pressure; BiPAP, bilevel positive airway pressure; HFNC, high flow nasal canula.

[a]Possible maximum score, 16

[b]Including capillary refill time >3seconds, mottled skin, cold peripheries or other signs of shock

Sensitivity analysis using birth weight

In the complete case analysis, AUC for mortality was 0.78 (95% CI 0.74-0.82) among 2804 infants who fulfilled NSIC excluding 197 enrolled in NeoOBS, and 0.82 (95% CI 0.78-0.85) among all 3746 admissions. Using MICE, the AUC for mortality was 0.81 (95% CI 0.78-0.83) among 2804 infants who fulfilled NSIC, excluding the 197 enrolled in NeoOBS, and 0.83 (95% CI 0.81-0.85) among all 3746 admissions. Performance of different NSS cutoffs is shown on Supplementary Table 8 and Supplementary Figure 1.

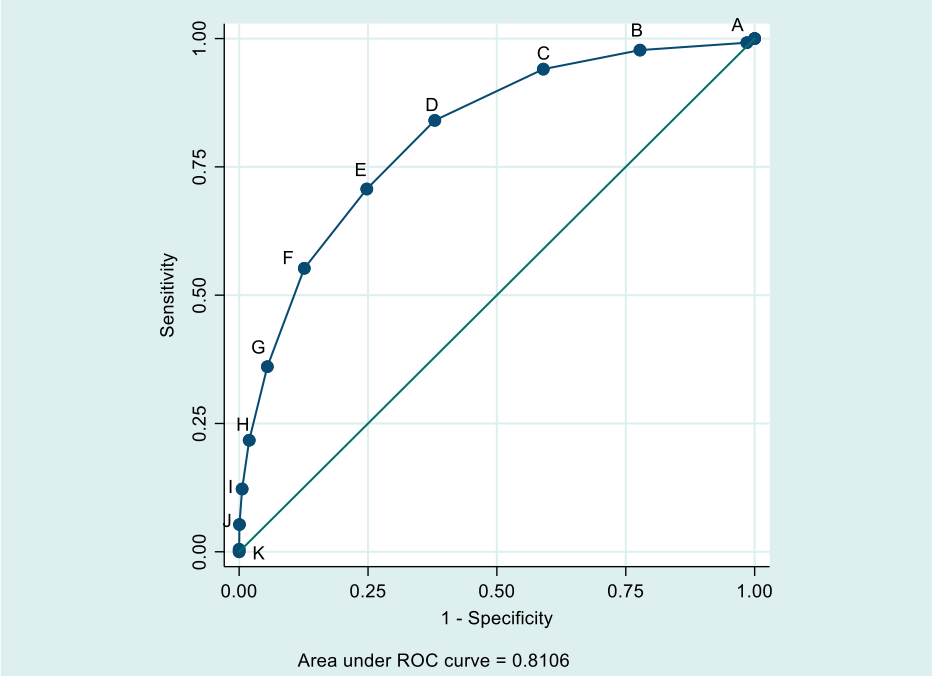


Figure 1. Performance of the NeoSEP severity score (using admission weight)

Area under receiver operating characteristic curve for NSS for mortality. Scores: (A) ≥1, (B) ≥2, (C) ≥3, (D) ≥4, (E) ≥5, (F) ≥6, (G) ≥7, (H) ≥8, (I) ≥9, (J) ≥10, (K) ≥11.

Table 4. Validation of the NeoSep severity score among all admissions (using admission weight)

Score	Meeting criteria N=3746 n (%)	Deaths N=621 n (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95%CI)
≥1	3694 (99)	616 (99)	99 (98-100)	1.5 (1.1-2.0)	17 (16-18)	90 (79-97)	1.0 (1.0-1.0)	0.5 (0.2-1.3)
≥2	3037 (81)	607 (98)	98 (96-99)	22 (21-24)	20 (19-22)	98 (97-99)	1.3 (1.2-1.3)	0.1 (0.1-0.2)
≥3	2428 (65)	584 (94)	94 (92-96)	41 (39-43)	24 (22-26)	97 (96-98)	1.6 (1.5-1.7)	0.1 (0.1-0.2)
≥4	1708 (46)	522 (84)	84 (81-87)	62 (60-64)	31 (28-33)	95 (94-96)	2.2 (2.1-2.3)	0.3 (0.2-0.3)
≥5	1213 (32)	439 (71)	71 (67-74)	75 (74-77)	36 (34-39)	93 (92-94)	2.9 (2.6-3.1)	0.4 (0.3-0.4)
≥6	738 (20)	343 (55)	55 (51-59)	87 (86-89)	47 (43-50)	91 (90-92)	4.4 (3.9-4.9)	0.5 (0.5-0.6)
≥7	395 (11)	224 (36)	36 (32-40)	95 (94-95)	57 (52-62)	88 (87-89)	6.6 (5.5-7.9)	0.7 (0.6-0.7)
≥8	196 (5.2)	135 (22)	22 (19-25)	98 (98-99)	69 (62-75)	86 (85-87)	11 (8.3-15)	0.8 (0.8-0.8)
≥9	94 (2.5)	76 (12)	12 (9.8-15)	99 (99-100)	81 (71-88)	85 (84-86)	21 (13-35)	0.9 (0.9-0.9)
≥10	35 (0.9)	33 (5.3)	5.3 (3.7-7.4)	100 (100-100)	94 (81-99)	84 (83-85)	83 (20-345)	0.9 (0.9-1.0)
≥11	3 (0.1)	3 (0.5)	0.5 (0.1-1.4)	100 (100-100)	100 (29-100)	84 (82-85)	-	1.0 (1.0-1.0)

Data are n (%)

Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

DISCUSSION

The NSS was derived from a large, prospective, multi-country cohort study to inform enrolment of infants in clinical trials. It also has potential to help in identification of infants needing urgent care. We validated this tool in predicting mortality in an external population of 3746 young infants of whom 17% died during hospitalisation. The NSS demonstrated fair discrimination for mortality among hospitalised infants, including those who were likely to have SBI based on the presence of NSIC. Presence of ≥1 of the NSS signs had 99% sensitivity for mortality, thereby demonstrating the tool's utility in identifying infants at risk of death. A potential screening rule for high-risk infants requiring prioritization in clinical care would require the presence of ≥4 NSS factors at admission, given that this cutoff had the best performance (sensitivity 84%, specificity 62%) in discriminating mortality at admission.

Young infants now comprise majority of admissions and deaths among children in LMICs[31] and reduction of mortality in this age group, particularly during the perinatal period, has lagged behind that in older children.[32] Delayed treatment, overtreatment, or failure to treat with appropriate antibiotics is associated with clinical deterioration, organ dysfunction, prolonged hospitalization,[33] antimicrobial resistance,[13] increased healthcare costs,[34] and mortality.[33] Several studies have identified individual clinical signs that predict severe disease and mortality in young infants in LMICs,[14 35] including a recently published analysis of data from the African Neonatal Sepsis Trial conducted in 5 sites in the Democratic Republic of Congo, Kenya and Nigeria that included 7129 infants aged <60 days presenting at first-level health facilities with possible serious infection.[36] Infants with signs of critical illness (convulsions, inability to feed or no movement at all) had the highest case fatality ratio (17%) compared to infants with fast breathing (0.2% and 2.0% in infants age 7-59 days and 0-6 days respectively) or clinical severe infection (2.3%; severe chest indrawing, axillary temperature ≥38^{o}C or <35.5^{o}C, poor feeding or movement only when stimulated). In addition, the risk of mortality was higher in infants presenting with signs of critical illness but managed as outpatients compared to infants who were hospitalised, underscoring the importance of appropriate medical care of seriously ill infants. Despite knowledge of signs associated with mortality in young infants, identification of infants needing urgent individualised care in LMICs remains challenging, and there is need of context-specific criteria or tools that can reliably guide clinical decision making in these settings.

The NSS was derived from a model based on characteristics and clinical parameters of infants enrolled in NeoObs and clinically suspected to have SBI at study enrolment, and internally validated in 15% of infants enrolled. We demonstrated fair discrimination ability of the score for mortality among infants meeting NSIC, similar to NeoOBS study results (AUC 0.76 [0.69-0.8]) in its validation set). Similar results were obtained among all admissions, including those not meeting NSIC, hence supporting applicability of this tool in the general population of hospitalised infants who often present with nonspecific signs. Importantly, the score had similar performance when admission weight or birth weight was used. Missing birth weight is a common finding in hospitalised infants in our setting as this is influenced by factors such as place of birth or poor documentation of health records within health facilities. The high proportion of missing birth weights may result in bias, and we therefore used admission weight for our primary analysis. Seventy-eight percent of infants in our study were hospitalised within the first week of life hence it is likely that admission weight was a valid proxy measure for birth weight.[37] Our cut-off of ≥4 NSS factors was different from that of ≥5 NSS factors proposed by the NeoObs study (submitted for publication). This difference may be due to different population characteristics and follow-up (28-days in NeoObs versus duration of hospitalisation in our study) since the population in Kilifi is rural and smaller than that of most of the hospitals that participated in NeoObs. In addition, respiratory support at KCH is limited to oxygen supplementation, with no capacity for positive airway pressure therapy or invasive ventilation.

The NSS is the first mortality prediction rule in young infants developed and validated in the LMICs. Paediatric prognostic scores developed in high-income countries such as the paediatric Sequential Organ Failure Assessment (pSOFA) score have limited applicability in LMICs as they mostly included older children age >60 days in emergency departments or intensive care settings and incorporated variables not routinely available in LMICs e.g. regular measurement of mean arterial pressure.[38] The Neonatal Sequential Organ Failure Assessment (nSOFA) score was developed[15] and validated[16] in preterm VLBW neonates in the USA. In a multicentre validation study of 20152 neonates admitted to neonatal intensive care units (NICU) between 2009-2020, nSOFA score had good to excellent discrimination with an AUC 0.88-0.89 in the first 24 hours of admission and 0.93-0.95 by day 28 of all-cause mortality across all birth weights.[39] In addition, similar to our study, nSOFA was also found to be useful in mortality risk stratification; neonates with a score >4 had higher mortality

than neonates with a score ≤4 at evaluation (13% vs 67%, *p<0.001*), +6 hours (15% vs 64%, *p=0.002*), and +12 hours (7% vs 71%, *p<0.001*).[15] However, applicability of nSOFA in our setting is limited as its components (e.g. use of mechanical ventilation and inotropes) are unavailable in most public hospitals, and its performance among term neonates and infants aged 29-59 days needs further evaluation. The NSS relies on simple parameters that can be easily assessed by clinicians in LMICs without detailed maternal history (e.g., of potential risk factors), detailed clinical examination, or any laboratory tests.

Since the NSS was derived from a cohort of infants with higher likelihood of SBI based on the presence of NSIC, we investigated performance in discriminating confirmed SBI. The NSIC had fair sensitivity with a high NPV for SBI, particularly in infants ≤48 hours old who comprised the majority of admissions and deaths in our study. However, the criteria had low specificity and PPV, and 11% of infants with SBI were misclassified as not having SBI. Current WHO[9] and Kenya paediatric guidelines[23] recommend empiric antibiotics in all infants presenting with signs suggestive of pSBI. Based on the performance of the NSIC, the criteria can be used to identify infants who have SBI and guide continuation of antibiotic treatment in the absence of confirmatory cultures. However, it is important to note that the signs were not 100% sensitive for SBI, hence clinical assessment of infants' progress while undergoing treatment for suspected SBI is vital to minimise residual diagnostic uncertainty. Existing prediction rules for SBI were developed and validated in high-income countries to identify febrile infants at low risk of SBI thereby not requiring hospitalization or intravenous antibiotics.[19] [20] For example, the Paediatric Emergency Care Applied Research Network (PECARN) rule (well-appearing, temperature ≥38^{o}C, urinalysis negative, absolute neutrophil count ≤4.1 x10^{3}cells/µL, and procalcitonin ≤1.71 ng/mL) was derived and validated in a cohort of 1,821 infants age <60 days reviewed at 26 emergency departments, and had 98% sensitivity, 60% specificity, 21% PPV, and 100% NPV.[19] The PECARN rule excluded infants with antibiotic pre-treatment or critically ill infants who form majority of admissions in our setting. We found 3% of infants lacking laboratory signs and meeting NSIC had SBI hence the criteria can be utilised to identify infants with IBI where laboratory tests are unavailable to clinicians, and support management of infants being treated for suspected SBI.

Limitations

Our retrospective study had several limitations. The NSS was derived from a population of infants who met NSIC and were followed up for 28-day mortality across a wide range of hospitals. Our analysis focused on inpatient fatalities with variable lengths of hospitalization. Missed laboratory results due to difficult venepuncture, clotted samples, or selective blood gas analysis measurement may have biased our assessment of the NSIC. Presence of clinical surrogates for missing laboratory signs e.g. signs of respiratory distress for acidosis,[40] may have mitigated this bias. We were unable to assess the extent to which subjective assessment of clinical signs affected our results. Most deaths (94%) occurred prior to performing an LP hence this may have resulted in an underestimation of the prevalence of SBI. In addition, we lacked data on antibiotic pre-treatment which may influence culture positivity.

Conclusion

In this validation study, the NSS appeared to be a useful prognostic tool for inpatient mortality in young infants that can guide prioritization and allocation of treatment and resources, particularly among at-risk infants presenting with a score of ≥4. Compared to the NSIC, the NSS did not miss the infants it was designed to detect (those who died during hospitalisation) thereby showing promise in optimising clinical care and outcomes. While the NSS has potential for use in tailoring antibiotic treatment based on risk of mortality at admission, the NSIC can be used to support clinicians in identifying infants likely to have SBI in the absence of cultures. However, 11% of confirmed SBI cases were not detected by NSIC therefore clinicians need to maintain a high index of suspicion during initial treatment. Strengthening of laboratory capacity and training of clinicians in recognition of signs is important for improved infant care and survival. Our results support the use of the NSS in clinical practice and research alongside additional further external validation of the NSS in LMICs in different settings and sample frames with varying SBI prevalence.

REFERENCES

1. Mulholland EK, Adegbola RA. Bacterial infections--a major cause of death among children in Africa. *The New England journal of medicine* 2005;352(1):75-7. doi: 10.1056/NEJMe048306 [published Online First: 2005/01/07]
2. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *The New England journal of medicine* 2005;352(1):39-47. doi: 10.1056/NEJMoa040275 [published Online First: 2005/01/07]
3. Oordt-Speets AM, Bolijn R, van Hoorn RC, et al. Global etiology of bacterial meningitis: A systematic review and meta-analysis. *PLoS One* 2018;13(6):e0198772. doi: 10.1371/journal.pone.0198772 [published Online First: 2018/06/12]
4. Ramakrishnan M, Ulland AJ, Steinhardt LC, et al. Sequelae due to bacterial meningitis among African children: a systematic literature review. *BMC medicine* 2009;7:47. doi: 10.1186/1741-7015-7-47 [published Online First: 2009/09/16]
5. 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. *The Lancet Infectious diseases* 2019;19(11):1219-34. doi: 10.1016/S1473-3099(19)30414-1 [published Online First: 2019/09/17]
6. Downie L, Armiento R, Subhi R, et al. Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics--systematic review and meta-analysis. *Archives of disease in childhood* 2013;98(2):146-54. doi: 10.1136/archdischild-2012-302033 [published Online First: 2012/11/13]
7. Hamer DH, Darmstadt GL, Carlin JB, et al. Etiology of bacteremia in young infants in six countries. *The Pediatric infectious disease journal* 2015;34(1):e1-8. doi: 10.1097/INF.0000000000000549 [published Online First: 2014/11/13]
8. Connell TG, Rele M, Cowley D, et al. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics* 2007;119(5):891-6. doi: 10.1542/peds.2006-0440 [published Online First: 2007/05/03]
9. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed: World Health Organization, 2013:412.
10. World Health Organization. Integrated Management of Childhood Illness: management of the sick young infant aged up to 2 months. IMCI chart booklet. Geneva, Switzerland: WHO, 2019. Available at: https://apps.who.int/iris/rest/bitstreams/1242053/retrieve
11. Weber MW, Carlin JB, Gatchalian S, et al. Predictors of neonatal sepsis in developing countries. *The Pediatric infectious disease journal* 2003;22(8):711-7. doi: 10.1097/01.inf.0000078163.80807.88 [published Online First: 2003/08/13]
12. Young Infants Clinical Signs Study G. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *Lancet* 2008;371(9607):135-42. doi: 10.1016/S0140-6736(08)60106-3 [published Online First: 2008/01/15]
13. Li G, Bielicki JA, Ahmed A, et al. Towards understanding global patterns of antimicrobial use and resistance in neonatal sepsis: insights from the NeoAMR network. *Archives of disease in childhood* 2020;105(1):26-31. doi: 10.1136/archdischild-2019-316816 [published Online First: 2019/08/26]
14. Opiyo N, English M. What clinical signs best identify severe illness in young infants aged 0-59 days in developing countries? A systematic review. *Archives of disease in childhood* 2011;96(11):1052-9. doi: 10.1136/adc.2010.186049 [published Online First: 2011/01/12]
15. Wynn JL, Polin RA. A neonatal sequential organ failure assessment score predicts mortality to late-onset sepsis in preterm very low birth weight infants. *Pediatric*

research 2020;88(1):85-90. doi: 10.1038/s41390-019-0517-2 [published Online First: 2019/08/09]
16. Fleiss N, Coggins SA, Lewis AN, et al. Evaluation of the Neonatal Sequential Organ Failure Assessment and Mortality Risk in Preterm Infants With Late-Onset Infection. *JAMA Netw Open* 2021;4(2):e2036518. doi: 10.1001/jamanetworkopen.2020.36518 [published Online First: 2021/02/05]
17. Zeigler AC, Ainsworth JE, Fairchild KD, et al. Sepsis and Mortality Prediction in Very Low Birth Weight Infants: Analysis of HeRO and nSOFA. *Am J Perinatol* 2021 doi: 10.1055/s-0041-1728829 [published Online First: 2021/05/11]
18. Gulla KM, Sachdev A. Illness severity and organ dysfunction scoring in Pediatric Intensive Care Unit. *Indian J Crit Care Med* 2016;20(1):27-35. doi: 10.4103/0972-5229.173685 [published Online First: 2016/03/10]
19. Kuppermann N, Dayan PS, Levine DA, et al. A Clinical Prediction Rule to Identify Febrile Infants 60 Days and Younger at Low Risk for Serious Bacterial Infections. *JAMA pediatrics* 2019;173(4):342-51. doi: 10.1001/jamapediatrics.2018.5501 [published Online First: 2019/02/19]
20. Nigrovic LE, Mahajan PV, Blumberg SM, et al. The Yale Observation Scale Score and the Risk of Serious Bacterial Infections in Febrile Infants. *Pediatrics* 2017;140(1):e20170695. doi: 10.1542/peds.2017-0695 [published Online First: 2017/08/02]
21. Tuzun F, Ozkan H, Cetinkaya M, et al. Is European Medicines Agency (EMA) sepsis criteria accurate for neonatal sepsis diagnosis or do we need new criteria? *PLoS One* 2019;14(6):e0218002. doi: 10.1371/journal.pone.0218002 [published Online First: 2019/06/07]
22. European Medicines Agency. Report on the Expert Meeting on Neonatal and Paediatric Sepsis. 8 June 2010, EMA London. Accessed on 8 October 2021. Available online: https://www.ema.europa.eu/en/documents/report/report-expert-meeting-neonatal-paediatric-sepsis_en.pdf.
23. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
24. Berkley JA, Mwangi I, Ngetsa CJ, et al. Diagnosis of acute bacterial meningitis in children at a district hospital in sub-Saharan Africa. *Lancet* 2001;357(9270):1753-7. doi: 10.1016/S0140-6736(00)04897-2 [published Online First: 2001/06/14]
25. Manickam N, Knorr A, Muldrew KL. Neonatal meningoencephalitis caused by Bacillus cereus. *The Pediatric infectious disease journal* 2008;27(9):843-6. doi: 10.1097/INF.0b013e31816feec4 [published Online First: 2008/08/06]
26. Ramarao N, Belotti L, Deboscker S, et al. Two unrelated episodes of Bacillus cereus bacteremia in a neonatal intensive care unit. *American journal of infection control* 2014;42(6):694-5. doi: 10.1016/j.ajic.2014.01.025 [published Online First: 2014/04/15]
27. Mirza IA, Khalid A, Hameed F, et al. Chryseobacterium Indologenes as a Novel Cause of Bacteremia in a Neonate. *J Coll Physicians Surg Pak* 2019;29(4):375-78. doi: 10.29271/jcpsp.2019.04.375 [published Online First: 2019/03/31]
28. Mehta R, Pathak A. Emerging Chryseobacterium indologenes Infection in Indian Neonatal Intensive Care Units: A Case Report. *Antibiotics (Basel)* 2018;7(4) doi: 10.3390/antibiotics7040109 [published Online First: 2018/12/19]
29. Seale AC, Obiero CW, Jones KD, et al. Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya? *The Pediatric infectious disease journal* 2017;36(11):1073-78. doi: 10.1097/INF.0000000000001699 [published Online First: 2017/07/22]
30. Wilson ML, Mitchell M, Morris A, et al. Principles and procedures for blood cultures; approved guideline. *CLSI document M47-A Clinical and Laboratory Standards Institute, Wayne, PA* 2007

31. Irimu G, Aluvaala J, Malla L, et al. Neonatal mortality in Kenyan hospitals: a multisite, retrospective, cohort study. *BMJ Glob Health* 2021;6(5) doi: 10.1136/bmjgh-2020-004475 [published Online First: 2021/06/02]
32. United Nations Inter-agency Group for Child Mortality Estimation (UNIGME) LTiCMR, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation', United Nations Children's Fund, New York, 2020.
33. Alsadoon A, Alhamwah M, Alomar B, et al. Association of Antibiotics Administration Timing With Mortality in Children With Sepsis in a Tertiary Care Hospital of a Developing Country. *Frontiers in pediatrics* 2020;8:566. doi: 10.3389/fped.2020.00566 [published Online First: 2020/10/06]
34. Naylor NR, Atun R, Zhu N, et al. Estimating the burden of antimicrobial resistance: a systematic literature review. *Antimicrobial resistance and infection control* 2018;7:58. doi: 10.1186/s13756-018-0336-y [published Online First: 2018/05/02]
35. Liang LD, Kotadia N, English L, et al. Predictors of Mortality in Neonates and Infants Hospitalized With Sepsis or Serious Infections in Developing Countries: A Systematic Review. *Frontiers in pediatrics* 2018;6:277. doi: 10.3389/fped.2018.00277 [published Online First: 2018/10/26]
36. Nisar YB, Tshefu A, Longombe AL, et al. Clinical signs of possible serious infection and associated mortality among young infants presenting at first-level health facilities. *PLoS One* 2021;16(6):e0253110. doi: 10.1371/journal.pone.0253110 [published Online First: 2021/07/01]
37. DiTomasso D, Cloud M. Systematic Review of Expected Weight Changes After Birth for Full-Term, Breastfed Newborns. *J Obstet Gynecol Neonatal Nurs* 2019;48(6):593-603. doi: 10.1016/j.jogn.2019.09.004 [published Online First: 2019/10/15]
38. Matics TJ, Sanchez-Pinto LN. Adaptation and Validation of a Pediatric Sequential Organ Failure Assessment Score and Evaluation of the Sepsis-3 Definitions in Critically Ill Children. *JAMA pediatrics* 2017;171(10):e172352. doi: 10.1001/jamapediatrics.2017.2352 [published Online First: 2017/08/08]
39. Wynn JL, Mayampurath A, Carey K, et al. Multicenter Validation of the Neonatal Sequential Organ Failure Assessment Score for Prognosis in the Neonatal Intensive Care Unit. *The Journal of pediatrics* 2021;236:297-300 e1. doi: 10.1016/j.jpeds.2021.05.037 [published Online First: 2021/05/23]
40. Mzumara G, Leopold S, Marsh K, et al. Identifying prognostic factors of severe metabolic acidosis and uraemia in African children with severe falciparum malaria: a secondary analysis of a randomized trial. *Malaria journal* 2021;20(1):282. doi: 10.1186/s12936-021-03785-0 [published Online First: 2021/06/27]

Supplementary Tables

Supplementary Table 1. NeoOBS study inclusion criteria

Clinical criteria	Laboratory criteria
• Grunting • Apnoea • Abnormal heart rate (>180/min or <100/min) • Severe chest in-drawing or increased oxygen requirement or need for respiratory support • Abnormal temperature (>37.5°C or <36.5°C) • Capillary refill time (CRT) >3 sec or mottled skin or other evidence of shock • Irritability • Convulsions • Abdominal distension • Abnormal posturing • Hypotonia/floppiness • Lethargy or drowsiness • Cyanosis • Bulging fontanelle • No movement or movement only when stimulated • Difficulty feeding or feeding intolerance • Multiple or severe skin pustules • Petechial rash • Pus from umbilical stump	• White blood cells (WBC) < 4.0 or > 20.0 x 10^9 cells/L • Absolute neutrophil count (ANC) <1.5 x 10^9 cells/L • Immature-to-total (IT) polymorph ratio of > 0.2[a] • C-reactive protein (CRP) >10 mg/L or >1 mg/dL[a] • Acidosis: Base excess (BE) < –10 mmol/L or blood lactate > 2 mmol/L To be identified with significant sepsis, infants must meet at least 2 criteria, 1 of which must be clinical [a] These tests were not done at Kilifi County Hospital hence were not part of the laboratory criteria at our site.

Supplementary Table 2. NeoSEP severity score

Factor	Score value if present	Maximum possible score value per factor[a]
Time in hospital: ≤10 days	1	1
Gestational age: <37 weeks	1	1
Birth weight:		2
• >2 kg	0	
• 1-2 kg	1	
• <1 kg	2	
Congenital anomalies	2	2
Temperature		2
• <35.5ºC	1	
• 35.5-37.9ºC	0	
• 38-38.9ºC	1	
• ≥39ºC	2	
Maximum respiratory support:		3
• None	0	
• Oxygen supplementation	2	
• CPAP, BiPAP, HFNC	3	
• Invasive ventilation	3	
Abdominal distension	1	1
Difficulty in feeding	1	1
Evidence of shock[b]	1	1
Lethargy/no or reduced movement:		2
• Lethargy only	1	
• No movement or movement only on stimulation +/- lethargy	2	

Abbreviations: Kg, kilogram; ºC, degree Celsius; CPAP, continuous positive airway pressure; BiPAP, bilevel positive airway pressure; HFNC, high flow nasal canula.
[a]Possible maximum score, 16
[b]Including capillary refill time >3seconds, mottled skin, cold peripheries or other signs of shock

Supplementary Table 3. Blood and cerebrospinal fluid culture isolates

	Blood culture (n=554 infants)	CSF culture (n=23 infants)
Presumed pathogens	**n=160 infants**	**n=15 infants**
Gram positive		
Staphylococcus aureus	17	1
Group B Streptococcus	12	4
Enterococcus species[a]	6	0
Streptococcus pneumoniae	3	1
Streptococcus pyogenes	3	0
Group D Streptococcus	2	0
Paenibacillus thiaminolyticus	2	1
Gram negative		
Klebsiella pneumoniae	57	5
Escherichia coli	22	0
Acinetobacter species[b]	15	0
Enterobacter cloacea	14	1
Pseudomonas aeruginosa	6	1
Salmonella species	5	0
Haemophilus influenzae	4	1
Aeromonas hydrophila	2	0
Stenotrophomonas maltophilia	2	0
Morganella morganii	1	0
Serratia marcescens	1	0
Total isolates	174	15[c]
Likely pathogenic	**n=5 infants**	**n=0 infants**
Gram positive		
Bacillus cereus	4	0
Gram negative		
Chryseobacterium indologenes	1	0
Total isolates	5	0
Likely nonpathogenic	**n=389 infants**	**n=5 infants**
Gram positive		
Coagulase negative Staphylococcus[d]	296	1
Coryneforms	47	0
Bacillus species[e]	30	3
Viridans Group Streptococcus[f]	10	1
Micrococcus species[g]	6	0
Others[h]	13	0
Gram negative		
Pseudomonas species[i]	7	0
Others[j]	10	0
Total isolates	419	5

Supplementary Table 3. Blood and cerebrospinal fluid culture isolates (continued)

	Blood culture (n=554 infants)	CSF culture (n=23 infants)
[a]Enterococcus species (n=4), *Enterococcus faecalis* (n=1), *Enterococcus faecium* (n=1)		
[b]Acinetobacter species (n=7), *Acinetobacter baumannii* (n=6), *Acinetobacter calcoaceticus/baumannii* (n=2)		
[c]Six had similar CSF and blood culture isolates: Group B Streptococcus (n=3), *Paenibacillus thiaminolyticus* (n=1), *Enterobacter cloacea* (n=1) and *Haemophilus influenzae* n=1)		
[d]*Staphylococcus epidermidis* (n=148), *Staphylococcus haemolyticus* (n=76), *Staphylococcus hominis* (n=63), *Staphylococcus capitis* (n=4), *Staphylococcus arlettae* (n=2), *Staphylococcus gallinarum* (n=1), *Staphylococcus pasteuri* (n=1), *Staphylococcus warneri* (n=1), *Staphylococcus xylosus* (n=1)		
[e]*Bacillus* species (n=30), *Bacillus pumilus* (n=2), *Bacillus megaterium* (n=1)		
[f]*Streptococcus viridans* (n=55), *Streptococcus mitis* (n=2), *Streptococcus gallolyticus* (n=1), *Streptococcus milleri* (n=1), *Streptococcus oralis* (n=1), *Streptococcus vestibularis* (n=1)		
[g]*Micrococcus* species (n=4), *Micrococcus luteus* (n=1), *Micrococcus lylae* (n=1)		
[h]Other Gram positive likely nonpathogenic: *Brevibacterium paucivorans* (n=1), *Corynebacterium* species (n=1), *Corynebacterium amycolatum* (n=1), *Corynebacterium mucifaciens* (n=1), *Corynebacterium simulans* (n=1), Group F Streptococcus (n=1), *Kocuria palustris* (n=1), *Microbacterium oleivorans* (n=1), *Microbacterium trichothecenolyticum* (n=1), Streptococcus species (n=4)		
[i]*Pseudomonas stutzeri* (n=5), *Pseudomonas oryzihabitans* (n=2)		
[j]Other Gram negative, likely nonpathogenic: *Arthrobacter globiformis* (n=1), *Comamonas kerstersii* (n=1), *Cupriavidus gilardii* (n=1), *Klebsiella oxytoca* (n=1), *Leclercia adecarboxylata* (n=1), Pantoea species (n=2), *Pantoea septica* (n=1), Unidentified gram-negative fastidious rod (n=2)		

Supplementary Table 4. Comparison of baseline characteristics before and during NeoOBS study

	All (n=3746)	Before NeoOBS (n=2284)	During NeoOBS (n=1462)	P value
Infant characteristics				
Age, days	1 (0-5)	1 (0-5)	1 (0-5)	0.097
Age categories				
≤48 h	2542 (68)	1535 (67)	1007 (69)	0.547
>48 h – 7 d	397 (11)	249 (11)	148 (10)	
>7 d – 59 d	807 (22)	500 (22)	307 (21)	
Weight, kg	2.7 (1.8-3.2)	2.7 (1.9-3.2)	2.7 (1.8-3.2)	0.427
Length, cm	48 (43-50)	48 (43-50)	47 (43-50)	0.305
MUAC, cm	9.5 (8.0-11)	9.5 (8.0-11)	9.5 (8.0-11)	0.183
Head circumference, cm	34 (31-36)	34 (32-36)	34 (31-36)	0.247
Male	2159 (58)	1315 (58)	844 (58)	0.772
Born at KCH	1861 (50)	1099 (48)	762 (52)	0.026
Clinical signs and symptoms				
Abnormal temperature (>37.5^{o}C or <36.5^{o}C) or temperature instability[a]	2813 (75)	1752 (77)	1061 (73)	0.009
Respiratory signs[b]	2582 (69)	1564 (69)	1018 (70)	0.601
No or reduced movement	1442 (39)	858 (38)	584 (40)	0.229
Lethargy or drowsiness	479 (13)	269 (12)	210 (14)	0.047
Abnormal heart rate (>180/min or <100/min)	472 (13)	304 (13)	168 (11)	0.192
Evidence of shock[c]	428 (11)	263 (12)	165 (11)	0.726
Convulsions	261 (7.0)	165 (7.2)	96 (6.6)	0.433
Inability to feed	253 (6.8)	172 (7.5)	81 (5.5)	0.047
Cyanosis	191 (5.1)	104 (4.6)	87 (6.0)	0.118
Apnoea	122 (3.3)	81 (4.0)	41 (2.8)	0.345
Irritability	90 (2.4)	66 (2.9)	24 (1.6)	0.039
Abnormal posturing	86 (2.3)	58 (2.5)	28 (1.9)	0.346
Abdominal distension	81 (2.2)	53 (2.3)	28 (1.9)	0.528
Multiple or severe skin pustules	80 (2.1)	55 (2.4)	25 (1.7)	0.266
Grunting	40 (1.1)	18 (0.8)	22 (1.5)	0.083
Bulging fontanel	30 (0.8)	21 (0.9)	9 (0.6)	0.443
Pus from umbilical stump	23 (0.6)	19 (0.8)	4 (0.3)	0.065
Hypotonia or floppiness	20 (0.5)	17 (0.7)	3 (0.2)	0.060
Petechial rash	0 (0)	0 (0)	0 (0)	0.436

Supplementary Table 4. Comparison of baseline characteristics before and during NeoOBS study (continued)

	All (n=3746)	Before NeoOBS (n=2284)	During NeoOBS (n=1462)	P value
Laboratory signs				
WBC <4 or >20 x10^9 cells/L[d]	926 (25)	568 (25)	358 (24)	0.602
ANC <1.5 x10^9 cells/L[d]	257 (6.9)	156 (6.8)	101 (6.9)	0.556
Acidosis[d e]	1,492 (40)	930 (41)	562 (38)	0.254
Outcome				
Alive	3125 (83)	1914 (84)	1211 (83)	0.437
Died	621 (17)	370 (16)	251 (17)	

Data are median (IQR), n (%)

Abbreviations: kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; KCH, Kilifi County Hospital; sec, second; min, minute; ^oC, degree Celsius; WBC, white blood cells; ANC, absolute neutrophil count; mmol/L, millimoles per litre.

[a] Includes temperature gradient. Overall. 1,645 (44%) infants had temperature <36.5^oC, 1,114 (30%) had temperature >37.5^oC

[b] Includes severe chest in-drawing, difficulty in breathing, irregular or deep respiration, abnormal respiratory rate (<30 or >60 breaths per minute), hypoxia (oxygen saturation ≤90%), increased oxygen requirement or requirement for ventilation support

[c] Evidence of shock include capillary refill time (CRT) > 3 sec, weak or bounding pulse, or mottled skin

[d] Complete blood count was offered to all infants at admission, but 6% (219/3,746) had missing results due to difficult venepuncture or clotted samples. Blood gas analysis was selectively done in 1,797/3,746 (48%) seriously ill infants who were hospitalised in the high dependency unit.

[e] Base excess <-10 mmol/L or blood lactate >2 mmol/L

Supplementary Table 5. Comparison of individual NeoOBS study inclusion criteria signs between infants with serious bacterial infection and infants without serious bacterial infection

	SBI (n=175)	No SBI (n=3571)	P value
Clinical criteria			
Abnormal temperature (>37.5°C or <36.5°C) or temperature instability[a]			
No	33 (19)	896 (25)	0.178
Yes	142 (81)	2671 (75)	
Missing	0 (0)	4 (0.1)	
Respiratory signs[b]			
No	53 (30)	1107 (31)	0.891
Yes	122 (70)	2460 (69)	
Missing	0 (0)	4 (0.1)	
No or reduced movement			
No	96 (55)	2168 (61)	0.099
Yes	79 (45)	1363 (38)	
Missing	0 (0)	40 (1.1)	
Lethargy or drowsiness			
No	152 (87)	3075 (86)	0.483
Yes	23 (13)	456 (13)	
Missing	0 (0)	40 (1.1)	
Abnormal heart rate (>180/min or <100/min)			
No	128 (73)	3142 (88)	<0.001
Yes	47 (27)	425 (12)	
Missing	0 (0)	4 (0.1)	
Shock[c]			
No	145 (83)	3133 (88)	0.028
Yes	30 (17)	398 (11)	
Missing	0 (0)	40 (1.1)	
Convulsions			
No	164 (94)	3283 (92)	0.528
Yes	11 (6.3)	250 (7.0)	
Missing	0 (0)	38 (1.1)	
Inability to feed			
No	163 (93)	3290 (92)	0.474
Yes	12 (6.9)	241 (6.8)	
Missing	0 (0)	40 (1.1)	
Cyanosis			
No	170 (97)	3345 (94)	0.168
Yes	5 (2.9)	186 (5.2)	
Missing	0 (0)	40 (1.1)	
Apnoea			
No	161 (92)	3423 (96)	0.002
Yes	14 (8.0)	108 (3.0)	
Missing	0 (0)	40 (1.1)	

Supplementary Table 5. Comparison of individual NeoOBS study inclusion criteria signs between infants with serious bacterial infection and infants without serious bacterial infection (continued)

	SBI (n=175)	No SBI (n=3571)	P value
Irritability			
No	170 (97)	3446 (97)	0.392
Yes	5 (2.9)	85 (2.4)	
Missing	0 (0)	40 (1.1)	
Abnormal posturing			
No	164 (94)	3456 (97)	0.002
Yes	11 (6.3)	75 (2.1)	
Missing	0 (0)	40 (1.1)	
Abdominal distension			
No	167 (95)	3458 (97)	0.038
Yes	8 (4.6)	73 (2.0)	
Missing	0 (0)	40 (1.1)	
Multiple or severe skin pustules			
No	168 (96)	3458 (97)	0.111
Yes	7 (4.0)	73 (2.0)	
Missing	0 (0)	40 (1.1)	
Grunting			
No	174 (99)	3492 (98)	0.482
Yes	1 (0.6)	39 (1.1)	
Missing	0 (0)	40 (1.1)	
Bulging fontanel			
No	171 (98)	3505 (98)	0.034
Yes	4 (2.3)	26 (0.7)	
Missing	0 (0)	40 (1.1)	
Pus from umbilical stump			
No	173 (99)	3510 (98)	0.224
Yes	2 (1.1)	21 (0.6)	
Missing	0 (0)	40 (1.1)	
Hypotonia or floppiness			
No	175 (100)	3511 (98)	0.437
Yes	0 (0)	20 (0.6)	
Missing	0 (0)	40 (1.1)	
Petechial rash			
No	175 (100)	3531 (99)	0.260
Yes	0 (0)	0 (0)	
Missing	0 (0)	40 (1.1)	

Supplementary Table 5. Comparison of individual NeoOBS study inclusion criteria signs between infants with serious bacterial infection and infants without serious bacterial infection (continued)

	SBI (n=175)	No SBI (n=3571)	P value
Laboratory signs			
WBC <4 or >20 x10^9 cells/L[d]			
No	120 (69)	2481 (69)	0.016
Yes	52 (30)	874 (24)	
Missing	3 (1.7)	216 (6.1)	
ANC <1.5 x10^9 cells/L[d]			
No	131 (75)	3136 (88)	<0.001
Yes	41 (23)	216 (6.1)	
Missing	3 (1.7)	219 (6.1)	
Acidosis[d, e]			
No	17 (9.7)	294 (8.2)	<0.001
Yes	94 (54)	1398 (39)	
Missing	64 (37)	1879 (53)	

Data are median (IQR), n (%)

Abbreviations: SBI, serious bacterial infection; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; KCH, Kilifi County Hospital; sec, second; min, minute; ^oC, degree Celcius; WBC, white blood cells; ANC, absolute neutrophil count; mmol/L, millimoles per litre.

[a] Includes temperature gradient. Axillary temperature <36.5^oC was recorded in 78/175 (45%) infants with SBI and 1567/3571 (44%) infants without SBI, while temperature >37.5^oC was in 61/175 (35%) infants with SBI and 1053/3571 (29%) infants without SBI.

[b] Includes severe chest in-drawing, difficulty in breathing, irregular or deep respiration, abnormal respiratory rate (<30 or >60 breaths per minute), hypoxia (oxygen saturation ≤90%), increased oxygen requirement or requirement for ventilation support
Forty-seven (27%) infants with SBI had abnormal heart rate (>180/min [n=36] or <100/min [n=11]) compared to 425 (12%) infants without SBI (>180/min [n=342] or <100/min [n=83]), *p<0.001*.

[c] Evidence of shock include capillary refill time (CRT) > 3 sec, weak or bounding pulse, or mottled skin

[d] Complete blood count was offered to all infants at admission, but some had missing results due to clotted samples. Blood gas analysis was not done in all infants and was limited to those who were seriously ill hence this sample is biased.

[e] Base excess <-10 mmol/L or blood lactate >2 mmol/L

[f] ≥2 of above clinical and/or laboratory features of which 1 must be clinical

Supplementary Table 6. Performance of NeoOBS study inclusion criteria, comparing infants aged ≤48h with infants aged >48h-59d

NSIC	SBI[a]	No SBI	P value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Overall (n=3746)	n=175	n=3571					
No	19 (11)	733 (21)	0.002	89 (84-93)	21 (19-22)	5.2 (4.4-6.1)	98 (96-99)
Yes	156 (89)	2838 (79)					
≤48 hr (n=2542)	n=99	n=2443					
No	10 (10)	420 (17)	0.065	90 (82-95)	17 (16-19)	4.2 (3.4-5.2)	98 (96-99)
Yes	89 (90)	2023 (83)					
>48h-59d (n=1204)	n=76	n=1128					
No	9 (12)	313 (28)	0.002	88 (79-94)	28 (25-31)	7.6 (6.0-10)	97 (95-99)
Yes	67 (88)	815 (72)					

Abbreviations: SBI, serious bacterial infection; CI, confidence interval.

Supplementary Table 7. Association of NeoOBS study inclusion criteria with mortality

	Died (n=621)	Survived (n=3125)	Univariable Odds Ratio (95% CI)	P value	Multivariable Odds Ratio (95% CI)	P value
NSIC						
No	5 (0.8)	747 (24)	1.0	-	1.0	-
Yes	616 (99)	2378 (76)	39 (16-94)	<0.001	32 (13-77)	<0001
Age category						
≤48h	507 (82)	2035 (65)	1.0	-	1.0	-
>48h-7d	42 (6.8)	355 (11)	0.5 (0.3-0.7)	<0.001	0.7 (0.5-1.0)	0.044
>7-59d	72 (12)	735 (24)	0.4 (0.3-0.5)	<0.001	0.5 (0.4-0.7)	<0.001
Weight, kg	2.1 (1.2-2.9)	2.8 (2.0-3.3)	0.5 (0.4-0.6)	<0.001	1.4 (1.0-1.9)	0.082
Length, cm	45 (37-49)	48 (44-50)	0.9 (0.8-0.9)	<0.001	0.9 (0.9-1.0)	0.006
Head circumference, cm	32 (27-35)	35 (32-36)	0.8 (0.8-0.9)	<0.001	1.0 (0.9-1.0)	0.078
MUAC, cm	8.5 (7.0-10)	9.6 (8.3-11)	0.7 (0.6-0.7)	<0.001	0.8 (0.7-0.9)	0.004
Sex						
Female	262 (42)	1308 (42)	1.0	-	-	-
Male	354 (57)	1805 (58)	1.0 (0.8-1.2)	0.813	-	-
Missing	5 (0.8)	12 (0.4)	2.1 (0.7-6.0)	0.172	-	-
SBI					-	-
No	558 (90)	3013 (96)	1.0	-	1.0	-
Yes	63 (10)	112 (3.6)	3.0 (2.2-4.2)	<0.001	2.5 (1.7-3.5)	<0.001

Data are N (%) or median (IQR)

Abbreviations: NSIC, NeoOBS study inclusion criteria; d, days; h, hours; CI, confidence interval; kg, kilogram; cm, centimetre; MUAC, mid-upper arm circumference; SBI, serious bacterial infection.

*Univariable logistic model for infants who died vs. survived

†Multivariable logistic model for infants who died vs. survived, including variables with P<0.1

Supplementary Table 8. Validation of the NeoSEP severity score using birth weight (sensitivity analysis)

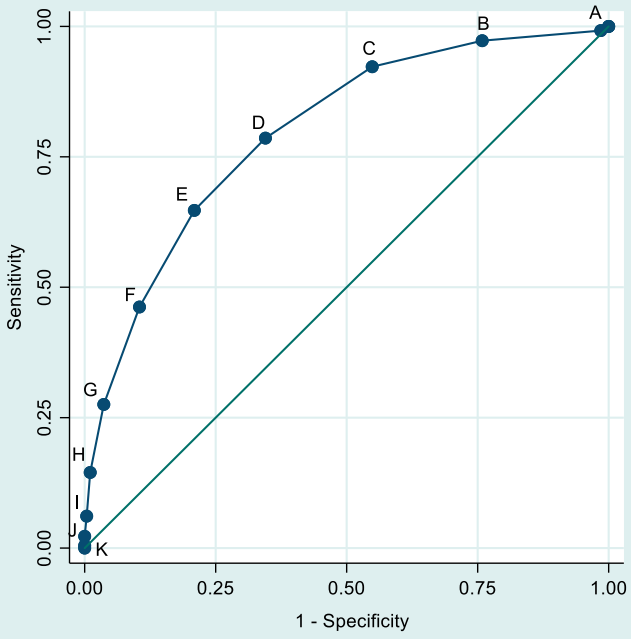
Score	Meeting criteria N=3746 n (%)	Deaths N=621 n (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95%CI)
≥1	3694 (99)	616 (99)	99 (98-100)	1.5 (1.1-2.0)	17 (16-18)	90 (79-97)	1.0 (1.0-1.0)	0.5 (0.5-0.5)
≥2	2975 (79)	604 (97)	97 (96-98)	24 (23-26)	20 (19-22)	98 (97-99)	1.3 (1.3-1.3)	0.1 (0.1-0.2)
≥3	2288 (61)	573 (92)	92 (90-94)	45 (43-47)	25 (23-27)	97 (96-98)	1.7 (1.6-1.8)	0.2 (0.1-0.2)
≥4	1566 (42)	488 (79)	79 (75-82)	66 (64-67)	31 (29-34)	94 (93-95)	2.3 (2.1-2.4)	0.3 (0.3-0.4)
≥5	1056 (28)	402 (65)	65 (61-69)	79 (78-81)	38 (35-41)	92 (91-93)	3.1 (2.8-3.4)	0.4 (0.4-0.5)
≥6	614 (16)	287 (46)	46 (42-50)	90 (88-91)	47 (43-51)	89 (88-90)	4.4 (3.9-5.1)	0.6 (0.6-0.6)
≥7	285 (7.6)	171 (28)	28 (24-31)	96 (96-97)	60 (54-66)	87 (86-88)	7.6 (6.1-9.4)	0.8 (0.7-0.8)
≥8	123 (3.3)	90 (14)	15 (12-18)	99 (99-99)	73 (64-81)	85 (84-87)	14 (9.3-20)	0.9 (0.8-0.9)
≥9	50 (1.3)	38 (6.1)	6.1 (4.4-8.3)	100 (99-100)	76 (62-87)	84 (83-85)	16 (8.4-30)	0.9 (0.9-1.0)
≥10	14 (0.4)	14 (2.3)	2.3 (1.2-3.8)	100 (100-100)	100 (77-100)	84 (83-85)	-	1.0 (1.0-1.0)
≥11	3 (0.1)	3 (0.5)	0.5 (0.1-1.4)	100 (100-100)	100 (29-100)	84 (82-85)	-	1.0 (1.0-1.0)

Data are n (%)

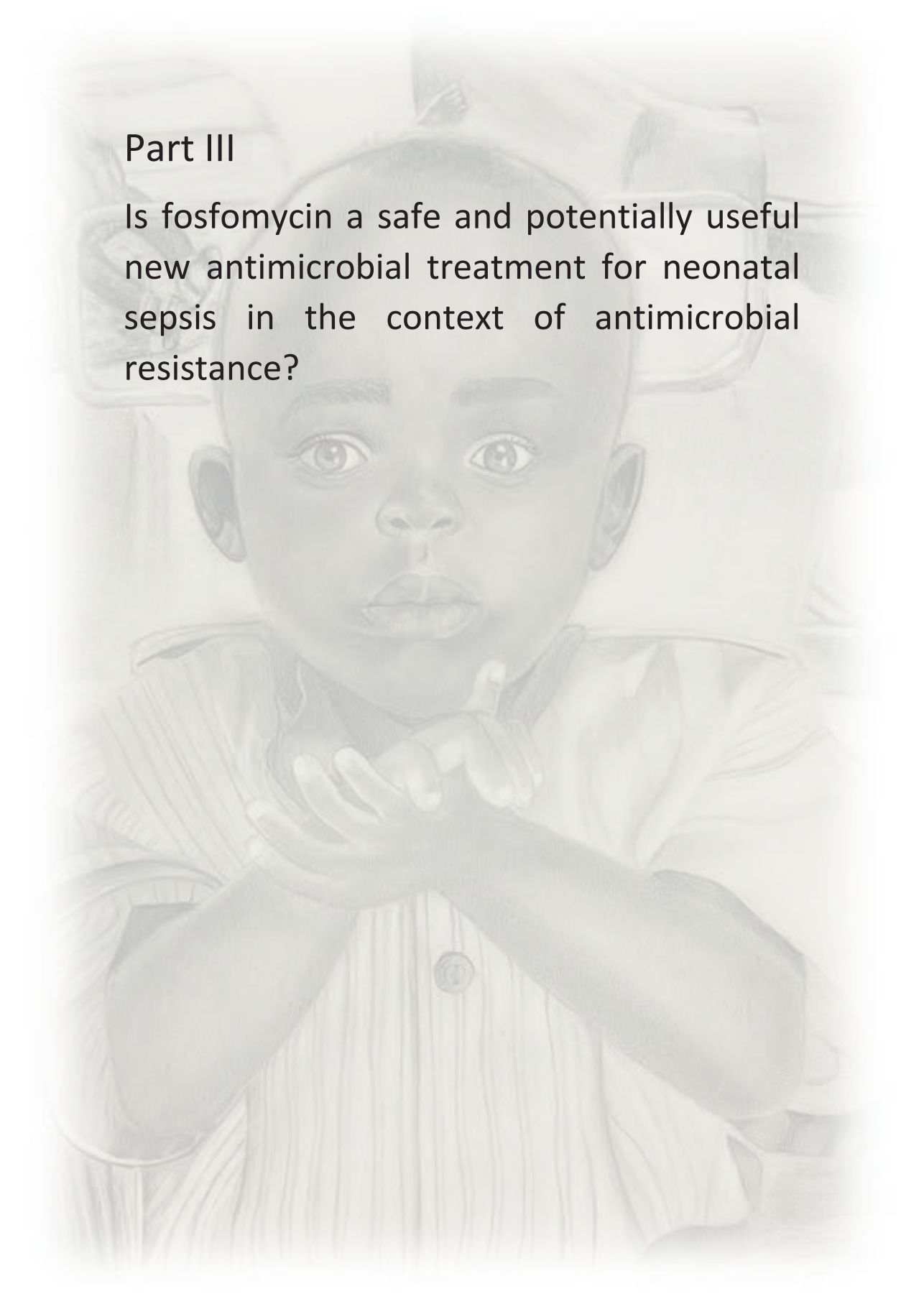
Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

Supplementary Figures

Supplementary Figure 1. Performance of the NeoSEP severity score using birth weight



Area under receiver operating characteristic curve for NSS for mortality. Scores: (A) ≥1, (B) ≥2, (C) ≥3, (D) ≥4, (E) ≥5, (F) ≥6, (G) ≥7, (H) ≥8, (I) ≥9, (J) ≥10, (K) ≥11.



Part III

Is fosfomycin a safe and potentially useful new antimicrobial treatment for neonatal sepsis in the context of antimicrobial resistance?

Chapter 7

Randomised controlled trial of fosfomycin in neonatal sepsis: pharmacokinetics and safety in relation to sodium overload

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ABSTRACT

Objective To assess pharmacokinetics and changes to sodium levels in addition to adverse events associated with fosfomycin among neonates with clinical sepsis.

Design A single-centre open-label randomised controlled trial

Setting Kilifi County Hospital, Kenya

Patients 120 neonates aged ≤28 days admitted being treated with standard-of-care (SOC) antibiotics for sepsis: ampicillin and gentamicin between March 2018 and February 2019.

Intervention We randomly assigned half the participants to receive additional intravenous then oral fosfomycin at 100mg/kg two times per day for up to 7 days (SOC-F) and followed up for 28 days.

Main outcome(s) and measure(s) Serum sodium, adverse events and fosfomycin pharmacokinetics.

Results 61 and 59 infants aged 0-23 days were assigned to SOC-F and SOC respectively. There was no evidence of impact of fosfomycin on serum sodium or gastrointestinal side-effects. We observed 35 adverse events (AEs) among 25 SOC-F participants and 50 AEs among 34 SOC participants during 1,560 and 1,565 infant-days observation respectively (2.2 vs 3.2 events/100 infant-days; incidence rate difference -0.95 events/100 infant-days [95%CI -2.1 to 0.20]). Four SOC-F and 3 SOC participants died. From 238 pharmacokinetic samples, modelling suggests an intravenous dose of 150mg/kg two times per day is required for pharmacodynamic target attainment in most children, reduced to 100mg/kg two times per day in neonates aged <7 days or weighing <1500g.

Conclusion and relevance Fosfomycin offers potential as an affordable regimen with a simple dosing schedule for neonatal sepsis. Further research on its safety is needed in larger cohorts of hospitalised neonates, including very preterm neonates or those critically ill. Resistance suppression would only be achieved for the most sensitive of organisms so fosfomycin is recommended to be used in combination with another antimicrobial**.**

Trial registration ClinicalTrials.gov: NCT03453177

INTRODUCTION

Antimicrobial resistance (AMR) disproportionally impacts populations in low- and middle-income countries (LMICs). Reductions in mortality have been less in neonates than older children, and at least one-quarter of neonatal deaths are attributable to infection.[1] AMR contributes to this burden, with multidrug-resistant (MDR) pathogens accounting for ~30% of global neonatal sepsis deaths.[2]

The World Health Organization (WHO) recommends ampicillin, penicillin or cloxacillin (if *Staphylococcus aureus* infection is suspected) plus gentamicin (first-line), and third-generation cephalosporins (second-line) for empiric treatment of neonatal sepsis.[3] With spread of extended spectrum β-lactamase (ESBL) and carbapenemase enzymes,[4] clinical isolates are commonly reported non-susceptible to this regimen.[5] Carbapenem-sparing is important in controlling MDR,[6] and reintroduction of legacy antibiotics has been advocated to address the lack of new affordable antibiotics.[7]

Fosfomycin is an off-patent phosphonic acid derivative identified as 'critically important' by the WHO.[8] Fosfomycin is bactericidal[9] and exhibits activity against Gram-positive and -negative bacteria, including methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus* spp*.,* ESBL-producers, and may penetrate biofilms.[10] Fosfomycin demonstrates *in vitro* synergy with aminoglycosides and carbapenems[11 12] and is commonly used for MDR urinary tract infections in adults.[13]

Current paediatric intravenous fosfomycin dosing recommendations are divergent, ranging between 100-400mg/kg/day, without published oral dosing regimens. Four neonatal studies estimate an elimination half-life of 2.4-7 hours following 25-50mg/kg intravenously.[14 15] Protein binding was minimal and maximum concentration was in-line with adult data.[16 17] Bactericidal effects are thought to correlate with either time above the minimum inhibitory concentration (MIC)[16] or area under the curve (AUC) to MIC ratio.[18 19]

Case reports totalling 84 neonates treated with intravenous fosfomycin 120-200mg/kg/day suggest it is well-tolerated.[20-24] Toxicity among adults and older children appears low.[25] However, parenteral fosfomycin contains 14.4mmol/330mg sodium per gram - a potential safety concern in neonates

whose sodium reabsorption is inversely proportional to gestational age (GA).[26] Furthermore, oral fosfomycin contains a high fructose load (~1600mg/kg/day), which may predispose to gastrointestinal side effects and impact fluid balance.[27 28]

We aimed to assess pharmacokinetics (PK) and changes to sodium levels in addition to adverse events (AEs) associated with intravenous followed by oral fosfomycin in neonates with clinical sepsis.

METHODS

Participants and study design

We conducted an open-label randomised controlled trial of standard of care (SOC) antibiotics alone, versus SOC plus intravenous then oral fosfomycin, in neonates with clinical sepsis at Kilifi County Hospital (KCH), Kenya.

Screening and eligibility

All neonates admitted to KCH were screened. Inclusion criteria were: age ≤28 days, weight >1500g, gestation >34 weeks and meeting criteria for intravenous antibiotics per WHO[3] and Kenyan[29] guidelines. Neonates were excluded if requiring cardiopulmonary resuscitation, grade 3 hypoxic ischemic encephalopathy,[30] sodium ≥150mmol/L, creatinine ≥150µmol/L, jaundice requiring exchange transfusion, allergy or contraindication to fosfomycin, a specific indication for another antibiotic class, admitted from another hospital or not residing within Kilifi county (Figure 1).

Participants were enrolled within 4 hours of the first dose of SOC antibiotics, until September 2018 when a protocol amendment extended this to within 24 hours to include overnight admissions. Written informed consent was sought by trained field assistants in the carer's preferred language.

Enrolment and randomization

A randomisation schedule with random block sizes was used to assign participants (1:1) to continue SOC antibiotics only or receive SOC plus (up to) 7-days of fosfomycin (SOC-F) (Figure S1 in Supplement 2). Concealment was by sequentially numbered opaque sealed envelopes.

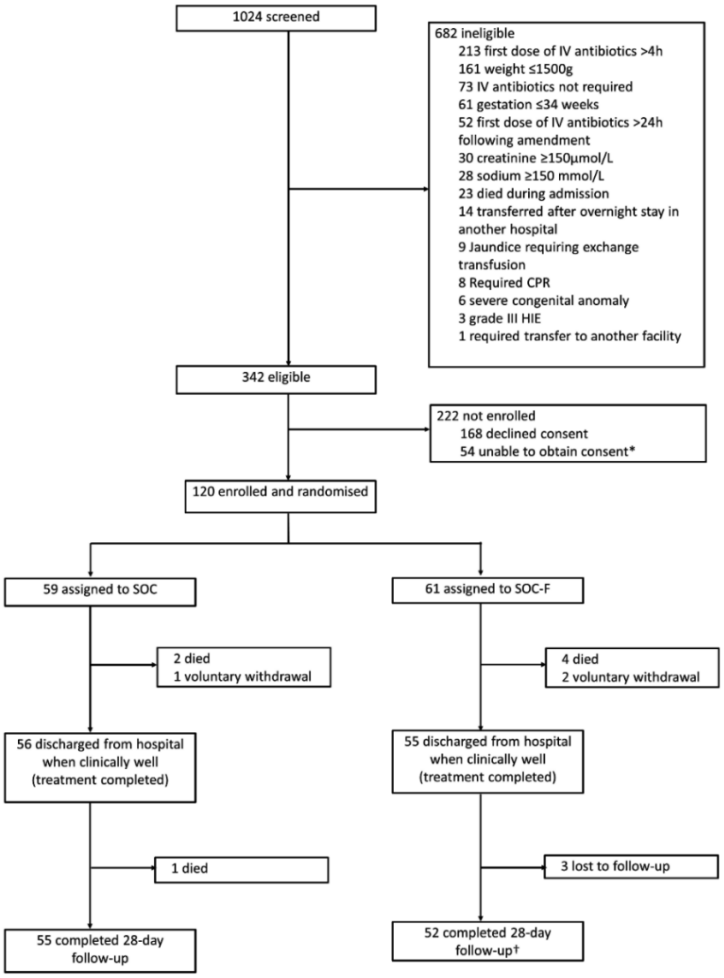

Figure 1. Trial Flowchart.
CPR, cardiopulmonary resuscitation; HIE, hypoxic ischaemic encephalopathy; IV, intravenous; SOC, standard of care; SOC-F, standard of care plus fosfomycin. *Reasons include mother postcaesarean section (46) or seriously ill (6), absconded from hospital (3), discharged against advice (3), abandoned by mother (1) and already enrolled into another study (1). †One SOC-F participant died after completing follow-up (on day 106). This original figure was created by CWO for this manuscript.

Study treatment

SOC entailed ampicillin or cloxacillin (if Staphylococcal infection was suspected) plus gentamicin as first-line antibiotics, or third-generation cephalosporins (e.g. ceftriaxone) as second-line antibiotics according to WHO and Kenya paediatric guidelines.[3 29] Participants randomised to SOC-F also received intravenous fosfomycin for at least 48 hours, switching to oral when tolerating feeds sufficiently to presume adequate absorption of oral medications. Fosfomycin (intravenous or oral) was administered for 7 days or until discharge, whichever occurred first. Fomicyt™ 40 mg/ml fosfomycin sodium solution for intravenous infusion (Infectopharm GmbH, Germany) and Fosfocina® 250mg/5ml fosfomycin calcium suspension for oral administration (Laboratorios ERN, Spain) were given at 100 mg/kg/dose two times per day.

Follow-up, safety monitoring and outcomes

Participants were followed-up for 28 days. All participants were cared for in the same high dependency unit to standardise AE monitoring. Complete blood count and biochemistry (including sodium) were done at admission, days 2 and 7, and were repeated if clinically indicated. AEs were coded according to MedDRA v22.0. Severity was classified according to DAIDS v2.1. AEs were followed-up until clinical resolution or judged to be chronic and stable while receiving care. "Anticipated" AEs were defined *a priori* as those expected to occur commonly in this population, including likely deteriorations of conditions present at birth (Trial Protocol in Supplement 1).

Pharmacokinetics

Patients allocated to SOC-F were randomly assigned to one early (5, 30 or 60 minutes) and one late (2, 4 or 8 hours) PK sample after both the first intravenous and first oral fosfomycin doses. A non-systematic fifth sample was collected for participants still hospitalised on day 7. Opportunistic cerebrospinal fluid (CSF) samples were collected from clinically indicated lumbar punctures (LP). Sample processing and fosfomycin measurement are described in Supplement 2.

Statistical methods

We reviewed admission data between 2015-2016 and calculated a mean sodium of 139 mmol/L (standard deviation [SD] 7.6, range 106-198) among

1,785 neonates weighing >1500g. Excluding 132 neonates who had serum sodium of >150mmol/L (our exclusion criteria) resulted in a mean sodium of 137 mmol/L (SD 5.2) among the remaining 1,653 neonates. A sample size of 45 per arm was subsequently calculated to ensure a 5mmol/L difference in plasma sodium at day 2 could be determined with >85% power based on local prior sodium distribution data.

For PK, a sample size of 45 provided >85% power to estimate PK parameters for clearance, volume of distribution and bioavailability with 95% confidence intervals (95%CI) with precision of ≥20% using simulation-estimation. For this, an adult disposition model, with age and size scaling to neonates with added first-order absorption and assumed bioavailability was used.[32] To allow for missed samples, we aimed to recruit 60 neonates per arm.

Differences in baseline parameters were tested using Chi-squared test, Student's t-test or Wilcoxon rank-sum test. Differences in sodium, potassium, creatinine and alanine aminotransferase (ALT) at day 2 and 7 were tested using analysis of covariance (ANCOVA) adjusting for baseline values. For AEs, serious adverse events (SAEs) and adverse drug reactions (ADRs), we estimated incidence rate ratios (IRR) and rate differences (IRD) between arms with two-sided exact CIs using STATA version 15.1 (StataCorp, College Station, TX, USA).

Model-based estimation of PK parameters was undertaken using first-order conditional estimation with interaction in NONMEM v7.4.[31] Full details of PK model development and simulations are provided elsewhere.[31]

Ethical review and oversight

The protocol was approved by KEMRI Scientific and Ethical Review Unit (KEMRI/SERU/CGMRC/097/3513), Kenya Pharmacy and Poisons Board (PPB/ECCT/17/10/01/2017(200)) and Oxford Tropical Research Ethics Committee (26-17). The trial was registered (NCT03453177). DND*i*/GARDP undertook on-site monitoring and an independent Data Safety and Monitoring Board provided oversight.

RESULTS

Enrolment

Between March 19, 2018, and February 6, 2019, 120 neonates (61 SOC-F, 59 SOC) were enrolled (Figure 1), 42 (35%) before the protocol amendment. Median (IQR) age, weight and GA were 1 day (IQR 0-3), 2,750g (2,370-3,215) and 39 weeks (38-40) respectively. Baseline characteristics and laboratory parameters are presented in Table 1 and Table S1 in Supplement 2.

Two neonates had detected bacteraemia (Table S2 in Supplement 2). Two of 55 neonates who underwent an LP had laboratory-confirmed meningitis (*Streptococcus agalactiae* bacteraemia with CSF leukocytes ≥20 cells/µL [SOC-F]; positive CSF antigen test for *Streptococcus pneumoniae* and CSF leukocytes ≥20 cells/µL [SOC]).

Treatment fidelity and follow-up

One SOC-F neonate erroneously received only SOC antimicrobials and was excluded from PK analyses. Two SOC-F and one SOC neonate withdrew consent - data are included up to withdrawal. All except two SOC participants (cloxacillin plus gentamicin [n=1] and ceftriaxone [n=1]) received ampicillin plus gentamicin at admission. Table S3 in Supplement 2 shows antibiotic combinations administered in participants who received antibiotics other than ampicillin plus gentamicin at admission or following change of treatment. Ten SOC-F participants switched to second-line therapy due to clinical deterioration or meningitis, five prior to the fourth PK sample (Table S3 in Supplement 2). Overall, 60 participants received at least one intravenous fosfomycin dose and 58 at least one oral dose.

Six (4 SOC-F, 2 SOC) participants died in hospital (Figure 1). One SOC participant died three days post-discharge (day 22). One SOC-F participant missed follow-up and was later found to have died on day 106 (outside the study follow-up period); data were included up to day 28. Three SOC-F infants were lost to follow-up. Total infant/days of observation were 1,560 and 1,565 for SOC-F and SOC respectively, of which 422 and 314 were in hospital.

Table 1. Baseline characteristics

	SOC (n=59)	SOC-F (n=61)	All (n=120)	SOC vs SOC-F P value
Age (days)	1 (0-4)	1 (0-3)	1 (0-3)	
Gestational age (weeks)	38 (37-40)	40 (38-40)	39 (38-40)	0.079
Sex				
Female	24 (41)	24 (39)	48 (40)	0.881
Male	35 (59)	37 (61)	72 (60)	
Anthropometry				
Weight (g)	2700 (2080-3200)	2800 (2500-3230)	2750 (2370-3215)	0.154
Head circumference (cm)	34.0 (32.5-36.0)	34.7 (33.6-36.0)	34.6 (33.0-36.0)	0.173
Length (cm)	48.0 (44.4-49.5)	48.0 (46.0-49.5)	48.0 (45.0-49.5)	0.371
Admitted from				
KCH maternity	24 (41)	28 (46)	52 (43)	0.846
Other health facility	20 (34)	19 (31)	39 (33)	
Home	15 (25)	14 (23)	29 (24)	
Clinical symptoms				
Fever	21 (36)	22 (36)	43 (36)	0.957
Difficulty in breathing	39 (66)	40 (66)	79 (66)	0.951
Difficulty feeding	10 (17)	11 (18)	21 (18)	0.876
Seizures	8 (14)	11 (18)	19 (16)	0.502
Vomiting	1 (1.7)	1 (1.6)	2 (1.7)	0.981
Clinical signs				
Axillary temperature (°C)	36.8 (36.3-37.4)	37 (35.7-37.6)	36.9 (35.9-37.5)	0.580
Heart rate (bpm)	147 (136-161)	147 (138-158)	147 (138-159)	0.471
Respiratory rate (bpm)	54 (45-68)	56 (48-68)	56 (48-68)	0.953
Oxygen saturation (%)	96 (86-97)	95 (88-98)	96 (88-98)	0.484
Capillary refill ≥2 sec	12 (20)	14 (23)	26 (22)	0.728
Respiratory distress[a]	43 (73)	37 (61)	80 (67)	0.156
Jaundice	6 (10)	11 (18)	17 (14)	0.217
Skin lesions[b]	4 (6.8)	3 (4.9)	7 (5.8)	0.664
Abdominal distension	5 (8.5)	1 (1.6)	6 (5.0)	0.086
Impaired consciousness[c]	2 (3.4)	9 (15)	11 (9.2)	0.031
Abnormal posture	1 (1.7)	3 (4.9)	4 (3.3)	0.223
Abnormal tone	8 (14)	13 (21)	21 (18)	0.264
Bulging fontanel				
Agitated	9 (15)	11 (18)	20 (17)	0.683
Lethargic	10 (17)	17 (28)	27 (23)	0.152

Data are n (%) or median (q25 – q75). Abbreviations: g, gram; cm, centimetre; bpm, beats per minute or breaths per minute. [a] Nasal flaring, lower chest wall indrawing and/or grunting.
[b] Pustules, vesicles, petechiae and/or cellulitis.
[c] Responsive to pain only or unresponsive.

Biochemical Safety

On day 2, the mean (SD) plasma sodium values were 137mmol/L (4.6) in SOC-F vs 136mmol/L (3.7) in SOC participants; mean difference +0.7mmol/L (95%CI -1.0 to +2.4). On day 7, mean (SD) sodium values were and 136mmol/L (4.2) vs 139mmol/L (3.3); mean difference -2.9mmol/L (95% CI -7.5 to +1.8) (Table 2).

On day 2, mean (SD) potassium concentration was marginally (yet not clinically significantly) lower in SOC-F than SOC infants: 3.5mmol/L (0.7) vs 3.9mmol/L (0.7), difference -0.4mmol/L (95%CI -0.7 to -0.1). There was no evidence of difference between arms in other laboratory parameters (Table 2).

Adverse events

We observed 35 AEs in 25 SOC-F participants and 50 AEs in 34 SOC participants; 2.2 events/100 infant and 3.2 events/100 infant-days respectively: IRR 0.7 [95%CI 0.4 to 1.1], IRD -0.9 events/100 infant-days [95%CI -2.1 to +0.2, p=0.11].

Twelve SAEs occurred among 11 SOC-F participants and 14 SAEs among 12 SOC participants (0.8 events/100 infant-days in SOC versus 1.0 events/100 infant-days; IRR 0.8 [95%CI 0.4 to 1.8], IRD -0.2 events/100 infant-days [95%CI -0.9 to +0.5, p=0.59]. Hypoglycaemia was the most common AE (5 SOC-F and 6 SOC); 4 cases in each arm were Grade 3 or 4 (Table S4 in Supplement 2). Three SOC-F and 4 SOC participants had moderate or severe thrombocytopenia and were well at day 28 without platelet transfusion. AEs classified as “anticipated” occurred in 13 SOC-F and 13 SOC participants (Table S5 in Supplement 2). Three SOC participants were re-admitted to hospital (pneumonia [n=2] and febrile illness of unknown origin [n=1]); all were discharged home alive. One SOC-F participant had a mild perineal rash and another SOC-F participant experienced moderate diarrhoea 13 days post-discharge; both resolved without sequelae. Excluding mortality, 50 AEs resolved while 27 were either resolving, had not changed, or had resolved with sequelae (Table S6 in Supplement 2). No AEs were related to study medication.

Table 2. Descriptive summary of blood chemistry parameters by randomised treatment arm

		Day 0		Day 2		Day 7	
Parameter	**Statistic**	**SOC (n=59)**	**SOC + Fosfomycin (n=61)**	**SOC (n=59)**	**SOC + Fosfomycin (n=61)**	**SOC (n=6)**	**SOC + Fosfomycin (n=7)**
Sodium (mmol/L)	Range (min-max)	126-145	125-149	126-143	126-149	136-144.8	128-141
	Mean (SD)	135.4 (4.1)	136.4 (5.3)	135.7 (3.8)	136.6 (4.6)	138.6 (3.3)	135.7 (4.2)
	Median (IQR)	136 (132-138)	136 (133-140)	136 (133.5-138)	136 (134-140)	137.9 (136-139)	136 (134-139)
	n (missing)	59 (0)	61 (0)	48 (11)	54 (7)	6 (0)	7 (0)
Creatinine (µmol/L)	Range (min-max)	32-147	35-142	39-135	33-122	40-77	40-74
	Mean (SD)	92.3 (28)	88.5 (24.1)	73.7 (24.1)	72.2 (20)	59.2 (12.7)	62 (11.4)
	Median (IQR)	96.5 (70-113)	89 (74-109)	72 (54.5-87)	70 (57-83)	59.5 (53-66)	65 (57-72)
	n (missing)	58 (1)	61 (0)	52 (7)	55 (6)	6 (0)	7 (0)
Potassium (mmol/L)	Range (min-max)	2.9-6.2	2.7-6.2	2.8-5.7	2.3-4.8	2.5-4.9	2.9-5.2
	Mean (SD)	4.3 (0.6)	4.3 (0.7)	3.9 (0.7)	3.5 (0.7)	4.1 (0.9)	3.9 (0.9)
	Median (IQR)	4.3 (3.9-4.6)	4.2 (3.8-4.7)	3.9 (3.4-4.4)	3.5 (3-4)	4.3 (3.8-4.9)	4 (3-4.4)
	n (missing)	59 (0)	61 (0)	48 (11)	55 (6)	6 (0)	7 (0)
Alanine transaminase (U/L)	Range (min-max)	23-238	25-244	15-475	16-152	44-83	23-64
	Mean (SD)	90.6 (58.4)	81.8 (46.5)	73.1 (78.3)	59.9 (32.5)	64.8 (18.3)	44.7 (14.2)
	Median (IQR)	74 (54-99)	68 (45-115)	51 (38.5-70)	56.5 (35-77)	66 (49.5-80)	46.5 (35-53)
	n (missing)	37 (22)	46 (15)	48 (11)	50 (11)	4 (2)	6 (1)

Abbreviations: Fosfo=Fosfomycin; IQR=inter quartile range; n=number; SD=standard deviation; SOC=Standard of care.

Pharmacokinetics

Sixty participants had at least one intravenous PK sample collected. Fifty-five participants contributed complete sets of 4 samples, and 5 participants had partial sets. Six participants had a sample collected on day 7. Overall, 238 plasma (119 for intravenous and 119 for oral fosfomycin) and 15 CSF samples were analysed. No sample had fosfomycin levels below the limit of quantification.[31]

Population PK model development and simulation results are described in detail elsewhere.[31] Briefly, a 2-compartment PK disposition model with an additional CSF compartment provided a good fit to the data, with clearance and volume at steady-state for a typical participant (weight [WT] 2805g, postnatal age [PNA] 1 day, postmenstrual age [PMA] 40 weeks) being 0.14L/h (0.05L/h/kg) and 1.07L (0.38L/kg) respectively. In addition to fixed allometric and expected PMA maturation based on renal function,[32] PNA was associated with increasing clearance over the first week of life. The model-based population estimate of oral bioavailability was 0.48 (95%CI 0.35 to 0.78) and CSF/plasma ratio was 0.32 (95%CI 0.27 to 0.41).

Simulated steady state plasma concentration-time curves are illustrated in Figure S2 in Supplement 2. Probability of target attainment (PTA) for AUC:MIC thresholds for bacteriostasis, 1-log kill and resistance suppression are given in Figures 2 & 3 for the studied population (weight >1500g), and extrapolated using data from smaller neonates. Given the rapid increase in clearance over the first week of life, simulations were further stratified by PNA (Table S7 in Supplement 2).

Resistance suppression could not be consistently achieved with any simulated dosing regimens for organisms with MIC >0.5mg/L (Figures 2 & 3). For 100mg/kg two times per day intravenously, bacteriostasis could be achieved with 100% PTA for an MIC of 32mg/L in all four simulated strata (Figure 2). Regarding 1-log kill, PTA for 100mg/kg two times per day intravenously for an MIC of 32mg/L was 0.84 and 0.96 for Groups 1 and 3 with PNA ≤7days, but PTA was lower at 0.19 and 0.60 for Groups 2 and 4 with PNA >7 days. At 150 and 200mg/kg two times per day intravenously, PTA for 1-log kill was 0.64 and 0.90 in Group 2, and 0.91 and 0.98 in Group 4 respectively.

Oral dosing with 100mg/kg two times per day in Groups 2 and 4 yielded PTA values for bacteriostasis of 0.85 and 0.96 respectively (Figure 3), and PTAs for Groups 1-4 were 0.15, 0.004, 0.41 and 0.05 respectively for 1-log kill at an MIC of 32mg/L.

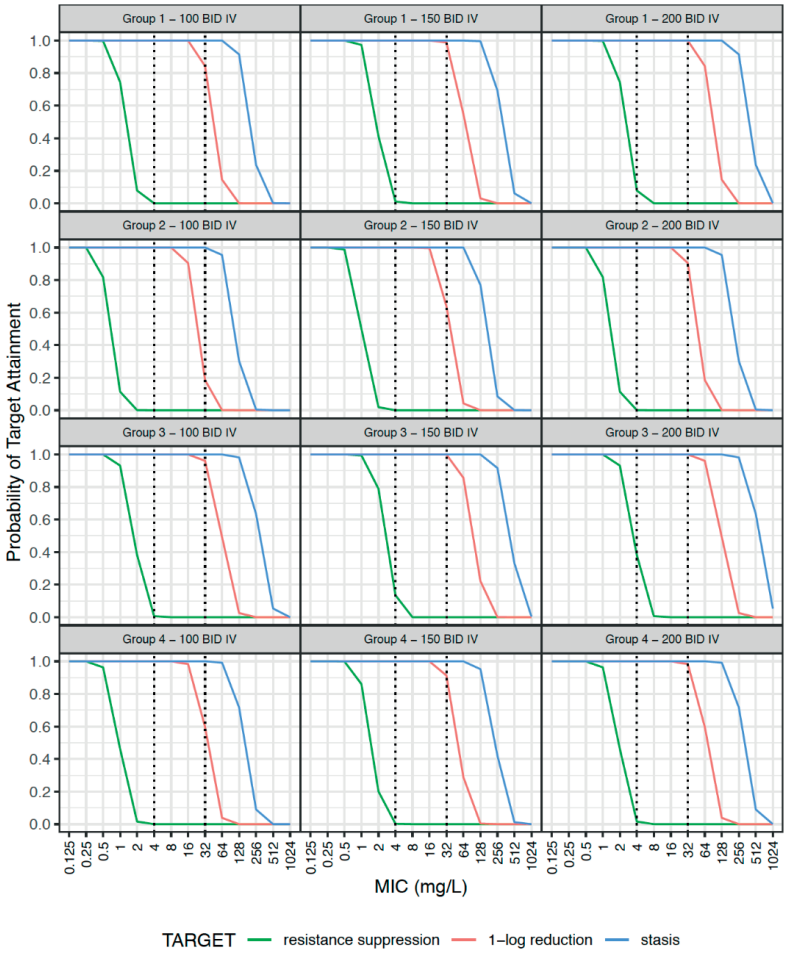

Figure 2: Probability Target Attainment for intravenous fosfomycin dosing.

Neonatal Sub-Populations. Group 1: WT>1.5kg+PNA<=7days (n=4391), Group 2: WT>1.5kg+PNA>7days (n=2798), Group 3: WT<=1.5kg+PNA<=7days (n=1534), Group 4: WT<=1.5kg+PNA>7days (n=1277). Groups 1 and 2 represent patients similar to those fitting our inclusion criteria. Groups 3 and 4 represent an extrapolation to pre-term neonates that were not studied in our population. This original figure was created by ZK for this manuscript. BID, two times per day; IV, intravenous; MIC, minimum inhibitory concentration; PNA, postnatal age; WT, weight.

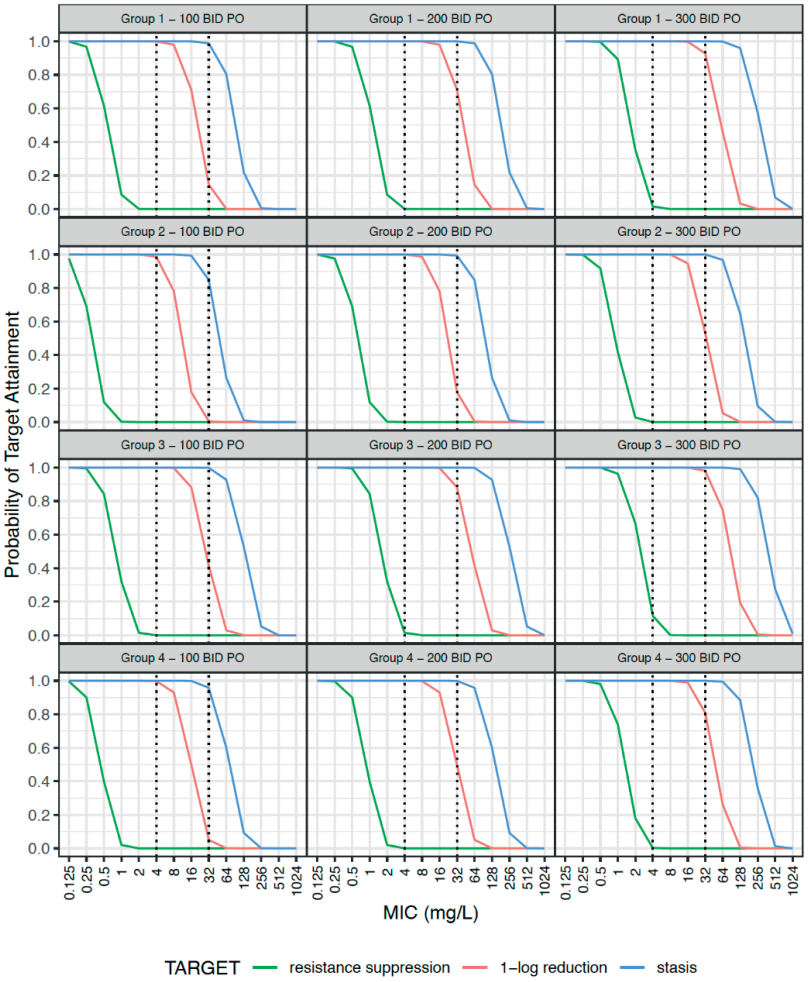

Figure 3: Probability Target Attainment for oral fosfomycin dosing.

Neonatal Sub-Populations. Group 1: WT>1.5kg+PNA<=7days (n=4391), Group 2: WT>1.5kg+PNA>7days (n=2798), Group 3: WT<=1.5kg+PNA<=7days (n=1534), Group 4: WT<=1.5kg+PNA>7days (n=1277). Groups 1 and 2 represent patients similar to those fitting our inclusion criteria. Groups 3 and 4 represent an extrapolation to pre-term neonates using external data that were not studied in our population. This original figure was created by ZK for this manuscript. BID, two times per day; MIC, minimum inhibitory concentration; PNA, postnatal age; PO, oral; WT, weight.

DISCUSSION

We provide evidence for the use of fosfomycin in infants at 100mg/kg/dose two times per day, without evidence of plasma sodium disturbance (intravenous) or osmotic diarrhoea (oral) when compared to SOC. Our primary safety objective, to detect differences in plasma sodium levels between the two treatment arms on day 2, was adequately powered. Although our sample size was too small to determine group differences for other safety events, all neonates were closely monitored, and events reported contribute towards evidence supporting the potential use of fosfomycin as an alternative empiric treatment for sepsis in this vulnerable group. However, confirmation of these results in larger and sicker cohorts will be important.

We aimed to enrol neonates aged ≤28 days and did not selectively include suspected early onset sepsis. However, 86% neonates were hospitalised within the first week of life, confirming the high burden of early neonatal morbidity reported in similar LMICs.[33-36] High levels of resistance of pathogens causing early- and late-onset sepsis (including ESBL *Escherichia coli* and *Klebsiella pneumoniae*) to empiric antimicrobials have been observed,[37-39] potentially acquired in the maternity department. Broad-spectrum antimicrobial coverage that includes fosfomycin as first-line treatment in such settings may improve outcomes and spare the use of carbapenems.

In common with many antimicrobials,[40] PNA was a key covariate in describing fosfomycin clearance. This effect was distinct from GA and weight and represents rapid glomerular filtration maturation postnatally. Locally, 90% of invasive Enterobacterales had fosfomycin MIC ≤32µg/ml[15] and for neonates aged >7 days it is likely that >100mg/kg/dose intravenously is required for bactericidal activity (Figure 2). For a 32µg/ml target, 150mg/kg two times per day is suggested for intravenous treatment if PNA >7 days. Once stabilised and if there is a requirement to move to oral fosfomycin, doses can be selected with consideration of a neonate's WT, PMA, PNA and the likely pathogen MIC but should take account the bioavailability reported here. Studies are needed to further assess the safety profile and efficacy of this higher dose recommended by our PK model.

Current guidance on neonatal parenteral fluid and electrolyte intake suggests limiting sodium supplementation to 2-3mmol/kg/day with PNA >3 days, with

preterm neonates requiring up to 5mmol/kg/day.[41] The studied fosfomycin intravenous formulation, at 100mg/kg/dose two times per day, provides 2.8 mmol/kg/day sodium. SOC-F neonates achieved median sodium levels <140mmol/L with only one neonate exceeding 145mmol/L (149mmol/L). Sodium intake using this fosfomycin formulation at 150mg/kg two times per day is calculated at 4.2mmol/kg/day. Thus, higher doses as per revised European Medicines Agency recommendations[42] will require monitoring electrolytes to confirm safety. In addition, studies are needed in neonates with shock or renal failure who need close monitoring of electrolytes and fluid balance and will likely require dose adjustment.

Since resistance suppression could only be achieved for the most sensitive of organisms, and fosfomycin-inactivating enzymes may exist in transferrable plasmids,[43] fosfomycin is recommended to be used in combination with another antibiotic. The potential utility of fosfomycin plus amikacin for neonatal sepsis was recently studied by assessing *in vitro* activity and pharmacodynamic interactions using checkerboard assays and a 16-arm dose-ranged hollow-fibre infection model.[44] This combination had enhanced bactericidal activity, prevented the emergence of resistance, and achieved sterility with lower combination exposures, compared to monotherapy with either antibiotic. This study concluded that fosfomycin plus amikacin combination is suitable for further clinical assessment. Simulation-based PK/pharmacodynamic (PD) assessments of ampicillin and gentamicin on 373 residual samples collected from 59 SOC-F participants suggested good Gram-positive cover (MIC ≤0.25 mg/L) but poor coverage against Enterobacterales (MIC ≤2 mg/L), underscoring the need for alternative antibiotic combinations in settings with high resistant rates. Although analysis of fosfomycin interaction with ampicillin, gentamicin or ceftriaxone was not done in this study, previous studies have shown that it has synergistic activity with β-lactams, aminoglycosides, and cephalosporins.[45]

Trials evaluating fosfomycin combinations in neonatal sepsis are urgently needed[46] and our data provide the basis on which to evaluate efficacy within a combination in multiple settings compared to current SOC, either empirically or to treat microbiologically confirmed MDR infections. We are planning a multisite randomised clinical trial to assess novel antimicrobial combinations (including fosfomycin) for optimal treatment of sepsis in settings with high AMR rates and variable SOC antimicrobial choices.[47] This trial will be preceded by a

run-in confirmatory PK study of fosfomycin at the higher dose identified in the current study and will generate further data on fosfomycin safety in a large population of neonates at moderate to high risk of mortality across different LMIC settings. Robust evidence of sepsis epidemiology and management in infants aged <60 days from a recently concluded observational study (NCT03721302) is contributing towards the design of this trial.

Limitations include single-centre recruitment and exclusion of the sickest neonates at enrolment, which was judged important given the very limited prior information. Our narrow eligibility criteria excluded neonates at highest risk of poor outcomes, including very preterm neonates or those critically ill or with conditions likely to cause hypernatraemia such as severe hypoxic ischaemic encephalopathy. Future trials need to include these vulnerable groups that may benefit most from optimal antibiotic treatment.

Our sample size was not intended to determine antimicrobial efficacy or comprehensively establish safety. Enrolment rate increased (42 enrolled/519 screened versus 79/505) after extension of recruitment window from 4 to 24 hours, based on guidelines on clinical evaluation of antimicrobial agents for AMR.[48] We believe that this did not impact our results. Our study highlights challenges faced by researchers conducting early phase clinical trials in resource-limited settings including difficulties in obtaining informed consent from parents/guardians of vulnerable neonates. We implemented strategies to optimise consent such as ensuring that key decision makers within each family were involved during the process. The small CSF dataset provides evidence of appreciable concentrations in CSF; however further data is required for firm dosing recommendations for meningitis.

Strengths of our trial include a low loss to follow-up, standardised observational data, a high ascertainment of PK samples, and robust timing and dosing information – a logistically challenging exercise in neonates in any setting.[49] Total observation days for neonates in both treatment arms were similar and sufficient number of neonates with available day 2 plasma sodium samples and complete sets of 4 PK samples contributed to this analysis, despite unbalanced losses due to consent withdrawals, loss to follow-up or deaths.

Increasing AMR in a population who may die rapidly due to inadequate antimicrobial coverage is concerning given limited new antibiotics in the

pipeline. Fosfomycin offers significant potential as part of a safe, easily administered and affordable regimen.

What is already known on this topic

Antimicrobial resistance poses a threat to neonatal survival and there is an urgent need for affordable new treatment options.

Intravenous fosfomycin presents a significant sodium load and oral fosfomycin preparations contain a large amount of fructose, but limited safety data exists in neonates.

Paediatric and neonatal dosing recommendations for intravenous fosfomycin are divergent and there are no published oral dosing regimens.

What this study adds

Intravenous and oral fosfomycin had no evidence of impact on serum sodium or gastrointestinal side-effects at 100mg/kg two times per day respectively.

Intravenous fosfomycin 150mg/kg two times per day is likely required for pharmacodynamic target attainment in most children, reduced to 100mg/kg two times per day in neonates aged <7 days or weighing <1500g.

Fosfomycin has potential for affordable treatment of neonatal sepsis in combination with other antimicrobials whilst sparing carbapenems in the context of increasing antimicrobial resistance.

Supplementary Data

Supplementary materials are available at ADC Fetal & Neonatal Edition online (https://adc.bmj.com/content/archdischild/early/2022/01/24/archdischild-2021-322483/DC3/embed/inline-supplementary-material-3.pdf?download=true). Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Data sharing

Trial datasets are deposited at https://dataverse.harvard.edu/dataverse/kwtrp and are available on request through the KEMRI/Wellcome Trust Research Programme Data Governance Committee dgc@kemri-wellcome.org

Notes

Contributors

CWO, PW, ASW, KK, JFS, SE, MS and JAB contributed to the study design. CWO, PW, SM, JT, BN, EC, SE and JAB contributed to the planning, conduct and reporting of the trial. SM developed the database. CWO, SM, RO, TE and JAB had full access to all the data in the study, performed the clinical data analysis, and take responsibility for the integrity of the data and the accuracy of the data analysis. KK undertook analysis of PK samples. ZK, SG and JFS analysed the PK data. CWO, PW and JAB prepared the draft manuscript, and all authors contributed to and reviewed the manuscript. JAB provided oversight of the trial.

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Potential conflicts of interest

No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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REFERENCES

1. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: progress, priorities, and potential beyond survival. *Lancet* 2014;384(9938):189-205. doi: 10.1016/S0140-6736(14)60496-7 [published Online First: 2014/05/24]
2. Laxminarayan R, Matsoso P, Pant S, et al. Access to effective antimicrobials: a worldwide challenge. *Lancet* 2016;387(10014):168-75. doi: 10.1016/S0140-6736(15)00474-2 [published Online First: 2015/11/26]
3. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed: World Health Organization, 2013:412.
4. Centers for Disease C, Prevention. Vital signs: carbapenem-resistant Enterobacteriaceae. *MMWR Morb Mortal Wkly Rep* 2013;62(9):165-70. [published Online First: 2013/03/08]
5. Downie L, Armiento R, Subhi R, et al. Community-acquired neonatal and infant sepsis in developing countries: efficacy of WHO's currently recommended antibiotics--systematic review and meta-analysis. *Archives of disease in childhood* 2013;98(2):146-54. doi: 10.1136/archdischild-2012-302033 [published Online First: 2012/11/13]
6. Moxon CA, Paulus S. Beta-lactamases in Enterobacteriaceae infections in children. *J Infect* 2016;72 Suppl:S41-9. doi: 10.1016/j.jinf.2016.04.021 [published Online First: 2016/05/18]
7. Folgori L, Ellis SJ, Bielicki JA, et al. Tackling antimicrobial resistance in neonatal sepsis. *The Lancet Global health* 2017;5(11):e1066-e68. doi: 10.1016/S2214-109X(17)30362-5 [published Online First: 2017/10/14]
8. The World Health Organization. Critically important antimicrobials for human medicine. 3rd Revis. 2011 2016 [Available from: https://apps.who.int/iris/bitstream/10665/255027/1/9789241512220-eng.pdf accessed 19th November 2020.
9. Kahan FM, Kahan JS, Cassidy PJ, et al. The mechanism of action of fosfomycin (phosphonomycin). *Ann N Y Acad Sci* 1974;235(0):364-86. doi: 10.1111/j.1749-6632.1974.tb43277.x [published Online First: 1974/05/10]
10. Hendlin D, Stapley EO, Jackson M, et al. Phosphonomycin, a new antibiotic produced by strains of streptomyces. *Science* 1969;166(3901):122-3. doi: 10.1126/science.166.3901.122
11. Souli M, Galani I, Boukovalas S, et al. In vitro interactions of antimicrobial combinations with fosfomycin against KPC-2-producing Klebsiella pneumoniae and protection of resistance development. *Antimicrob Agents Chemother* 2011;55(5):2395-7. doi: 10.1128/AAC.01086-10 [published Online First: 2011/02/16]
12. Falagas ME, Kastoris AC, Kapaskelis AM, et al. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. *The Lancet Infectious diseases* 2010;10(1):43-50. doi: 10.1016/S1473-3099(09)70325-1 [published Online First: 2010/02/05]
13. Raz R. Fosfomycin: an old--new antibiotic. *Clin Microbiol Infect* 2012;18(1):4-7. doi: 10.1111/j.1469-0691.2011.03636.x [published Online First: 2011/09/15]
14. Guggenbichler JP, Kienel G, Frisch H. [Fosfomycin, a new antibiotic drug (author's transl)]. *Padiatr Padol* 1978;13(4):429-36. [published Online First: 1978/01/01]
15. Molina MA, Olay T, Quero J. Pharmacodynamic data on fosfomycin in underweight infants during the neonatal period. *Chemotherapy* 1977;23 Suppl 1:217-22. doi: 10.1159/000222051 [published Online First: 1977/01/01]

16. Bergan T. Degree of absorption, pharmacokinetics of fosfomycin trometamol and duration of urinary antibacterial activity. *Infection* 1990;18 Suppl 2:S65-9. doi: 10.1007/BF01643430 [published Online First: 1990/01/01]
17. Bergan T, Thorsteinsson SB, Albini E. Pharmacokinetic profile of fosfomycin trometamol. *Chemotherapy* 1993;39(5):297-301. doi: 10.1159/000239140 [published Online First: 1993/09/01]
18. Lepak AJ, Zhao M, VanScoy B, et al. In Vivo Pharmacokinetics and Pharmacodynamics of ZTI-01 (Fosfomycin for Injection) in the Neutropenic Murine Thigh Infection Model against Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2017;61(6) doi: 10.1128/AAC.00476-17 [published Online First: 2017/04/12]
19. Docobo-Perez F, Drusano GL, Johnson A, et al. Pharmacodynamics of fosfomycin: insights into clinical use for antimicrobial resistance. *Antimicrob Agents Chemother* 2015;59(9):5602-10. doi: 10.1128/AAC.00752-15 [published Online First: 2015/07/01]
20. Taylor CG, Mascaros E, Roman J, et al. Enteropathogenic E. coli gastroenterocolitis in neonates treated with fosfomycin. *Chemotherapy* 1977;23 Suppl 1:310-4. doi: 10.1159/000222068 [published Online First: 1977/01/01]
21. Rossignol S, Regnier C. [Fosfomycin in severe infection in neonatology]. *Ann Pediatr (Paris)* 1984;31(5):437-44. [published Online First: 1984/05/01]
22. Guillois B, Guillemin MG, Thoma M, et al. [Neonatal pleuropulmonary staphylococcal infection with multiple abscesses of the liver]. *Ann Pediatr (Paris)* 1989;36(10):681-4. [published Online First: 1989/12/01]
23. Gouyon JB, Francois C, Semama D, et al. [Nosocomial Staphylococcus epidermidis and Staphylococcus aureus septicemias in neonates]. *Ann Pediatr (Paris)* 1990;37(1):21-5. [published Online First: 1990/01/01]
24. Algubaisi S, Buhrer C, Thomale UW, et al. Favorable outcome in cerebral abscesses caused by Citrobacter koseri in a newborn infant. *IDCases* 2015;2(1):22-4. doi: 10.1016/j.idcr.2014.11.004 [published Online First: 2016/01/23]
25. Iarikov D, Wassel R, Farley J, et al. Adverse Events Associated with Fosfomycin Use: Review of the Literature and Analyses of the FDA Adverse Event Reporting System Database. *Infect Dis Ther* 2015;4(4):433-58. doi: 10.1007/s40121-015-0092-8 [published Online First: 2015/10/07]
26. Nash PL. Potassum and sodium homeostasis in the neonate. *Neonatal Netw* 2007;26(2):125-8. doi: 10.1891/0730-0832.26.2.125 [published Online First: 2007/04/04]
27. Akcam M. Oral fructose solution as an analgesic in the newborn: a randomized, placebo-controlled and masked study. *Pediatr Int* 2004;46(4):459-62. doi: 10.1111/j.1442-200x.2004.01932.x [published Online First: 2004/08/18]
28. Bueno M, Yamada J, Harrison D, et al. A systematic review and meta-analyses of nonsucrose sweet solutions for pain relief in neonates. *Pain Res Manag* 2013;18(3):153-61. doi: 10.1155/2013/956549 [published Online First: 2013/06/12]
29. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
30. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33(10):696-705. doi: 10.1001/archneur.1976.00500100030012 [published Online First: 1976/10/01]
31. Kane Z, Gastine S, Obiero C, et al. IV and oral fosfomycin pharmacokinetics in neonates with suspected clinical sepsis. *The Journal of antimicrobial chemotherapy* 2021;76(7):1855-64. doi: 10.1093/jac/dkab083 [published Online First: 2021/04/16]

32. Rhodin MM, Anderson BJ, Peters AM, et al. Human renal function maturation: a quantitative description using weight and postmenstrual age. *Pediatric nephrology (Berlin, Germany)* 2009;24(1):67-76. doi: 10.1007/s00467-008-0997-5 [published Online First: 2008/10/11]
33. Obiero CW, Mturi N, Mwarumba S, et al. Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital. *Archives of disease in childhood* 2021;106(2):130-36. doi: 10.1136/archdischild-2020-318913 [published Online First: 2020/08/21]
34. Dandona R, Kumar GA, Bhattacharya D, et al. Distinct mortality patterns at 0-2 days versus the remaining neonatal period: results from population-based assessment in the Indian state of Bihar. *BMC medicine* 2019;17(1):140. doi: 10.1186/s12916-019-1372-z [published Online First: 2019/07/20]
35. Irimu G, Aluvaala J, Malla L, et al. Neonatal mortality in Kenyan hospitals: a multisite, retrospective, cohort study. *BMJ Glob Health* 2021;6(5) doi: 10.1136/bmjgh-2020-004475 [published Online First: 2021/06/02]
36. Investigators of the Delhi Neonatal Infection Study c. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. *The Lancet Global health* 2016;4(10):e752-60. doi: 10.1016/S2214-109X(16)30148-6 [published Online First: 2016/09/17]
37. 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. *The Lancet Infectious diseases* 2019;19(11):1219-34. doi: 10.1016/S1473-3099(19)30414-1 [published Online First: 2019/09/17]
38. Blomberg B, Jureen R, Manji KP, et al. High rate of fatal cases of pediatric septicemia caused by gram-negative bacteria with extended-spectrum beta-lactamases in Dar es Salaam, Tanzania. *Journal of clinical microbiology* 2005;43(2):745-9. doi: 10.1128/JCM.43.2.745-749.2005 [published Online First: 2005/02/08]
39. Mohsen L, Ramy N, Saied D, et al. Emerging antimicrobial resistance in early and late-onset neonatal sepsis. *Antimicrobial resistance and infection control* 2017;6:63. doi: 10.1186/s13756-017-0225-9 [published Online First: 2017/06/21]
40. Germovsek E, Kent A, Metsvaht T, et al. Development and Evaluation of a Gentamicin Pharmacokinetic Model That Facilitates Opportunistic Gentamicin Therapeutic Drug Monitoring in Neonates and Infants. *Antimicrob Agents Chemother* 2016;60(8):4869-77. doi: 10.1128/AAC.00577-16 [published Online First: 2016/06/09]
41. Jochum F, Moltu SJ, Senterre T, et al. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Fluid and electrolytes. *Clin Nutr* 2018;37(6 Pt B):2344-53. doi: 10.1016/j.clnu.2018.06.948 [published Online First: 2018/08/02]
42. European Medicines Agency. Product Information as approved by the CHMP on 26 March 2020, pending endorsement by the European Commission 2020 [Available from: https://www.ema.europa.eu/en/documents/referral/fosfomycin-article-31-referral-annex-iii_en.pdf accessed 18th June 2020.
43. Wang H, Min C, Li J, et al. Characterization of fosfomycin resistance and molecular epidemiology among carbapenem-resistant Klebsiella pneumoniae strains from two tertiary hospitals in China. *BMC Microbiol* 2021;21(1):109. doi: 10.1186/s12866-021-02165-7 [published Online First: 2021/04/12]
44. Darlow CA, Docobo-Perez F, Farrington N, et al. Amikacin Combined with Fosfomycin for Treatment of Neonatal Sepsis in the Setting of Highly Prevalent Antimicrobial

Resistance. *Antimicrob Agents Chemother* 2021;65(7):e0029321. doi: 10.1128/AAC.00293-21 [published Online First: 2021/05/12]
45. Kastoris AC, Rafailidis PI, Vouloumanou EK, et al. Synergy of fosfomycin with other antibiotics for Gram-positive and Gram-negative bacteria. *Eur J Clin Pharmacol* 2010;66(4):359-68. doi: 10.1007/s00228-010-0794-5 [published Online First: 2010/02/27]
46. Li G, Standing JF, Bielicki J, et al. The Potential Role of Fosfomycin in Neonatal Sepsis Caused by Multidrug-Resistant Bacteria. *Drugs* 2017;77(9):941-50. doi: 10.1007/s40265-017-0745-x [published Online First: 2017/05/01]
47. Li G, Bielicki JA, Ahmed A, et al. Towards understanding global patterns of antimicrobial use and resistance in neonatal sepsis: insights from the NeoAMR network. *Archives of disease in childhood* 2020;105(1):26-31. doi: 10.1136/archdischild-2019-316816 [published Online First: 2019/08/26]
48. Japanese Pharmaceuticals and Medical Devices Agency. Report on Clinical Evaluation of Antimicrobial Agents for AMR; 2019. https://www.pmda.go.jp/files/000233507.pdf Accessed 18th June 2020.
49. O'Hara K, Martin JH, Schneider JJ. Barriers and Challenges in Performing Pharmacokinetic Studies to Inform Dosing in the Neonatal Population. *Pharmacy (Basel)* 2020;8(1):16. doi: 10.3390/pharmacy8010016 [published Online First: 2020/02/09]

Discussion

Chapter 8

Summary and general discussion

Significant progress to reduce child mortality using effective interventions such as vaccines, therapeutics, improved nutrition, and sanitation has been achieved over the last three decades despite regional disparities.[1] However, reduction of mortality in neonates has been slower compared to older children and evidence-based strategies are urgently needed to optimize care and outcomes[2] among vulnerable children in sub-Saharan Africa where disease burden is highest and healthcare resources are scarce.[3-5] Compared to most public hospitals in Kenya, children hospitalised at KCH receive better quality of healthcare because of reliable clinical and diagnostic support from the KWTRP. I leveraged the KWTRP clinical and surveillance platform to address challenges faced by clinicians taking care of young children in public hospitals where diagnostic capacity and treatment options are limited. In this thesis, different aspects of the aetiology, diagnosis and treatment of sepsis and meningitis among neonates and young children have been explored. I investigated pathogens causing EONS, determined the performance of clinical and laboratory criteria in predicting sepsis, meningitis and mortality in young children, and examined the safety and PK profile of fosfomycin for potential future use in treatment of neonatal sepsis. **Chapter 1** provides a general introduction to this thesis while **Chapter 2** describes our research setting. Subsequent chapters describe the studies done to answer specific research questions.

Part I: A molecular assay to help define the causes of early onset neonatal sepsis (EONS)

In **Chapter 3**, I explored the use of a customised multiplex TAC RT-qPCR platform to identify organisms associated with EONS. I reported a novel approach using cord blood at birth to detect organisms associated with admission with sepsis within the first 48 hours of life since microbial causes of EONS are often already acquired in utero.[6] Sampling of cases and suitable controls under similar circumstances was important to allow for group comparisons and interpretation of results. The importance of obtaining parallel samples from suitable controls has been demonstrated in previous studies investigating the aetiology of infection especially since background DNAemia in healthy controls is common.[7] Examples of infection aetiology studies that faced methodological challenges in case definition and selection of controls, and detected multiple organisms in both groups include: ANISA (randomly selected healthy community controls lacking WHO-defined pSBI; case definition was similar to our analysis except for exclusion of tachypnoea due to low specificity; results found that controls were

older than cases, and cumulative enrolment of controls did not always track pSBI episodes),[7] SANISA (hospitalised controls lacking pSBI based on predefined clinical and laboratory criteria),[8] and Pneumonia Aetiology Research for Child Health (PERCH) (randomly selected age-group matched community controls with/without respiratory symptoms but not meeting WHO-defined severe or very severe pneumonia).[9] As seen in these studies and our analysis, selection of appropriate comparator groups in infection aetiology studies is vital for causal attribution informing treatment and prevention strategies. Case definition may have contributed to inclusion of neonates lacking true sepsis in ANISA, SANISA and our study, hence development of a consensus definition of neonatal sepsis is imperative to standardise study design and harmonise results across different settings.[10]

In addition to the above methodological challenges, analysis of results is often complex when multiple organisms and/or sample types are tested. ANISA,[7] SANISA,[8] and PERCH,[9] estimated probabilities of aetiology distribution using Bayesian latent class analysis that integrated case and control data across multiple specimens and diagnostic tests, and accounted for pathogens not tested for by the methods used and differences in test sensitivity and specificity. However, despite use of refined analytical methods, a large proportion of disease remained unattributed in these studies, and it was not possible to distinguish primary pathogens responsible for infection in children who tested positive for multiple pathogens. Our study design faced similar analytical challenges as it tested for multiple organisms in cord blood samples. Attribution of organisms to infection or death is complex and sample positivity may be influenced by several factors such as specimen contamination,[8 11] true subclinical detection of circulating non-viable genetic material or low copies of organisms insufficient to cause disease, or assay specificity performance. We explored different analytical methods to investigate association of organisms with pSBI and death, and case attribution to organisms tested. Ordinary logistic regression models were not helpful in comparing cases and controls for those organisms that tested negative among the controls (models did not yield odds ratios). To compare controls, pSBI cases who survived and pSBI cases who died, we initially used Bayesian ordered logistic regression. However, this required knowledge of informative prior distribution of organisms in our population and assumptions made may bias results. Frequentist analysis using ordered logistic regression models was used in the final analysis to determine cumulative odds

of pSBI and death, and eventually obtain attributable fractions of different pathogens.

Our results demonstrate the high burden of early neonatal morbidity and mortality in LMICs,[12 13] underscoring the importance of prompt diagnosis and treatment that includes specialised neonatal care. Seriously ill neonates are admitted to the HDU at our study hospital and receive close monitoring and appropriate management. However, capacity for intensive care of those neonates who develop acute organ dysfunction due to sepsis is limited. We included neonates born at KCH who had available cord blood samples and restricted our study population to KHDSS residents in order to establish outcomes among healthy neonates (control group). Our results may not be generalizable to outborns who are also vulnerable to poor health outcomes and may die soon after birth in the community.[14 15]

Our study is the first to investigate the diagnostic utility of cord blood in detecting pathogens causing EONS in sub-Saharan Africa, including organisms associated with early mortality such as GBS.[16] Studies done in Ethiopia,[17] Nigeria,[18 19] and Mali[20] assessed the reference ranges of cord blood haematological parameters among healthy term neonates but did not explore its use in detecting causes of sepsis among hospitalised neonates. Our retrospective study relied on available archived cord blood samples obtained using aseptic techniques soon after delivery. *K. oxytoca* and *P. aeruginosa* were common in cord blood samples obtained from pSBI cases and healthy controls, regarded as contaminants, and not associated with pSBI and death. Our results were influenced by environmental sample contamination, and potential residual confounding by maternal, neonatal or pathogen-specific factors associated with infection but not adequately assessed in our retrospective design. Future prospective studies testing paired cord blood and peripheral venous blood samples using advanced diagnostics such as pathogen-specific biomarkers and metagenomic approaches may be more informative with minimal sample contamination.

Although TAC RT-qPCR allows for detection of multiple pathogens at the same time, the panels are customised to include specific targets *a priori*[21] and this may be a potential source of bias. The choice of the 21 targets included in our TAC was based on the range of organisms tested in previous studies investigating the causes of neonatal sepsis.[7 8] Using TAC RT-qPCR, we identified important pathogens associated with pSBI and death. However, it is possible

that we missed organisms not prioritised by our TAC panel. Expansion of TAC panels and/or use of advanced molecular diagnostics in future studies will allow interrogation of other pathogens of interest. In addition, we detected organisms not currently recognised as significant pathogens in our setting i.e., *Ureaplasma* spp. and enterovirus. *Ureaplasma* spp. is associated with adverse pregnancy outcomes in premature neonates e.g. bronchopulmonary dysplasia, intraventricular haemorrhage and necrotizing enterocolitis.[22] Research on the use of macrolides for the eradication of *Ureaplasma* spp. respiratory tract colonization in preterms[23] and reduction of the risk of bronchopulmonary dysplasia[24-26] and death[26] has been inconclusive. The WHO guidelines do not include antibiotic options targeting treatment of *Ureaplasma* spp. infection in at-risk neonates and resistance of *Ureaplasma* serovars to macrolides and other antibiotics is increasing.[27] The clinical significance of *Ureaplasma* spp. detection in our setting needs further investigation alongside research on non-culture dependent diagnostics and treatment of sick neonates keeping in mind limited antimicrobial options.[28] Viral targets in our TAC panel included enterovirus and CMV which have been associated with severe sepsis-like illness[29] [30] and neurodevelopmental impairment[31] in studies done in other settings. Six pSBI cases (of which half died) and no healthy controls tested positive for enteroviruses while CMV was the commonest virus detected in both cases and controls. Lack of peripartum screening and virologic diagnostic methods, and costly treatment options such as antivirals (e.g., pleconaril) and intravenous immunoglobulin[29] limits knowledge of the true burden of neonatal viral infection in sub-Saharan Africa.

Part II: Which clinical features predict serious bacterial infection (SBI [sepsis and/or meningitis]) and mortality in newborns and young children following changes in disease epidemiology and patient profile?

Diagnosis of SBI and initiation of empiric antibiotics in resource-limited settings depends on recognition of a set of signs and symptoms at admission. **Chapters 4** and **5** describe two studies that investigated the performance of previously identified and WHO-recommended clinical signs and symptoms in distinguishing children with meningitis from children without meningitis given changes in disease epidemiology and patient profile following widespread conjugate vaccination. The analysis done in **Chapter 4** included young infants aged <60 days old while **Chapter 5** included children aged between 60 days and 13 years. I approached these analyses separately, as done in the previous analysis at KCH, because of variable meningitis aetiology, clinical presentation,

management, and risk of poor outcomes.[32 33] We reported a lower prevalence of meningitis among young infants (**Chapter 4**) and older children (**Chapter 5**) than done in previous studies at our centre.[34 35] This demonstrates the effectiveness of conjugate vaccines on meningitis morbidity, including the impact of herd immunity on invasive disease in older children.[36] Despite a declining prevalence, we observed a high meningitis case fatality showing that the risk of poor outcomes is still high as seen in other similar settings.[37] We may have underestimated meningitis cases occurring in early deaths prior to an LP. However, inclusion/exclusion of these children in the analysis did not alter our results. Post-mortem LP has been shown to be useful in identifying meningitis cases among seriously ill children who die soon after admission but is not often possible due to challenges in obtaining consent from parents/guardians.[38]

Although, several scoring algorithms for predicting meningitis in young children exist, applicability in our setting is limited by differences in patient population (e.g. children presenting with meningeal signs only,[39 40] age 1 month-18 years,[40 41] hospitalised or reviewed at the outpatient department,[40 41] or children with CSF pleocytosis only[41]), bacterial meningitis case definitions, and use of tests such as CRP which are not routinely available in our setting.[40] Variable meningitis case definitions using different cut-offs for CSF WBC, CSF protein and CSF:blood glucose ratio (Addendum 1) have been used to classify children as having either definitive, probable, or possible meningitis, limiting generalisability of results across different settings. For our analyses, meningitis was defined based on CSF results as done in the previous studies conducted at KCH.[34 35] Most public hospitals do not do LPs for CSF analysis due to lack of laboratory resources and qualified staff. Gross inspection and basic CSF analysis (e.g. microscopy [Gram staining and cell count] and glucose measurement) have been shown to aid detection of children with meningitis.[42] Capacity for basic CSF analysis needs to be prioritised in public hospitals, and adherence to clinical guidelines emphasized for better treatment outcomes.

Our results show that although meningitis has become less common than previously reported, it is now more difficult to identify as signs and symptoms are less typical than previously found.[34 35] Prehospital antibiotic exposure (data not available) and missed LPs in early deaths or children who had LPs deferred at admission but not done later due to lack of consent or early clinical resolution of symptoms may have contributed to missing or misclassifying meningitis cases. However, we believe that this was minimised by the level of care provided by trained clinicians at KCH that included regular reviews,

paediatrician oversight, and reliable diagnostic support. Based on the results of these two studies, clinicians should have a high index of suspicion and a low threshold for performing an LP and starting treatment in children presenting with the signs we investigated. Training and supervision of clinicians on recognition of clinical signs and symptoms is crucial. We do not recommend a change in the current WHO[33] and Kenya paediatric guidelines[43] as they are still useful in identifying children with meningitis. Changes in the meningitis epidemiology secondary to non-vaccine serotype replacement,[44] increasing AMR,[45] and underlying co-morbidities may contribute to atypical clinical presentation in children in the coming years. Continued robust surveillance of meningitis in sub-Saharan Africa is therefore of paramount importance. Viral testing is not done routinely at our centre, and this is the case in public hospitals in sub-Saharan Africa. Future research assessing the performance of clinical criteria in predicting meningitis confirmed using advanced molecular diagnostics for testing bacterial and viral pathogens in CSF is warranted.

In **Chapter 6**, I validated a novel score predicting mortality in young infants (NeoSep severity score [NSS]) and assessed the performance of a set of clinical and laboratory signs (NeoObs study inclusion criteria [NSIC]) in distinguishing young infants with SBI from those without SBI. We first determined infants with SBI based on the blood and CSF culture results. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was installed in Kilifi in January 2018 and implemented in February 2018 to support conventional cultures in rapid identification of clinical isolates of microorganisms.[46] This has resulted in identification of rare organisms whose clinical significance in children in our setting is not clear. Addendum 2 shows a list of culture isolates of unclear significance. I conducted extensive literature review to determine the pathogenicity of these organisms in neonates. *Paenibacillus thiaminolyticus*, a known environmental bacterium and rarely identified in our setting was detected in two blood culture samples, of which one neonate had the same bacteria in CSF. This organism was considered likely pathogenic based on cases of neonatal sepsis and meningitis in other settings (Addendum 2), and isolation from both blood and CSF samples at KCH. *Bacillus cereus* and *Chryseobacterium indologenes* were considered likely pathogenic based on numerous reports of neonatal bacteraemia or meningitis as shown on Addendum 2. Environmental bacteria with no case reports among infants e.g., *Arthrobacter globiformis*, or known commensals causing infection in the presence of underlying predisposing factors such as use of invasive medical

devices and procedures e.g., Coagulase-negative *Staphylococci*, were considered to be likely nonpathogenic or contaminants. Infants who had presumed pathogens of likely pathogenic organisms identified in blood or CSF samples were classified as having SBI. Our laboratory is currently updating its microbiology database as rare organism are identified, and careful review and discussions about the clinical significance of each organism is paramount as this impact antibiotic use and treatment outcomes.

KCH was one of the 19 study sites in 11 countries that participated in the NeoObs study, contributing data used to derive the NSS and NSIC. One-hundred and ninety-seven (6.1%) of 3,204 infants were enrolled at KCH based on the NSIC. Although the sites were predominantly located in LMICs (mostly in Africa and Asia), they had variable infant characteristics, resource availability, and empiric antibiotic use. Of note, KCH provides medical services to a small, mostly rural demographic, has a lower bed-capacity than the other sites, and does not have intensive care facilities found in some of the study hospitals e.g., use of central venous catheters and invasive ventilation. It was therefore important to assess the performance of these criteria in our setting given the heterogenous nature of the study sites involved. In addition, existing criteria for SBI developed and validated in high-income countries (Addendum 1) have limited applicability in our setting. Our analysis found that NSIC discriminated SBI among infants and can support clinicians to identify infants needing empiric antibiotics in the absence of confirmatory cultures. Although these criteria include laboratory parameters, they can be used in the absence of laboratory support since a minimum of two clinical signs is sufficient to raise suspicion of SBI. The question regarding continuation of antibiotics in infants who experience clinical resolution of symptoms at 48-72 hours remains. NSIC were assessed at admission and applicability of these criteria among hospitalised infants who develop clinical deterioration is not known. The same applies to utility of the NSS in predicting mortality in young infants during hospitalisation. Our analysis focused on inpatient deaths (17%) which differed from the 28-day mortality (11%) reported in the NeoObs study. Despite this difference, we found that NSS was a useful prognostic tool for mortality risk in young infants, may guide prioritization of resources and antibiotic use, and can inform selection of high-risk infants for clinical trials. Admission weight served as a reliable proxy measure of birth weight and can be used in similar settings where most young infant admissions lack documented birth weight due to various reasons. Infants presenting with an NSS ≥4 in our setting would benefit from prompt initiation

of antibiotics, supportive treatment and close monitoring. Further assessment of the NSS and NSIC in clinical practice, external validation in other LMICs, and improved healthcare capacity and delivery are warranted.

Part III: Is fosfomycin a safe and potentially useful new antimicrobial treatment for neonatal sepsis in the context of antimicrobial resistance (AMR)?

The final part of this thesis presents the results of a clinical trial investigating the safety and PK of fosfomycin in neonates hospitalised with clinical sepsis (**Chapter 7**). Fosfomycin was administered for the first time in our setting alongside empiric antibiotics prescribed according to WHO[33] and Kenya national paediatric guidelines.[43] Similar to observations made in the other studies involving neonates and young infants, majority of the clinical trial participants were hospitalised during the first week of life, highlighting the high burden of early neonatal morbidity reported in other similar settings.[47] Randomisation of neonates into one of the treatment arms effectively minimised the risk of bias as the baseline clinical and laboratory parameters were similar between the two arms upon study conclusion. Key safety results showed that administration of parenteral fosfomycin had no impact on sodium levels, while no gastrointestinal side effects were observed following oral fosfomycin administration. PK modelling done provided dosing recommendations for use among neonates hospitalised with sepsis based on postnatal age and body weight.

This was the first interventional study involving neonates conducted at our site. As expected, we faced several challenges while implementing the study in Kilifi, most of which were unique to our target population. Clinical trials investigating interventions such as drugs in neonates are crucial since data obtained from older children and adults is not sufficient to assure safety and efficacy in this vulnerable age group. Early postnatal life is characterised by structural and physiological changes (e.g., initiation of pulmonary ventilation and oxygenation, and changes in circulatory pathways and metabolism) necessary for adaptation to extrauterine life.[48] Neonatal physiology is distinct from that seen in older children and adults, and influences clinical response following drug administration.[49] In addition to the biochemical characteristics and route of drug administration, drug disposition and pharmacodynamics in neonates is affected by age-related maturational differences and changes in organ function resulting from illnesses such as sepsis and birth asphyxia.[49 50] Extrapolation of data from older age groups to neonates is often unreliable and unsafe, hence

quality drug trials are crucial to guide use of effective treatments among neonates of different postnatal age and weight. Obstacles faced in neonatal drug trials include additional regulatory requirements, limited/lack of data from early clinical trials in neonates, and ethical issues in the design of randomised controlled trials in vulnerable populations such as those related to clinical equipoise and choice of comparator groups.[51-53] Very few drug trials among neonates have been conducted over the past 3 decades despite rising AMR and limited availability of effective antibiotics.[54 55] In addition, most settings in sub-Saharan Africa lack capacity to conduct early-phase neonatal clinical trials.[55] Difficulties in obtaining parental informed consent was one of the key challenges we faced while conducting our PK trial in Kilifi and the main reasons for this were parental distress related to their newborns' acute illness and short time available for decision-making, unwillingness to participate in experimental research, hesitancy related to repeated PK blood sampling and inability to obtain consent from mothers who had just undergone caesarean section or were seriously ill after delivery. Several studies have been done to investigate ways in which consent and participation in neonatal trials can be optimised.[56-58] Measures used to improve the consenting process in our study included training of staff involved in the process, assessing the parents'/guardians' comprehension using a checklist, involving key family decision makers in the process, and ensuring that the consenting process was continuous from enrolment to study termination. Timing of PK sampling alongside other samples for clinical monitoring where possible reduced the frequency of sampling procedures thereby allaying parental anxiety and improving participant retention. Lessons learned during implementation of this clinical trial will be useful in assuring the success of a future antibiotic trial which is currently in the planning phase at our site.

Other challenges faced in the design of neonatal clinical trials are related to the entry criteria and selection of appropriate clinical endpoints and may affect generalizability of results across different settings.[54] As already described in the previous studies/chapters, neonatal sepsis lacks a consensus definition, making it challenging to select seriously ill neonates who would benefit most from life-saving interventions.[10] Neonates presenting with signs of sepsis were enrolled in our study based on clinical judgement. We started our study with a narrow recruitment window of 4 hours which was extended to 24 hours since the first dose of intravenous antibiotics according to guidelines on clinical evaluation of antimicrobial agents for AMR,[59] to include overnight admissions and enhance

enrolment rate. We are confident that this protocol amendment did not bias our results. Future neonatal antibiotic trials require objective, quantifiable and highly specific inclusion criteria useful in monitoring progress and outcomes.[54] The NSS derived from the NeoObs study will be used as inclusion criteria in a large multisite prospective antibiotic trial to enrol neonates at high risk of mortality. In addition, this study derived criteria to assess disease progression among neonates undergoing treatment for sepsis (not tested in this thesis but for assessment in future studies). Our sample size was small and had a narrow eligibility criterion that excluded the very sick and very small neonates to optimise PK sampling. Our study was also not adequately powered to assess efficacy and all safety parameters. A larger trial with clearly defined inclusion criteria and endpoints and including the very preterm and critically ill neonates is needed to confirm the fosfomycin PK parameters and dose regimens reported by our study and generate robust evidence of fosfomycin safety among neonates. *In vitro* studies have shown that fosfomycin combined with amikacin has enhanced bactericidal activity compared to fosfomycin alone[60] and further clinical assessment of this combination is needed since our modelling showed that resistance suppression could only be achieved for the most sensitive organisms using fosfomycin monotherapy. Our study plays a pivotal role in efforts towards developing novel effective antibiotic combinations for use in a vulnerable population facing increasing AMR with limited treatment options.

FUTURE PERSPECTIVES, CONCLUSIONS AND RECOMMENDATIONS

Reduction of sepsis and meningitis burden in sub-Saharan African children is dependent upon local and global efforts to improve diagnosis and management. The WHO is supporting Member States through advocacy, funding and technical assistance to facilitate high-quality primary research on sepsis epidemiology and strengthen laboratory capacity.[61] Other priority areas targeted by the WHO include AMR, surveillance, and use of point-of-care diagnostic and prognostic tests (e.g. biomarkers) for early recognition of sepsis. This approach aims to address critical research gaps related to sepsis including lack of a consensus definition, variable diagnostic criteria, and paucity of quality data from prospective studies conducted in high-burden countries. To combat meningitis, the WHO recently launched the “Defeating meningitis by 2030” global roadmap by which it aims to eliminate bacterial meningitis epidemics, reduce vaccine-preventable bacterial meningitis cases by 50% and deaths by 70%, and reduce disability and improve quality of life in survivors.[62] Key action pillars related to this thesis include prompt diagnosis and optimal treatment of meningitis cases,

efficient and effective disease surveillance, and achievement of high vaccination coverage. This strategy acknowledges the need for context-specific diagnosis and treatment guidelines and tools, and the threat that AMR poses on outcomes. Sustained access and availability of affordable vaccines is essential to avoid reversal of gains achieved in reduction of invasive infection following conjugate vaccination, and collaboration between GAVI, the Vaccine Alliance and countries transitioning from full support to self-financing status such as Kenya is important.[63] This thesis presents the results of research providing vital evidence of the diagnosis and treatment of sepsis and meningitis in neonates and young children at a rural Kenyan hospital. The results are generalizable to other healthcare settings in sub-Saharan Africa with similar disease burden. To support sustained efforts in reduction of child morbidity and mortality secondary to sepsis and meningitis, the work presented in this thesis may progress in several ways as explored below.

Diagnosis of sepsis and meningitis

Establishment of blood and CSF culture capacity in all public hospitals in Kenya by the Ministry of Health is crucial for diagnosis of sepsis and meningitis since culture remains the gold standard test. Support for basic CSF microscopy and glucose measurement will aid diagnosis of meningitis where cultures are unavailable. Clinicians should be encouraged to perform LPs in children presenting with signs suggestive of meningitis as gross CSF examination may raise suspicion of the likelihood of infection and guide initiation of antibiotics. Use of WHO and Kenya paediatric guidelines can be enhanced through multifaceted interventions including education, training, provision of job aides, supervision and feedback.[64] This should be coupled with periodic assessment of clinical guidelines, including revalidation of signs and symptoms discriminating sepsis and meningitis in children to ensure that these are updated promptly if needed.

Molecular tests are costly and logistically challenging for use in routine clinical care. However, use of molecular platforms within the context of research contributes data on the epidemiology of disease in populations. Expansion of TAC RT-qPCR panels may be considered in future studies to interrogate more organisms in clinical samples than we did in our study. Molecular rapid diagnostic tests such as MALDI-TOF need further exploration in our setting as they have been associated with considerable healthcare savings[65] and improved outcomes (significant reduction in risk of mortality, time to effective therapy

and duration of hospitalisation) when used alongside antimicrobial stewardship programmes among patients with bloodstream infections in high-income countries.[66] Metagenomic next-generation sequencing of blood and CSF has been shown to enhance pathogen detection and may be explored in future studies.[67-69] These platforms are also useful in identification of rare organisms of unknown pathogenicity in our setting.

Antimicrobial resistance (AMR) and antibiotic treatment of sepsis and meningitis

Surveillance of AMR is essential to monitor AMR burden and trends, identify emerging threats to current treatment regimens and inform interventions and policies. This can be done through implementation of global and regional initiatives such as the WHO Global Action Plan on AMR (2015)[70] and the Kenya National Action Plan (2017-2022)[71] which aim to control the emergence and spread of AMR by improving awareness of AMR, strengthening evidence base through surveillance and research, improving infection prevention and control practices, optimizing use of antimicrobials, and increasing investments in new interventions such as medicines, diagnostics and vaccines. This requires several interventions such as building of microbiology laboratory capacity, developing, implementing and regulating guidelines, ensuring access to quality antimicrobials, establishing antimicrobial stewardship programmes, and strengthening data collection and reporting.[70 71] The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) was launched in 2015 to standardize global AMR surveillance of epidemiological, clinical, and population-level data and inform strategies to curb AMR.[72] GLASS has a wide range of modules which include routine surveillance of clinical microbiological and antimicrobial consumption data, focused surveillance of emerging AMR and invasive fungal bloodstream infections, point prevalent surveys of antibiotic use, and estimation of the public health impact of AMR. Kenya enrolled in GLASS in May 2016. In 2020, GLASS reported high resistant rates of blood stream infection caused by *K. pneumonia* (40-50%) to third generation cephalosporins; AMR burden was highest in LMICs despite less data from these regions compared to high-income countries.[72] Kenya reported on implementation of the national surveillance system i.e. 5 participating hospitals and 5 laboratories performing antibiotic susceptibility testing with external quality assurance and an established national reference laboratory.[72] Kenya did not contribute data on AMR or antibiotic consumption suggesting that more work is needed to strengthen surveillance activities, capacity and data reporting. Current efforts

to strengthen laboratory capacity and support AMR surveillance activities in Kenya include initiatives by the East Africa Public Health Laboratory Networking Project (EAPHLNP),[73] the Fleming Fund,[74] and the United States Centres for Disease Control and Prevention (CDC) Global Disease Detection (GDD) program.[75]

De novo drug discovery and development approaches are costly and time-consuming and may not address the current urgent demand for effective antibiotics given the lack of approved new antibiotics for clinical use. Repurposing of existing and underused antibiotics bridges the gap between the drug development pipeline and post-development drug licensure. The WHO is repurposing older off-patent antimicrobials with retained spectrum of antimicrobial activity for treatment of sepsis in children. The Global Antibiotic Research and Development Partnership (GARDP), a joint initiative of the WHO and the Drugs for Neglected Diseases initiative (DNDi) in support of the Global Action Plan for AMR aims to develop an antimicrobial regimen for use in LMICs for the empiric treatment of neonatal sepsis in locations with increasing resistance to current WHO-recommended treatments.[76] [77] Fosfomycin, flomoxef and amikacin showed the greatest promise for further clinical evaluation in children with sepsis[77] based on criteria such as low cost, activity against MDR bacteria, availability of a license or extensive use in the neonatal context if no license exists, and minimal toxicity.[76] [77] These antibiotics will be investigated in new combinations in a large prospective multisite trial among hospitalised neonates with sepsis, and compared to existing combinations in order to provide a clinically relevant ranking of treatment regimens based on safety and efficacy.

Conclusion

This thesis provides data on different components of research on sepsis and meningitis in neonates and young children, addressing important gaps associated with poor outcomes. The data suggests that more studies using specific diagnostic tests for sepsis are needed since a large proportion of neonates still lacks a causal attribution. Atypical organisms and viruses e.g., *Ureaplasma* spp. and Enterovirus cause infection in neonates in sub-Saharan Africa and further research to understand their clinical significance and management is needed. Clinical algorithms consisting of signs and symptoms predicting sepsis, meningitis and mortality remain useful in guiding patient management. Periodic assessment of the performance of clinical criteria in

discriminating sepsis and meningitis is important to inform revision of management guidelines. Repurposing of legacy off-patent antibiotics shows promise as a means to address the urgent need for treatment combinations effective against resistant bacteria. Continued surveillance of the causes of sepsis and meningitis, antibiotic use and resistance is crucial for improved outcomes in vulnerable children in our region. Finally, strengthening of child health services in sub-Saharan Africa needs to be prioritised alongside development and implementation of cost-effective diagnostic approaches and therapeutic options for optimal child survival.

REFERENCES

1. United Nations Inter-agency Group for Child Mortality Estimation (UNIGME) LTiCMR, Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation', United Nations Children's Fund, New York, 2020.
2. Mangu CD, Rumisha SF, Lyimo EP, et al. Trends, patterns and cause-specific neonatal mortality in Tanzania: a hospital-based retrospective survey. *Int Health* 2021;13(4):334-43. doi: 10.1093/inthealth/ihaa070 [published Online First: 2020/09/26]
3. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet* 2020;395(10219):200-11. doi: 10.1016/S0140-6736(19)32989-7 [published Online First: 2020/01/20]
4. Zunt JR, Kassebaum NJ, Blake N, et al. Global, regional, and national burden of meningitis, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology* 2018;17(12):1061-82. doi: 10.1016/s1474-4422(18)30387-9
5. Global Health Data Exchange IHME Data. 2020 Accessed on 27 September 2021. Available online: http://ghdx.healthdata.org/gbd-results-tool.
6. Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. *Pediatr Clin North Am* 2013;60(2):367-89. doi: 10.1016/j.pcl.2012.12.003 [published Online First: 01/17]
7. Saha SK, Schrag SJ, El Arifeen S, et al. Causes and incidence of community-acquired serious infections among young children in south Asia (ANISA): an observational cohort study. *Lancet* 2018;392(10142):145-59. doi: 10.1016/S0140-6736(18)31127-9
8. Velaphi SC, Westercamp M, Moleleki M, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. *PLoS One* 2019;14(4):e0214077. doi: 10.1371/journal.pone.0214077 [published Online First: 2019/04/11]
9. 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(10200):757-79. doi: 10.1016/S0140-6736(19)30721-4 [published Online First: 2019/07/02]
10. McGovern M, Giannoni E, Kuester H, et al. Challenges in developing a consensus definition of neonatal sepsis. *Pediatric research* 2020;88(1):14-26. doi: 10.1038/s41390-020-0785-x [published Online First: 2020/03/04]
11. Underwood MA, Sohn K. The Microbiota of the Extremely Preterm Infant. *Clinics in perinatology* 2017;44(2):407-27. doi: 10.1016/j.clp.2017.01.005 [published Online First: 2017/05/10]
12. Sankar MJ, Natarajan CK, Das RR, et al. When do newborns die? A systematic review of timing of overall and cause-specific neonatal deaths in developing countries. *Journal of perinatology : official journal of the California Perinatal Association* 2016;36 Suppl 1(Suppl 1):S1-S11. doi: 10.1038/jp.2016.27 [published Online First: 2016/04/26]
13. Abdul-Mumin A, Cotache-Condor C, Owusu SA, et al. Timing and causes of neonatal mortality in Tamale Teaching Hospital, Ghana: A retrospective study. *PLoS One* 2021;16(1):e0245065. doi: 10.1371/journal.pone.0245065 [published Online First: 2021/01/14]
14. Amouzou A, Ziqi M, Carvajal-Aguirre L, et al. Skilled attendant at birth and newborn survival in Sub-Saharan Africa. *J Glob Health* 2017;7(2):020504. doi: 10.7189/jogh.07.020504 [published Online First: 2018/02/10]

15. Bitew ZW, Alemu A, Ayele EG, et al. Incidence Density Rate of Neonatal Mortality and Predictors in Sub-Saharan Africa: A Systematic Review and Meta-Analysis. *International journal of pediatrics* 2020;2020:3894026. doi: 10.1155/2020/3894026 [published Online First: 2020/10/31]
16. Seale AC, Bianchi-Jassir F, Russell NJ, et al. Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017;65(suppl_2):S200-S19. doi: 10.1093/cid/cix664 [published Online First: 2017/11/09]
17. Angelo A, Derbie G, Demtse A, et al. Umbilical cord blood hematological parameters reference interval for newborns from Addis Ababa, Ethiopia. *BMC pediatrics* 2021;21(1):275. doi: 10.1186/s12887-021-02722-z [published Online First: 2021/06/13]
18. Adewumi A, Titilope AA, Akinsegun AA, et al. Cord blood full blood count parameters in Lagos, Nigeria. *Pan Afr Med J* 2014;17:192. doi: 10.11604/pamj.2014.17.192.3680 [published Online First: 2014/11/15]
19. Adediran A, Gbadegesin A, Adeyemo TA, et al. Haemoglobin and ferritin concentrations in cord blood in a tertiary health centre in Nigeria. *Nig Q J Hosp Med* 2011;21(4):284-9. [published Online First: 2012/11/28]
20. Diallo DA, Diawara F, Guindo A, et al. [Reference values of neonatal erythrocyte and leukocyte count in Bamako, Mali]. *Mali Med* 2013;28(1):36-43. [published Online First: 2013/01/01]
21. Lin B, Diaz MH, Waller JL, et al. Optimization of Multiple Pathogen Detection Using the TaqMan Array Card: Application for a Population-Based Study of Neonatal Infection. *PLoS ONE* 2013;8(6):e66183. doi: 10.1371/journal.pone.0066183 [published Online First: 2013/06/28]
22. Viscardi RM. Ureaplasma species: role in neonatal morbidities and outcomes. *Archives of disease in childhood Fetal and neonatal edition* 2014;99(1):F87-92. doi: 10.1136/archdischild-2012-303351 [published Online First: 2013/08/21]
23. Viscardi RM, Terrin ML, Magder LS, et al. Randomised trial of azithromycin to eradicate Ureaplasma in preterm infants. *Archives of disease in childhood Fetal and neonatal edition* 2020;105(6):615-22. doi: 10.1136/archdischild-2019-318122 [published Online First: 2020/03/15]
24. Smith C, Egunsola O, Choonara I, et al. Use and safety of azithromycin in neonates: a systematic review. *BMJ open* 2015;5(12):e008194. doi: 10.1136/bmjopen-2015-008194 [published Online First: 2015/12/15]
25. Kim SH, Chun J, Ko KH, et al. Effect of antenatal azithromycin for Ureaplasma spp. on neonatal outcome at </=30 weeks' gestational age. *Pediatr Int* 2019;61(1):58-62. doi: 10.1111/ped.13738 [published Online First: 2018/11/22]
26. Nair V, Loganathan P, Soraisham AS. Azithromycin and other macrolides for prevention of bronchopulmonary dysplasia: a systematic review and meta-analysis. *Neonatology* 2014;106(4):337-47. doi: 10.1159/000363493 [published Online First: 2014/10/04]
27. Boujemaa S, Mlik B, Ben Allaya A, et al. Spread of multidrug resistance among Ureaplasma serovars, Tunisia. *Antimicrobial resistance and infection control* 2020;9(1):19. doi: 10.1186/s13756-020-0681-5 [published Online First: 2020/01/31]
28. Beeton ML, Spiller OB. Antibiotic resistance among Ureaplasma spp. isolates: cause for concern? *The Journal of antimicrobial chemotherapy* 2017;72(2):330-37. doi: 10.1093/jac/dkw425 [published Online First: 2016/11/01]
29. Chuang YY, Huang YC. Enteroviral infection in neonates. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2019;52(6):851-57. doi: 10.1016/j.jmii.2019.08.018 [published Online First: 2019/10/15]

30. Zhang M, Wang H, Tang J, et al. Clinical characteristics of severe neonatal enterovirus infection: a systematic review. *BMC pediatrics* 2021;21(1):127. doi: 10.1186/s12887-021-02599-y [published Online First: 2021/03/17]
31. Marsico C, Kimberlin DW. Congenital Cytomegalovirus infection: advances and challenges in diagnosis, prevention and treatment. *Italian journal of pediatrics* 2017;43(1):38. doi: 10.1186/s13052-017-0358-8 [published Online First: 2017/04/19]
32. Zainel A, Mitchell H, Sadarangani M. Bacterial Meningitis in Children: Neurological Complications, Associated Risk Factors, and Prevention. *Microorganisms* 2021;9(3):535. doi: 10.3390/microorganisms9030535 [published Online First: 2021/04/04]
33. WHO. Pocket book of hospital care for children: Guidelines for the management of common childhood illnesses. Second ed: World Health Organization, 2013:412.
34. Mwaniki MK, Talbert AW, Njuguna P, et al. Clinical indicators of bacterial meningitis among neonates and young infants in rural Kenya. *BMC infectious diseases* 2011;11:301. doi: 10.1186/1471-2334-11-301 [published Online First: 2011/11/03]
35. Berkley JA, Versteeg AC, Mwangi I, et al. Indicators of acute bacterial meningitis in children at a rural Kenyan district hospital. *Pediatrics* 2004;114(6):e713-9. doi: 10.1542/peds.2004-0007 [published Online First: 2004/12/03]
36. 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. *The Lancet Global health* 2018;6(7):e744-e57. doi: 10.1016/S2214-109X(18)30247-X [published Online First: 2018/06/16]
37. Nakamura T, Cohen AL, Schwartz S, et al. The Global Landscape of Pediatric Bacterial Meningitis Data Reported to the World Health Organization-Coordinated Invasive Bacterial Vaccine-Preventable Disease Surveillance Network, 2014-2019. *The Journal of infectious diseases* 2021;224(Supplement_3):S161-S73. doi: 10.1093/infdis/jiab217 [published Online First: 2021/09/02]
38. Berkley JA, Mwangi I, Mellington F, et al. Cerebral malaria versus bacterial meningitis in children with impaired consciousness. *QJM : monthly journal of the Association of Physicians* 1999;92(3):151-7. [published Online First: 1999/05/18]
39. Oostenbrink R, Moons KG, Derksen-Lubsen AG, et al. A diagnostic decision rule for management of children with meningeal signs. *Eur J Epidemiol* 2004;19(2):109-16. doi: 10.1023/b:ejep.0000017828.13995.76 [published Online First: 2004/04/13]
40. Oostenbrink R, Moons KG, Donders AR, et al. Prediction of bacterial meningitis in children with meningeal signs: reduction of lumbar punctures. *Acta paediatrica* 2001;90(6):611-7. [published Online First: 2001/07/07]
41. Nigrovic LE, Malley R, Kuppermann N. Meta-analysis of bacterial meningitis score validation studies. *Archives of disease in childhood* 2012;97(9):799-805. doi: 10.1136/archdischild-2012-301798 [published Online First: 2012/07/06]
42. Berkley JA, Mwangi I, Ngetsa CJ, et al. Diagnosis of acute bacterial meningitis in children at a district hospital in sub-Saharan Africa. *Lancet* 2001;357(9270):1753-7. doi: 10.1016/S0140-6736(00)04897-2 [published Online First: 2001/06/14]
43. MOH. Basic Paediatric Protocols for ages up to 5 years. Fourth ed, February 2016.
44. Sime WT, Aseffa A, Woldeamanuel Y, et al. Serotype and molecular diversity of nasopharyngeal Streptococcus pneumoniae isolates from children before and after vaccination with the ten-valent pneumococcal conjugate vaccine (PCV10)

in Ethiopia. *BMC infectious diseases* 2019;19(1):409. doi: 10.1186/s12879-019-4024-1 [published Online First: 2019/05/12]
45. Biset S, Benti A, Molla L, et al. Etiology of Neonatal Bacterial Meningitis and Their Antibiotic Susceptibility Pattern at the University of Gondar Comprehensive Specialized Hospital, Ethiopia: A Seven-Year Retrospective Study. *Infect Drug Resist* 2021;14:1703-11. doi: 10.2147/IDR.S307156 [published Online First: 2021/05/20]
46. Dingle TC, Butler-Wu SM. Maldi-tof mass spectrometry for microorganism identification. *Clin Lab Med* 2013;33(3):589-609. doi: 10.1016/j.cll.2013.03.001 [published Online First: 2013/08/13]
47. Irimu G, Aluvaala J, Malla L, et al. Neonatal mortality in Kenyan hospitals: a multisite, retrospective, cohort study. *BMJ Glob Health* 2021;6(5) doi: 10.1136/bmjgh-2020-004475 [published Online First: 2021/06/02]
48. Morton SU, Brodsky D. Fetal Physiology and the Transition to Extrauterine Life. *Clinics in perinatology* 2016;43(3):395-407. doi: 10.1016/j.clp.2016.04.001 [published Online First: 2016/08/16]
49. Johnson PJ. Neonatal pharmacology--pharmacokinetics. *Neonatal Netw* 2011;30(1):54-61. doi: 10.1891/0730-0832.30.1.54 [published Online First: 2011/02/15]
50. Noya FJD. Antibiotic usage in neonates. *Seminars in pediatric infectious diseases* 1998;9(4):322-29. doi: 10.1016/s1045-1870(98)80022-8
51. Djulbegovic B. The paradox of equipoise: the principle that drives and limits therapeutic discoveries in clinical research. *Cancer Control* 2009;16(4):342-47.
52. Wiles JR, Vinks AA, Akinbi H. Federal legislation and the advancement of neonatal drug studies. *The Journal of pediatrics* 2013;162(1):12-5. doi: 10.1016/j.jpeds.2012.08.034 [published Online First: 2012/11/01]
53. Laventhal N, Tarini BA, Lantos J. Ethical issues in neonatal and pediatric clinical trials. *Pediatr Clin North Am* 2012;59(5):1205-20. doi: 10.1016/j.pcl.2012.07.007 [published Online First: 2012/10/06]
54. Oeser C, Lutsar I, Metsvaht T, et al. Clinical trials in neonatal sepsis. *The Journal of antimicrobial chemotherapy* 2013;68(12):2733-45. doi: 10.1093/jac/dkt297 [published Online First: 2013/08/02]
55. O'Hara K, Martin JH, Schneider JJ. Barriers and Challenges in Performing Pharmacokinetic Studies to Inform Dosing in the Neonatal Population. *Pharmacy (Basel)* 2020;8(1):16. doi: 10.3390/pharmacy8010016 [published Online First: 2020/02/09]
56. Neyro V, Elie V, Thiele N, et al. Clinical trials in neonates: How to optimise informed consent and decision making? A European Delphi survey of parent representatives and clinicians. *PLoS One* 2018;13(6):e0198097. doi: 10.1371/journal.pone.0198097 [published Online First: 2018/06/14]
57. Aurich B, Vermeulen E, Elie V, et al. Informed consent for neonatal trials: practical points to consider and a check list. *BMJ Paediatr Open* 2020;4(1):e000847. doi: 10.1136/bmjpo-2020-000847 [published Online First: 2021/01/14]
58. Pant S, Elias MA, Woolfall K, et al. Parental and professional perceptions of informed consent and participation in a time-critical neonatal trial: a mixed-methods study in India, Sri Lanka and Bangladesh. *BMJ Glob Health* 2021;6(5) doi: 10.1136/bmjgh-2021-005757 [published Online First: 2021/05/23]
59. Japanese Pharmaceuticals and Medical Devices Agency. Report on Clinical Evaluation of Antimicrobial Agents for AMR; 2019. https://www.pmda.go.jp/files/000233507.pdf Accessed 18th June 2020.
60. Darlow CA, Docobo-Perez F, Farrington N, et al. Amikacin Combined with Fosfomycin for Treatment of Neonatal Sepsis in the Setting of Highly Prevalent Antimicrobial

Resistance. *Antimicrob Agents Chemother* 2021;65(7):e0029321. doi: 10.1128/AAC.00293-21 [published Online First: 2021/05/12]
61. Cassini A, Fleischmann-Struzek C, Naghavi M, et al. Future directions and priorities in sepsis epidemiology research: a call for action. *Bulletin of the World Health Organization* 2021;99(5):398-401. doi: 10.2471/BLT.20.276709 [published Online First: 2021/05/08]
62. Defeating meningitis by 2030: a global road map. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. Acessed on: 14th October 2021. Available from: https://apps.who.int/iris/rest/bitstreams/1352955/retrieve.
63. Ojal J, Griffiths U, Hammitt LL, et al. Sustaining pneumococcal vaccination after transitioning from Gavi support: a modelling and cost-effectiveness study in Kenya. *The Lancet Global health* 2019;7(5):e644-e54. doi: 10.1016/S2214-109X(18)30562-X [published Online First: 2019/04/20]
64. Ayieko P, Ntoburi S, Wagai J, et al. A multifaceted intervention to implement guidelines and improve admission paediatric care in Kenyan district hospitals: a cluster randomised trial. *PLoS Med* 2011;8(4):e1001018. doi: 10.1371/journal.pmed.1001018 [published Online First: 2011/04/13]
65. Pliakos EE, Andreatos N, Shehadeh F, et al. The Cost-Effectiveness of Rapid Diagnostic Testing for the Diagnosis of Bloodstream Infections with or without Antimicrobial Stewardship. *Clinical microbiology reviews* 2018;31(3) doi: 10.1128/CMR.00095-17 [published Online First: 2018/06/01]
66. Timbrook TT, Morton JB, McConeghy KW, et al. The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017;64(1):15-23. doi: 10.1093/cid/ciw649 [published Online First: 2016/11/03]
67. Wilson MR, Sample HA, Zorn KC, et al. Clinical Metagenomic Sequencing for Diagnosis of Meningitis and Encephalitis. *The New England journal of medicine* 2019;380(24):2327-40. doi: 10.1056/NEJMoa1803396 [published Online First: 2019/06/13]
68. Ge M, Gan M, Yan K, et al. Combining Metagenomic Sequencing With Whole Exome Sequencing to Optimize Clinical Strategies in Neonates With a Suspected Central Nervous System Infection. *Front Cell Infect Microbiol* 2021;11:671109. doi: 10.3389/fcimb.2021.671109 [published Online First: 2021/07/06]
69. Popescu CR, Tembo B, Chifisi R, et al. Whole blood genome-wide transcriptome profiling and metagenomics next-generation sequencing in young infants with suspected sepsis in a low-and middle-income country: A study protocol. *Gates Open Res* 2020;4:139. doi: 10.12688/gatesopenres.13172.2 [published Online First: 2021/01/19]
70. Global Action Plan on Antimicrobial Resistance. Available online https://apps.who.int/iris/rest/bitstreams/864486/retrieve; accessed 1 Mar 2022.
71. Government of Kenya. National Action Plan for the Prevention and Containment of Antimicrobial Resistance. June 2017. Available online https://www.flemingfund.org/wp-content/uploads/0cff5e08e6a64fcf93731d725b04792e.pdf; accessed 1 Mar 2022.
72. Global antimicrobial resistance and use surveillance system (GLASS) report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. Available online file:///C:/Users/Cobiero/Downloads/9789240027336-eng.pdf; accessed 1 Mar 2022.
73. East Africa Public Health Laboratory Networking Project. Available online https://www.eac.int/health/disease-prevention/east-africa-public-health-laboratory-networking-project; accessed 01 Mar 2022 [

74. The Fleming Fund. Available online https://www.flemingfund.org/grants/?type=country-grant&country=kenya; accessed 01 Mar 2022 [
75. Hunsperger E, Juma B, Onyango C, et al. Building laboratory capacity to detect and characterize pathogens of public and global health security concern in Kenya. *BMC public health* 2019;19(Suppl 3):477. doi: 10.1186/s12889-019-6770-9 [published Online First: 2020/04/25]
76. Folgori L, Ellis SJ, Bielicki JA, et al. Tackling antimicrobial resistance in neonatal sepsis. *The Lancet Global health* 2017;5(11):e1066-e68. doi: 10.1016/S2214-109X(17)30362-5 [published Online First: 2017/10/14]
77. Darlow CA, da Costa RMA, Ellis S, et al. Potential Antibiotics for the Treatment of Neonatal Sepsis Caused by Multidrug-Resistant Bacteria. *Paediatr Drugs* 2021 doi: 10.1007/s40272-021-00465-z [published Online First: 2021/08/27]

Addendum

SAMENVATTING

Sepsis (bloedvergiftiging) en meningitis (hersenvliesontsteking) behoren wereldwijd tot de belangrijkste doodsoorzaken bij pasgeborenen en jonge kinderen en de ziektelast is het hoogst in Afrika ten zuiden van de Sahara. Ten gevolge van beperkingen in de diagnostiek is het niet goed mogelijk inzicht te krijgen in de etiologie. Klinische beslissingsregels kunnen het gebruik van antibiotica sturen of helpen met de identificatie van kinderen met een hoog risico op slechte klinische uitkomst .. In dit verband is het belangrijk dat men zich realiseert dat irrationeel antibioticagebruik in het licht van toenemende antibiotica resistentie (AMR) en het beperkte aantal nieuwe antibiotica in ontwikkeling een bedreiging vormen voor de overleving van Afrikaanse kinderen.

Dit proefschrift heeft als doel een bijdrage te leveren aan verbetering van de besluitvorming van clinici die ernstig zieke jonge kinderen moeten behandelen in algemene ziekenhuizen, waar de mogelijkheden tot diagnostiek en behandeling vaak beperkt zijn.. Verschillende aspecten van de etiologie, diagnostiek en behandeling van sepsis en meningitis bij neonaten en jonge kinderen zijn onderzocht. **Hoofdstuk 1 en hoofdstuk 2** geven achtergrond informatie bij het verrichte onderzoek, waarna de daaropvolgende hoofdstukken de afzonderlijke studies beschrijven die antwoord geven op specifieke onderzoeksvragen.

In **Hoofdstuk 3** hebben we het gebruik van een op maat gemaakt multiplex TAC RT-qPCR platform onderzocht om organismen in navelstrengbloed te identificeren die geassocieerd zijn met vroeg optredende sepsis bij zuigelingen (Early Onset Neonatal Sepsis [EONS]). Bij patiënten en controles werden onder vergelijkbare omstandigheden monsters afgenomen om vergelijking tussen groepen mogelijk te maken. We hebben belangrijke pathogenen geassocieerd met een mogelijk ernstige bacteriële infectie (possible serious bacterial infection pSBI) en overlijden geïdentificeerd, waaronder Ureaplasma spp. en Enterovirus. Deze pathogenen zijn geassocieerd met ernstige sepsis en een slechte uitkomst die vaak niet gericht behandeld worden. Een van de uitdagingen was mogelijke contaminatie van de monsters, aangezien voor deze analyse gearchiveerde monsters werden gebruikt. Toekomstige prospectieve studies waarbij gepaarde navelstrengbloed- en perifere veneuze bloedmonsters

getest met geavanceerde diagnostica zoals pathogeen specifieke biomarkers en metagenomics, kunnen helpen bij minimale monstercontaminatie. Bovendien is verder onderzoek nodig naar de klinische betekenis en behandeling van pathogenen zoals Ureaplasma spp. gedetecteerd in onze studie en de klinische bruikbaarheid van navelstrengbloed in de diagnostiek in onze setting.

In **hoofdstukken 4 en 5** wordt de waarde van de eerder geïdentificeerde en door de World Health Organization (WHO) aanbevolen 'clinical signs and symptoms' bij het diagnostiseren van meningitis bij respectievelijk jonge zuigelingen (leeftijd <60 dagen) en kinderen tussen 60 dagen en 13 jaar beschreven waarbij tegenwoordig aan het grootste deel van de kinderen conjugaatvaccinaties worden gegeven. Wij rapporteren een lagere prevalentie van meningitis onder jonge zuigelingen (**Hoofdstuk 4**) en oudere kinderen (**Hoofdstuk 5**) in vergelijking met eerdere studies in ons centrum, wat de effectiviteit aantoont van conjugaatvaccinaties op meningitis morbiditeit, inclusief het effect van groeps-immuniteit bij invasieve ziekten bij oudere kinderen. Ondanks een dalende prevalentie vinden we een hoge mortaliteit bij meningitis, hetgeen aantoont dat het risico op een slechte uitkomst nog steeds hoog is, wat in overeenstemming is met bevindingen in andere settings. Bovendien tonen wij aan dat meningitis nu moeilijker klinisch te diagnosticeren is doordat atypische symptomen op de voorgrond staan. Belangrijk is om op te merken dat we mogelijk meningitis gevallen gemist hebben door antibiotica inname voor ziekenhuis-presentatie, vroegtijdig overlijden of uitgestelde lumbaal puncties (LP). Wij denken dat door de hoge kwaliteit van zorg en geavanceerde diagnostische mogelijkheden de kans hierop klein is geweest. Op basis van onze resultaten zouden clinici bij een hoge verdenkingsindex een lage drempel moeten hebben voor het uitvoeren van een LP en het starten van een behandeling bij kinderen die zich presenteren met de door ons onderzochte symptomen. Opleiding van clinici om klinische tekenen en symptomen te herkennen blijkt weer van cruciaal belang. Wij bevelen geen wijziging aan van de huidige pediatrische richtlijnen van de WHO en Kenia, aangezien deze nog steeds zeer waardevol zijn voor het identificeren van kinderen met meningitis. In algemene ziekenhuizen moet ons inziens prioriteit worden gegeven aan het opbouwen van capaciteit om de basale analyse van het hersenvocht (CSF) uit te kunnen voeren en moet de nadruk worden gelegd op het toepassen van de richtlijnen wat zal leiden tot een betere overleving van kinderen met meningitis.

Toekomstig onderzoek zou zich moeten richten op het evalueren van klinische criteria bij het voorspellen van meningitis en het opzetten van geavanceerde moleculaire diagnostiek voor het testen van bacteriële en virale pathogenen in CSF.

In **hoofdstuk 6** hebben we een nieuwe score onderzocht die de mortaliteit bij jonge zuigelingen voorspelt (NeoSep ernstige scorelijst [NSS]) en de waarde onderzocht van een set klinische en laboratorium variabelen (NeoObs studie inclusie criteria [NSIC]) waarmee jonge zuigelingen met ernstige bacteriële infectie onderscheiden kunnen worden van jonge zuigelingen zonder een dergelijke infectie. De NeoObs studie werd onder andere in Kilifi County Hospital (KCH) uitgevoerd. De kenmerken van de kinderen, de beschikbaarheid van middelen en het antibioticagebruik varieerden, vandaar de noodzaak om de prestaties van deze criteria te evalueren. Onze analyse toonde aan dat NSIC bij zuigelingen discrimineerde op SBI en clinici kan helpen bij het identificeren van zuigelingen die empirische antibiotica nodig hebben bij afwezigheid van confirmatieve kweken. Hoewel deze criteria laboratorium-parameters omvatten, kunnen ze toch worden gebruikt bij afwezigheid van laboratoriumondersteuning, aangezien een minimum van twee klinische symptomen voldoende is om verdenking van SBI te wekken. De vraag betreffende de voortzetting van antibiotica bij zuigelingen bij wie de klinische symptomen na 48-72 uur verdwenen zijn, is nog niet beantwoord. Evenmin is bekend of NSIC bruikbaar zijn bij kinderen die na opname in het ziekenhuis klinisch verslechteren, aangezien de NCIS alleen bij opname werden vastgesteld . Hetzelfde geldt voor de bruikbaarheid van de NSS bij het voorspellen van sterfte bij jonge zuigelingen tijdens de ziekenhuisopname. Onze analyse was gericht op sterfgevallen tijdens de opname (17%), wat verschilde van de sterfte na 28 dagen (11%) die in de NeoObs-studie werd gerapporteerd. Ondanks dit verschil vinden wij dat NSS een nuttig prognostisch instrument is voor het bepalen van sterfterisico bij jonge zuigelingen en een leidraad kan zijn voor de prioriteitsbepaling van middelen en antibioticagebruik. Daarbij kan het bijdragen aan de selectie van hoogrisico zuigelingen voor klinische studies. Het gewicht bij opname diende als een betrouwbare proxymaatstaf voor het geboortegewicht en kan gebruikt worden in vergelijkbare settings waar de meeste jonge zuigelingen bij opname om verschillende redenen geen gedocumenteerd geboortegewicht hebben. Zuigelingen met een NSS ≥4 in onze

setting zouden baat hebben bij een snelle start van antibiotica, ondersteunende behandeling en nauwgezette monitoring. Verdere beoordeling van de NSS en NSIC in de klinische praktijk, externe validatie in andere 'low- and middle-income countries' (LMICs), en verbeterde capaciteit van de gezondheidszorg zijn nodig.

Het laatste deel van dit proefschrift presenteert de resultaten van een klinische studie naar de veiligheid en farmacokinetiek (PK) van fosfomycine bij neonaten die met klinische tekenen van sepsis in het ziekenhuis opgenomen zijn (**Hoofdstuk 7**). In onze studie werd Fosfomycine gegeven naast empirische antibiotica, die volgens de lokale richtlijnen was voorgeschreven. De randomisatie van neonaten in één van de behandelingsarmen minimaliseerde de kans op bias, aangezien de klinische en laboratoriumparameters bij de start van de studie vergelijkbaar waren. Wat betreft bijwerkingen en toxiciteit bleek parenterale fosfomycine geen invloed te hebben op de natriumwaarde in het bloed en werden geen gastro-intestinale bijwerkingen waargenomen na orale toediening van fosfomycine. De uitgevoerde PK-modellering leverde doseringsaanbevelingen (obv leeftijd en lichaamsgewicht) op voor gebruik bij neonaten die met sepsis in het ziekenhuis waren opgenomen.

Dit was de eerste fosfomycine interventie studie met neonaten die in onze site werd uitgevoerd. Moeilijkheden bij het verkrijgen van geïnformeerde toestemming van de ouders was een van de belangrijkste uitdagingen waarmee wij werden geconfronteerd bij het uitvoeren van onze PK studie in Kilifi. De belangrijkste reden hiervoor was de ongerustheid van de ouders door de acute ziekte van hun kind en de beperkte tijd die beschikbaar was om over het geven van de toestemming na te denken. Daarnaast was er aarzeling door de herhaalde bloedafnames en was het onmogelijk om toestemming te verkrijgen van moeders die net een keizersnede hadden ondergaan of ernstig ziek waren na de bevalling. Ter verbetering van het toestemmingsproces in onze studie werden maatregelen getroffen waaronder het beter opleiding van het personeel dat bij het proces betrokken was, het betrekken van de belangrijkste besluitvormers in het gezin bij het proces en ervoor zorgen dat het toestemmingsproces doorlopend was van inschrijving tot beëindiging van het onderzoek. Door waar mogelijk de bloedafnamen voor het onderzoek te laten samenvallen met andere bloedafnamen voor klinische monitoring, werd de

frequentie verminderd, waardoor de angst van de ouders sterk afnam. Deze ervaringen zullen helpen bij het succesvol uitvoeren van toekomstige PK studies in onze site.

Andere uitdagingen bij het opzetten van neonatale klinische studies waren het bepalen van selectiecriteria en de selectie van geschikte klinische eindpunten. Zoals reeds beschreven in de vorige studies/hoofdstukken, is er geen consensus over de definitie van neonatale sepsis , wat het moeilijk maakt om ernstig zieke neonaten te selecteren die het meeste baat zouden hebben bij levensreddende interventies. Neonaten die tekenen van sepsis vertoonden, werden in onze studie opgenomen op basis van klinische beoordeling Bovendien werden uit deze studie criteria afgeleid om de ziekteprogressie te beoordelen bij neonaten die een behandeling voor sepsis ondergingen. Onze steekproef was klein en de studie had strikte toelatingscriterium waardoor zeer zieke en zeer kleine neonaten uitsloten werden van de studie. Onze studie had ook niet de power om werkzaamheid en alle veiligheidsparameters te kunnen evalueren. Een grotere studie met duidelijk gedefinieerde inclusiecriteria en eindpunten en met inbegrip van zeer premature en ernstig zieke neonaten is nodig om de fosfomycine PK parameters en doseringsschema's gerapporteerd door onze studie te bevestigen en robuust bewijs van fosfomycine veiligheid bij neonaten te genereren. Onze studie heeft een bijdrage geleverd aan het ontwikkelen van nieuwe effectieve antibioticacombinaties te gebruiken in een kwetsbare populatie bij een toenemende AMR en met beperkte behandelingsmogelijkheden. . De NSS afgeleid uit de NeoObs studie zal gebruikt worden als inclusie criteria in een grote Multi site prospectieve antibiotica studie om neonaten met een hoog risico op sterfte in op te nemen.

TOEKOMSTPERSPECTIEVEN, CONCLUSIES EN AANBEVELINGEN

Vermindering van sepsis en meningitis in Afrikaanse kinderen is afhankelijk van lokale en wereldwijde inspanningen om diagnose en behandeling te verbeteren. Deze dissertatie presenteert de resultaten van onderzoek dat essentieel bewijs levert voor de diagnose en behandeling van sepsis en meningitis bij pasgeborenen en jonge kinderen in een ziekenhuis op het platteland van Kenia. De resultaten zijn generaliseerbaar naar andere ‘settings’ in Afrika met een vergelijkbare ziektelast. Ter ondersteuning van duurzame inspanningen in het terugdringen van de morbiditeit en mortaliteit bij kinderen als gevolg van sepsis

en meningitis, kan het werk dat in deze dissertatie wordt gepresenteerd op verschillende manieren worden voortgezet, zoals hieronder wordt beschreven.

Diagnose van bloedvergiftiging en hersenvliesontsteking

Voor de diagnose van sepsis en meningitis is het van cruciaal belang dat het Ministerie van Volksgezondheid in alle algemene ziekenhuizen in Kenia de bloed- en CSF-bacteriële-kweekcapaciteit vergroot, aangezien de kweek voorlopig de gouden standaardtest blijft. Ondersteuning voor basismicroscopie van CSF en glucosemeting zal de diagnose van meningitis bevorderen wanneer geen kweken beschikbaar zijn. Artsen moeten worden aangemoedigd LP's uit te voeren bij kinderen die zich presenteren met tekenen die wijzen op meningitis, wat kan helpen bij het besluit rondom het starten van antibiotica. De toepassing van de pediatrische richtlijnen van de WHO en Kenia kan worden bevorderd door veelzijdige maatregelen, waaronder onderwijs, opleiding, supervisie en feedback. Dit moet gecombineerd worden met een periodieke her-beoordeling van de klinische richtlijnen, met inbegrip van symptomen die gebruikt worden in de diagnostiek van sepsis en meningitis. Geavanceerde moleculaire diagnostische platforms, waaronder metagenomics (next-generation sequencing-technieken), zullen de opsporing van pathogenen in de toekomst verbeteren.

Antimicrobiële resistentie (AMR) en antibiotische behandeling van sepsis en meningitis

Surveillance van AMR is essentieel om de AMR-druk en -trends te monitoren, opkomende bedreigingen voor de huidige behandelingsregimes te identificeren en beleidsmakers te informeren. Dit vereist adequate microbiologische laboratoriumcapaciteit, richtlijnen, toegang tot antimicrobiële middelen van goede kwaliteit en een degelijke gegevensverzameling en -rapportage systeem. Kenia heeft zich in mei 2016 ingeschreven in het Global Antimicrobial Resistance and Use Surveillance System (GLASS) van de WHO; de lokale surveillanceactiviteiten, -capaciteit en -rapportage moeten echter worden versterkt, aangezien Kenia er niet in is geslaagd om in 2020 gegevens over AMR of antibioticagebruik aan GLASS aan te leveren. Een nieuwe aanpak voor de ontwikkelen van van geneesmiddelen is duur en tijdrovend en kan wellicht niet voorzien in de huidige dringende behoefte aan nieuwe effectieve antibiotica.

Het hergebruik van bestaande en onderbenutte antibiotica kan mogelijk een tijdelijke oplossing bieden om nieuwe antibiotica door de ontwikkelingspijplijn en het vergunningverleningsproces te loodsen. Voor de behandeling van sepsis bij kinderen ondersteunt dee WHO het hergebruik van oudere antimicrobiële stoffen, waarvan het octrooi is verlopen en waarvan de antimicrobiële activiteit is behouden, voor de behandeling van sepsis bij kinderen. Het Global Antibiotic Research and Development Partnership (GARDP), een gezamenlijk initiatief van de WHO en het Drugs for Neglected Diseases initiative (DNDi) ter ondersteuning van het wereldwijde actieplan voor AMR, heeft tot doel een antimicrobiële regeling te ontwikkelen voor gebruik in LMIC's voor de empirische behandeling van neonatale sepsis op locaties met toenemende resistentie tegen de huidige door de WHO aanbevolen behandelingen. Voor verdere klinische evaluatie bij kinderen met sepsis bleken fosfomycine, flomoxef en amikacine bleken het meest veelbelovend op basis van criteria zoals lage kosten, minimale toxiciteit, activiteit tegen MDR bacteriën, beschikbaarheid van een licentie of uitgebreid gebruik in de neonatale context als er geen licentie bestaat.(**Hoofdstuk 1**). Deze antibiotica zullen in nieuwe combinaties onderzocht worden in een grote prospectieve multi site trial onder gehospitaliseerde neonaten met sepsis, en vergeleken worden met bestaande combinaties om zo een klinisch relevante rangschikking van behandelingsregimes op te stellen op basis van veiligheid en werkzaamheid.

Conclusie

Deze dissertatie verschaft inzicht in de verschillende aspecten van het onderzoek naar sepsis en meningitis bij pasgeborenen en jonge kinderen, waarbij belangrijke tekortkomingen aan de orde komen die verband houden met een slechte klinische uitkomst. De resultaten suggereren dat meer onderzoek nodig is met specifieke diagnostische assays voor sepsis, omdat bij een groot deel van de neonaten de etiologie onbekend blijft. Een goed voorbeeld zijn de bevinden rond de door atypische organismen en virussen (zoals Ureaplasma spp. en Enterovirus) veroorzaakte infecties bij pasgeborenen in Afrika. Klinische algoritmen bestaande uit symptomen die bij sepsis en meningitis mortaliteit voorspellen, blijven nuttig bij het sturen van de behandeling. Een periodieke evaluatie van de klinische criteria gebruikt voor het onderscheiden van sepsis en meningitis is van belang voor de herziening van

de richtlijnen. Hergebruik van antibiotica waarvan het octrooi is verlopen, lijkt veelbelovend als middel om tegemoet te komen aan de dringende behoefte aan behandelingscombinaties die effectief zijn tegen resistente bacteriën. Voortdurend toezicht op de oorzaken van sepsis en meningitis, antibioticagebruik en resistentie is van cruciaal belang voor het verbeteren van de uitkomst bij kwetsbare kinderen in Kenia. Ten slotte moet prioriteit worden gegeven aan de versterking van de gezondheidsdiensten voor kinderen in Afrika, naast de ontwikkeling en uitvoering van kosteneffectieve diagnostische benaderingen en therapeutische opties.

Supplementary material

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
Predictors of IBI among infants with WHO signs of pSBI	Lishman J. et al, 2021[1]	South Africa	Age 21-90d; Paediatric Emergency Centre, 2016 Retrospective cross-sectional review	WHO pSBI IMCI criteria[2]: 1. Inability to feed 2. Convulsions 3. Fast breathing (≥60 b/min) 4. Severe chest in-drawing 5. Fever (≥38^{o}C) 6. Low body temperature (<35.5^{o}C) 7. Movement only when stimulated or no movement at all Blood, CSF, urine and other relevant cultures	12/248 (4.8%) had IBI (bacteraemia or meningitis) 165/248 (67%) met WHO IMCI pSBI criteria; 51 (31%) had positive cultures and focal infections e.g., pneumonia, UTI, and STI (SBI). Sensitivity for SBI 82% (95%CI 71-91), specificity 39% (95%CI 32-46), PPV 31% (95%CI 28-35), NPV 87% (95%CI 79-92) 34% infants meeting pSBI criteria received IM antibiotics	Infants without confirmed SBI received antibiotics Not tested in neonates age <21d
Predictors of SBI in low-risk febrile infants	Dagan R. et al, 1985[3]	USA	Age <3mo, hospitalised between 1982-1984 Prospective cohort Derivation study	Rochester criteria for febrile infants at low risk of SBI 1. Previously healthy term infant without perinatal complications and with no previous antibiotic treatment 2. Normal physical examination findings 3. Peripheral WBC: 5–15 x 10^3 cells/µL 4. Band count: <1.5 x 10^3 cells/µL 5. Urinalysis: <10 WBC/HPF Bacterial cultures done	62% (144/233) infants met all low-risk criteria for SBI 1/144 (0.7%) infants in low-risk group had SBI compared with 22/89 (25%) infants in high-risk group, *P<0.001* NPV 99.3% for SBI 26/144 (18%) infants in the low-risk group and 7/89 (8%) infants in the high-risk group did not receive antibiotics, *P=0.03*	Not applicable in premature, afebrile or ill-appearing infants with abnormal examination findings, or infants with underlying conditions. Cultures unavailable; peripheral band count not done; sterile urine sampling difficult. Insufficient evidence to recommend outpatient management or hospitalization and observation prior to/without antibiotics.

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
	Baskin MN. et al, 1992[4]	USA	Febrile, age 28-89d, treated with IM ceftriaxone in the ED between 1987-1990 Prospective cohort Derivation study	Boston low-risk criteria: 1. Temperature ≥38°C, 2. Well-appearing with no focus of infection on physical examination, 3. Peripheral WBC: <20 ×10^3 cells/µL, 4. CSF WBC: <10 cells/µL, or 5. Urine WBC <10 cells/HPF or negative urinary leukocyte esterase Blood, CSF and urine cultures obtained.	27/503 (5.4%) SBI Clinical screening criteria did not enable discrimination between infants with and those without SBI.	Included low-risk infants only hence performance not assessed. Daily IM ceftriaxone pending culture results plus follow-up (three telephone calls and one return visit) is a feasible alternative to hospitalization. Not applicable in neonates.
	Jaskiewicz JA. et al, 1994[5]	USA	Age ≤60d hospitalised between 1984-1992 Retrospective cohort Validation study	Updated Rochester criteria: 1. Temperature ≥38°C 2. Well- appearing and previously healthy 3. No focal infection 4. Peripheral WBC: 5–15 x 10^3 cells/µL 5. Band count: <1.5 x 10^3 cells/µL 6. Urinalysis: ≤10 WBC/HPF 7. Stool microscopy (if diarrhoea): ≤5 WBC/HPF Blood, CSF and urine cultures	47% (437/931) well appearing infants met low risk criteria. 5 low-risk infants had SBI NPV of the low-risk criteria was 99% (95%CI 97-100) for SBI, and 100% (95%CI 98%-100) for bacteraemia. Over one-third low risk infants were not initially treated with antibiotics and remained well. Low-risk infants can be carefully observed without parenteral antimicrobials.	Similar as above Limited data on capacity of hospital or out-patient observation and follow-up of low-risk infants without antimicrobial treatment or single dose intramuscular antibiotic in sSA

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
	Baker MD. et al, 1993[6]	USA	Age 29-56 days screened at the ED between 1987-1992 Prospective cohort Derivation study	Philadelphia criteria: 1. Temperature ≥38.2°C, 2. Peripheral WBC ≥15 X10^3 cells/μL, 3. CSF WBC ≥8 cells/μL, 4. Positive CSF Gram stain, 5. Urine WBC ≥10 cells/HPF or positive bright-field microscopy, 6. Chest X-ray showing an infiltrate, 7. Clinical assessment (infant observation score >10[7]) Blood, CSF, urine, and stool cultures	65/747 (8.7%) infants of which 64 were identified by criteria; sensitivity 98% (95%CI 92-100), specificity 42% (95%CI 38-46), PPV 14% (95%CI 11-17), NPV 100% (95%CI 98-100). 286/287 (100%) infants who did not meet criteria lacked SBI and were observed without antibiotics. Modified criteria included band-to-neutrophil ratio, identifying all infants with SBI (sensitivity 100% NPV 100%).	Not applicable in neonates. Limited laboratory capacity in sSA.
	Lyons TW. et al, 2020[8]	USA and Canada	Age 29-60d, evaluated in 23 tertiary-care EDs, 2005-2013 Retrospective cohort Validation study Excluded critically ill infants in ICU	High risk predictors of IBI in well-appearing febrile infants Modified Boston criteria: 1. Peripheral WBC: ≥20 x10^3 cells/μL, 2. CSF WBC: ≥10 cells/μL, and 3. Urine WBC >10 cells/hpf or positive urinalysis (trace to 3+). Modified Philadelphia criteria: 1. Peripheral WBC ≥15 x10^3 cells/μL, 2. CSF WBC: ≥8 cells/μL,	Modified Boston criteria identified 133 of the 212/8,344 (2.5%) infants with IBI (bacteraemia or meningitis); sensitivity 63% (95%CI 56-69) and specificity 59% (95%CI 58-60). Modified Philadelphia criteria identified 157 of the 219/8,131 (2.7%) infants with IBI; sensitivity 72% (95%CI 65-78) and specificity 46% (95%CI 45-47).	Misclassification of a substantial number of infants, including those with bacteraemia or meningitis suggests need for newer tools to stratify risk of SBI in infants. May have missed SBI cases excluded due to critical illness. Recommend systematic LPs in all febrile infants; challenging in sSA

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
				3. Positive CSF Gram-stain result, and 4. Urine WBC >10 cells/hpf or positive urinalysis (trace - 3+). Blood and CSF cultures done	Modified Boston and Philadelphia criteria misclassified 17/53 (32%) and 13/56 (23.3%) infants with bacterial meningitis, respectively.	
	Bonadio WA. et al, 1993[9]	USA	Age 4-8 weeks, febrile, evaluated for sepsis in the ED	Milwaukee criteria: 1. Age 28-56d 2. Well appearing; no signs of focal infection 3. Peripheral WBC < 15 x10^3 cells/µL 4. CSF WBC < 10 cells/µL 5. Urine WBC < 5–10 cells/hpf or no bacteria, negative leukocyte esterase, negative nitrites 6. CXR without infiltrate	143/534 (27%) met Milwaukee protocol, were stable, received ceftriaxone 50mg/kg, were discharged and re-evaluated within 24h; 1 (0.7%) had SBI. 391 (73%) had compromised presentation, did not meet Milwaukee protocol, and were hospitalised for antibiotics pending culture; 23 (5.9%) had SBI. Sensitivity 96% and NPV 99% for SBI.	Not applicable in neonates, critical infants. Limited laboratory and radiology capacity in sSA.
	Lacour AG. et al, 2008[10]	Switzerland	Age 7d-36mo evaluated in the ED between 1998-2002 Retrospective cohort	Laboratory score: 1. Temperature >38^{o}C 2. Well-appearing without any source of infection O/E (Infant Observation Scale) 1. PCT (ng/mL): <0.5 (0 point); 0.5-2 (2 points); ≥2 (4 points)	54/202 (27%) had SBI PCT (OR 38, 95%CI 5.8-243), CRP (OR 7.8, 95%CI 2.0-30), and urine dipstick (OR 23, 95%CI 5.1-105) were significantly associated with SBI. Derivation set: sensitivity for SBI 94% (95%CI 82-99),	Wide age criteria may affect performance in different age categories in other settings. Small sample size, less precision. Limited laboratory test capacity in sSA.

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
			Derivation and validation study	2. CRP (mg/L): <40 (0 point); 40-99 (2 points); ≥100 (4 points) 3. Urine dipstick: no leukocyturia or nitrituria (0 point); leukocyturia of nitrituria (1 point) Blood, urine and CSF (if clinically indicated) cultures done	specificity 81% (95%CI 72-88), PPV 64% (95%CI 51-76) and NPV 98% (95%CI 92-99). Validation set: sensitivity 94% (95%CI 74-99), specificity 78% (95%CI 64-87), PPV 61% (95%CI 42-76) and NPV 97% (95%CI 87-100).	
	Gomez B. et al, 2016[11]	Spain, Italy and Switzerland	Age ≤90d with fever without source, 11 European EDs between 2012-2014 Prospective cohort Validation study	Step-by-Step low-risk criteria: 1. Temperature ≥38^{o}C 2. Well appearing 3. >21d old 4. No leukocyturia 5. PCT <0.5 ng/mL 6. CRP ≤20 mg/L 7. ANC ≤10 x10^{3}cells/µL Rochester criteria and Laboratory score (<3) defined above Blood, CSF, and urine cultures done	87/2,185 (4.0%) had IBI. Step-by-Step: sensitivity 92%, specificity 47%, PPV 6.7% and NPV 99% for ruling out IBI. Rochester criteria: sensitivity 82%, specificity 45%, PPV 5.7% and NPV 98%. Lab-score: sensitivity 60%, specificity 84%, PPV 13% and NPV 98% for ruling out IBI. 7, 16 and 35 infants with an IBI were misclassified by Step-by-Step, Rochester criteria, and 35 by the Lab-score respectively (*P<0.05*).	Clinical and lab criteria applied in a sequential manner; not applicable in sSA due to limited lab capacity

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/ limitations
	Nigrovic LE. et al, 2017[12]	USA	Age ≤60d, temperature ≥38°C 26 Eds; 2008-2013 Prospective cohort Validation study Excluded critically ill infants or those who had received antibiotics within 48h preceding ED presentation.	Yale Observation Scale (YOS) score: 6 behavioural domains assigned 1, 3 or 5 points if normal, moderately impaired, or severely impaired. Total score: 6 (most well-appearing) to 30 (most ill-appearing infant). 1. Quality of cry: 2. Reaction to parents 3. State variation 4. Colour 5. Hydration 6. Response to social overtures Unstructured clinician suspicion: clinician's estimate of infant's risk of having SBI in 1 of 5 risk categories: <1%, 1-5%, 6-10%, 11-50%, or >50%. Blood, CSF and urine cultures	444/4,591 (9.7%) had SBIs (bacteraemia, meningitis or UTIs) and 97/4,591 (2.1%) had IBI (bacteraemia or meningitis) YOS did not discriminate between infants with or without SBI (AUC 0.53; 95%CI 0.50-0.55) and had modest discriminative ability for IBI (AUC 0.61; 95%CI 0.56-0.67). Unstructured clinician suspicion had modest discriminative ability for SBI (AUC:0.61; 95%CI: 0.58–0.63) or IBI (AUC:0.66; 95%CI: 0.61–0.72). Neither the YOS score nor unstructured clinician suspicion (at either of the cut-off points) identified all 24 infants with bacterial meningitis.	Unsatisfactory performance may be due to wide age range (≤24mo) that included older infants in the original derivation and validation studies.
	Kupperma nn N. et al, 2019[13]	USA	Age ≤60d reviewed at 26 EDs; 2014-2018 Retrospective cohort; derivation and validation	PECARN rule: 1. Temperature ≥38°C 2. Well-appearing 3. Urinalysis negative 4. ANC ≤4.09 x10^3cells/μL 5. PCT ≤1.71 ng/mL	170/1,821 (9.3%) had SBI Derivation set: Sensitivity 99% (95%CI 93-100), specificity 63% (95%CI 60-66), PPV 21% (17-26), NPV 100% (95%CI 99-100), NLR 0.02 (95%CI 0.003-0.14), PLR 2.68 (95%CI 2.44-2.93).	Has potential to decrease LPs needed. However, PCT not available in sSA.

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
				Excluded critically ill infants, infants who received antibiotics in preceding 48h, prematurity (≤36 weeks gestation), pre-existing medical conditions, indwelling devices or soft-tissue infections. Blood, urine and CSF (if clinically indicated) cultures	Validation set: Sensitivity 98% (95%CI 91-100), specificity 60% (95%CI 57-63), PPV 21% (95%CI 17-25), NPV 100% (95%CI 98-100), NLR 0.04 (95%CI 0.01-0.15), PLR 2.44 (95%CI 2.23-2.67). Missed 1 infant with bacteraemia and two with UTI. No infant with meningitis was misclassified	
	Velasco R. et al, 2021[14]	Spain	Age ≤60d screened in the ED at a tertiary teaching hospital; 2007-2018 Retrospective cohort Validation study	Febrile infants without a source of infection meeting PECARN rule described above.	21% (256/1,247) had SBI and 3.1% (38/1,247) had IBI. 4.5% (26/576) low-risk infants had SBI; 5 had IBI of which 2 had meningitis. Sensitivity 90% (95%CI 86-93) and specificity 56% (95%CI 52-59) for SBI. AUC 0.73 (95%CI 0.70-0.75). Sensitivity for SBIs among infants with a <6hr history of fever (55% of all infants) was 89% (95% CI 82% to 93%).	Performance was less in study population compared to derivation study hence should be carefully applied in infants with a short history of fever. Data on fever duration not always available/reliable.
Predictors of meningitis only	Nigrovic LE. et al, 2012[15]	USA, Western Europe and Argentina	Age 1mo-18y Meta-analysis of 8 published validation	Bacterial meningitis score for low-risk children with CSF pleocytosis: 1. CSF Gram stain negative 2. CSF ANC <1000 cells/µL 3. CSF protein <80 mg/dL	1,242/5,312 (23%) had bacterial meningitis Sensitivity 99%. (95%CI 99-100), specificity 62% (95%CI 61-64), NPV 100% (95%CI 99-	Limited to children with CSF pleocytosis hence cannot be used in the absence of an LP. Not applicable in ill-appearing infants aged

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
			studies; 2002-2012. Hospitalised (6), ED (1) and nationwide meningitis registry (1); 88% overall admission	4. Peripheral blood ANC <10 x10^3 cells/µL 5. No seizure at or prior to initial presentation	100), PLR 2.6 (95%CI 2.5-2.7), NLR 0.01 (95%CI 0.01-0.02). 9 patients with bacterial meningitis were misclassified as 'very low risk' by the score; 3 were age <2mo.	≤2mo with clinical features suggestive of meningitis. Not applicable in children pre-treated with antibiotics
Predictors of severe disease	Weber MW. et al, 2003[16]	Ethiopia, the Gambia, Papau New Guinea, and the Philipines	Age <2mo, outpatient clinics at first level or referral hospitals between 1990-1993 Excluded neonates<1g birth weight or 48h old Cultures done	14 independent predictors of severe disease: General status: reduced feeding, no spontaneous movement, temperature >38°C, drowsiness/unconscious, history of a feeding problem, change in activity, agitation, and delayed capillary refill. Respiratory signs: lower chest wall indrawing, respiratory rate >60 b/min, grunting, and cyanosis. Meningitic signs: history of convulsions and bulging fontanel.	Presence of ≥1 of the above 14 signs: sensitivity for severe disease 87%, specificity 54%. More stringent combinations resulted in considerable loss of sensitivity. Reduction of the list to 9 signs resulted in slight loss of sensitivity and considerable gain in specificity. Fever plus any other sign: low sensitivity 25%	Not applicable in neonates with birth weight ≥1500g, post-natal age <48h Blood and CSF cultures not available in most hospitals Performance of signs may have changed over time with changes in disease epidemiology in sSA.

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
	YICSSG, 2008[17]	Bangladesh Bolivia, Ghana, India, Pakistan and South Africa	Age <60d with acute illness at 12 first-level health facilities. No cultures done.	12/31 symptoms and signs predicted severe illness requiring hospitalization in the first week of life: history of difficulty feeding (OR 10, 95%CI, 6·9–15), history of convulsions (15, 6·4–37), lethargy (3·5, 1·7–7·1), movement only when stimulated (6·9, 3·0–16), respiratory rate ≥60 breaths/min (2.7, 1·9–3·8), grunting (2·9, 1·1–7·5), severe chest indrawing (8·9, 4·0–20), temperature ≥37·5°C (3·4, 2·4–4·9) or <35·5°C (9·2, 4·6–19), prolonged capillary refill (11, 5·1–22), cyanosis (14, 1·6–117), and stiff limbs (15, 2·2–106).	≥1 of 12 signs: sensitivity 87% and specificity 74%. Reduction of the algorithm to 7 signs (history of difficulty feeding, history of convulsions, movement only when stimulated, respiratory rate ≥60 breaths/min, severe chest indrawing, temperature ≥37·5°C or <35·5°C), based on the prevalence of each sign or symptom resulted in similar results (sensitivity 85% and specificity 75%) in infants 0-6d old. In 5,712 infants 7–59-day-old: sensitivity 74% and specificity 79%.	Utility of signs not tested against confirmatory cultures
Predictors of mortality in infants with SBI	Singh P. et al, 2018[18]	India	Age 7-120d; ED at 3 tertiary referral hospitals; 2005-2008. Retrospec-tive cohort	≥1 WHO IMCI signs of pSBI (convulsions, tachypnoea [respiratory rate ≥60 b/min], severe chest indrawing, nasal flaring, grunting, bulging fontanel, ≥10 skin pustules or a big boil, axillary temperature <35.5°C or ≥37.5°C, lethargic, unconscious, or less than normal movements) + CRP ≥12mg/L	Cow's milk or formula feeding (OR 3.7, 95%CI 1.5-9.3), lethargy (OR 2.4, 95%CI 1.1-5.4), increased CRP (OR 1.9, 95%CI 1.1-3.3), female gender (OR 2.3, 95%CI 1.0-5.0), abdominal distension (OR 3.7, 95%CI 1.1-12) and bulging fontanel (OR 5.8, 95%CI 1.1-31) were associated with increased odds of death.	No confirmatory cultures. CRP not readily available. Excluded critically infants who may have had SBI.

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
	Wynn JL. et al, 2020[19]	USA	<33 weeks gestation, VLBW with LOS in NICU between 2012-2016. Retrospective cohort Derivation study	Neonatal sequential organ failure assessment (nSOFA) score (total=15): 1. Need for mechanical ventilation and oxygen requirement (peripheral oxygen saturation/fraction of inspired oxygen ratio) (score range 0–8) 2. Need for inotropic support/vasoactive drugs including corticosteroid support (score range 0–4), and 3. Presence and degree of thrombocytopenia indicating haematologic dysfunction (score range 0–3)	Neonates with nSOFA score of >4 had higher mortality than neonates with a score ≤4 at evaluation (13% vs 67%, *p<0.001*), +6 hours (15% vs 64%, *p=0.002*), and +12 hours (7% vs 71%, *p<0.001*). AUC 0.77 (95%CI 0.62–0.92; *p=0.001*), 0.78 (0.66–0.92; *p<0.001*) and 0.93 (0.86–0.997; *p<0.001*) at evaluation, +6hrs and +12hrs respectively. Combined sensitivity and specificity were maximized using an nSOFA score of ≥3 (evaluation [75%, 77%]; +6 hours [75%, 68%]; +12 hours [100%, 70%]).	Not applicable in term neonates, early-onset sepsis. Most hospitals in sSA do not have NICUs. Objective parameters not available in sSA public hospitals

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
	Fleiss N. et al, 2021[20]	USA	<33 weeks gestation, VLBW with LOS admitted to NICU at 7 academic medical centres; 2010-2019. Validation study	nSOFA score as described above	97/653 (15%) neonates died. ROC for mortality across centres was 0.71-0.95 (T0 hours), 0.77-0.96 (T6 hours), and 0.78-0.96 (T12 hours). Maximum nSOFA score at T0 or T6, had ROC of 0.88 (95%CI, 0.84-0.91). Association of nSOFA score with infection-related mortality was not altered by sex, pathogen or gestation <25 weeks.	Not applicable in term neonates, early-onset sepsis. Most hospitals in sSA do not have NICUs. Objective parameters not available in sSA public hospitals.
	Wynn JL. et al, 2021[21]	USA	Neonates admitted to NICU at 3 academic medical centres between 2009-2020. Retrospective cohort Validation study	nSOFA score as described above	603/20,152 (3%) neonates died within 28d Good-to-excellent discrimination of all-cause mortality across birth weights (especially ≥750 g). Overall nSOFA score in the first 24 hours had AUC 0.88-0.89 which by day 28 ranged between 0.93-0.95 overall. Discrimination of mortality stratified by birth weight: 0.57 to 0.94 for <750 g, 0.82 to 0.99 for 750-1,499 g, 0.88 to 0.95 for 1,500-2,499 g, and ≥0.92 for ≥2,500 g.	Not applicable in term neonates, early-onset sepsis. Most hospitals in sSA do not have NICUs. Objective parameters not available in sSA public hospitals.

Table 1. Clinical prediction rules of sepsis, meningitis and mortality in young infants (continued)

Indication	Author, yr	Country	Methods	Definition of SBI and criteria	Results	Application/limitations
	Ziegler AC. et al, 2021[22]	USA	VLBW infants admitted between 2011-2019. Retrospective cohort	The heart rate characteristics (HRC) index (HeRO score; an early warning system for LOS which also rises before necrotizing enterocolitis [NEC]) and the nSOFA score were analysed near blood cultures relative to diagnosis (LOS or NEC) and sepsis-associated mortality.	Both scores were higher in nonsurvivors than in survivors. HRC index increased before blood culture sampling hence useful as an early indicator of imminent sepsis. Mortality prediction using nSOFA most optimal 12 hr after the time of blood culture during treatment compared to other timepoints analysed (AUC 0.91).	Not applicable in early-onset sepsis, term or normal birth weight neonates. Most hospitals in sSA do not have NICUs. Cultures often unavailable.

Abbreviations: SBI, serious bacterial infection; sSA, sub-Saharan Africa; IBI, invasive bacterial infection; pSBI, possible serious bacterial infection; CSF, cerebrospinal fluid; WHO, World Health Organization; IMCI, Integrated Management of Childhood Illness; WBC, white blood cell; µL, microlitre; HPF, high-power field; ED, emergency department; UTI, urinary tract infection; STI, soft tissue infection; O/E, on examination; OR, odds ratio; PECARN, Paediatric Emergency Care Applied Research Network; NICU, neonatal intensive care unit; VLBW, very low birth weight; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; AUC, area under the receiver operating characteristic curve.

Table 2. Culture isolates of unclear clinical significance

Gram positive	No. (Sample)	Associated clinical syndromes
Arthrobacter globiformis *Likely non-pathogenic*	1 (BC) 0 d old neonate, survived	Found in soil Associated with Whipple's disease (a multisystemic infection characterized by fever, diarrhoea, lymphadenopathy, chronic arthritis, central nervous system involvement and uveoretinitis) in a 60-year old male patient with B27-negative spondyloarthropathy, meningitis, lymphadenopathy, and uveitis[23] No case reports in infants
Bacillus cereus *Likely pathogenic*	4 (BC) 0 d (n=3), 2 survived, 1 died 47 d (n=1), survived	An environmental bacterium Mainly associated with food poisoning but has been implicated in fatal non-gastrointestinal tract infections such as pneumonia, fulminant sepsis, and central nervous system infections, particularly in immunosuppressed individuals, intravenous drug abusers, and neonates.[24] Case reports of neonatal meningoencephalitis,[25 26] meningitis,[27] bacteraemia,[28-30] and fatal sepsis in premature neonates.[31] Outbreaks of neonatal respiratory tract infections associated with mechanical ventillation.[32 33]
Bacillus megaterium *Likely non-pathogenic*	1 (BC) 0 d, survived	Found in diverse habitats and rarely pathogenic to humans[34] Only four case reports of human infection in adults to date: pleuritis with pleural effuion,[34] post-surgical keratitis,[35] cutaneous infection,[36] and brain abscess[37] No case reports in infants
Bacillus pumilus *Likely non-pathogenic*	2 (BC) 0 d (n=2), survived	Soil commensal. Commonly isolated as a culture contaminant and rarely implicated as a pathogen.[38] Case report of severe sepsis in 2 neonates.[39] Case report of septic arthritis in a 6 year old healthy female child following a fall.[38] Refractory case of central venous catheter infection in an immunocompetent 8 year old female child with tufting enteropathy on long-term parenteral nutrition.[40]
Brevibacterium paucivorans *Likely non-pathogenic*	1 (BC) 0 d, died	Associated with raw milk and milk products and are also found on human skin. Rarely cause human infections. Rare cause of catheter related blood steam infection mainly in immunocompromised adult hosts secondary to malignancies or AIDS patients.[41 42] *Brevibacterium casei* associated with intravenous catheter-related bacteraemia in children with cancer.[43] No cases reported in infants.

Table 2. Culture isolates of unclear clinical significance (continued)

Gram positive	No. (Sample)	Associated clinical syndromes
Coagulase-negative *Staphylococci* *Likely non-pathogenic*	296 (BC), 1 (CSF) 35 died	Skin and epithelium commensal 995/9552 (10%) blood culture isolate in neonates in Kilifi. Not associated with case fatality of prolonged hospitalisation.[44]
Corynebacterium amycolatum *Likely non-pathogenic*	1 (BC) 6 d, died	Skin commensal. Bacteraemia in 3 adult patients with prolonged hospitalization, multi-instrumentation, and severe underlying disease.[45] Fatal sepsis in premature neonate. Labour characterised with premature rupture of the membrane, maternal fever and chorioamnionitis, and *C. amycolatum* was isolated from vaginal swab 24 hr before delivery.[46]
Corynebacterium mucifaciens *Likely non-pathogenic*	1 (BC) 14 d, survived	Skin commensal. Recent case reports of cavitatory pneumonia,[47] fatal bacteraemia,[48] septic cerebral embolus,[49] corneal ulcer,[50] and implantable cardioverter defibrillator infective endocarditis[51] in adults. No case reports in infants.
Corynebacterium simulans *Likely non-pathogenic*	1 (BC) 3 d, survived	Skin commensal. Acute pyogenic spondylitis in a 78-year-old male patient with diabetes mellitus.[52] No case reports in infants.
Group F Streptococcus *Likely non-pathogenic*	1 (BC) 0 d, survived	Resident flora of the oropharyx, gastrointestinal tract and perineum.[53] Related to *Streptococcus milleri* (part of viridans Streptococci) Associated with abscess formation, especially in patients with history of trauma, perforation, manipulation or underlying gastrointestinal pathology.[54] Bacteraemia is rare.[54 55] Also associated with tonsilitis, myocarditis,[56] and purulent pericarditis.[57] No cases reported in infants.
Kocuria palustris *Likely non-pathogenic*	1 (BC) 0 d, survived	Skin and mucous membranes commensals that are also ubiquitous in the environment. Associated with ulcerative keratitis.[58] Other species of *Kocuria* are associated with both superficial and invasive infections in immunocompetent and immunosuppressed individuals e.g. urinary tract infections, cholecystitis, catheter-associated bacteremia, dacryocystitis, canaliculitis, keratitis, native valve endocarditis, peritonitis, descending necrotizing mediastinitis, brain abscess and meningitis. Predisposing

Table 2. Culture isolates of unclear clinical significance (continued)

Gram positive	No. (Sample)	Associated clinical syndromes
		factors include prolonged cathetarization, congenital deformities, malignancies and peritoneal dialysis.[59] No cases reported in infants.
Microbacterium oleivorans *Likely non-pathogenic*	1 (BC) 0 d, died	Found in hydrocarbon-contaminated environments.[60] No human case reports.
Microbacterium trichothecenolyticum *Likely non-pathogenic*	1 (BC) 1 d, survived	Environmental bacterium.[61] No human case reports.
Paenibacillus thiaminolyticus *Presumed pathogen*	2 (BC), 1 (CSF) BC 5 d (n=2), 1 survived, 1 died CSF 5 d, survived (same bacteria in BC)	Environmental bacterium. Bacteria in this *Paenibacillus* genera are used in agriculture, medicine, process manufacturing and bioremediation, and have been implicated in spoilage of pasterurized dairy products and opportunistic human infections.[62] Not detectable on human skin.[63] First report of human infection by *Paenibacillus thiaminolyticus* was that of bacteraemia in an 80 year old male patient with a permacath for chronic haemodialysis due to renal failure. Two successive blood cultures were positive for this organisim and resolution of fever and peripheral leukocytosis followed administration of antibiotics (piperacillin plus an aminoglycoside) to which the bacteria was susceptible. [64] Isolated from CSF from a Ugandan infant suffereing from postinfectious hydrocephalus.[65] Sepsis and meningitis in a 25-day old premature infant who died during hospitalization.[66]
Streptococcus species *Likely non-pathogenic*	4 (BC) 1 d, died 2 d, died 29 d, survived 44 d, survived	Bacteria not further differentiated hence associated clinical syndromes not listed.

Table 2. Culture isolates of unclear clinical significance (continued)

Gram negative	No. (Sample)	Associated clinical syndromes
Aeromonas hydrophila *Presumed pathogen*	2 (BC) 3 d, died 1 d, survived	Found in the aquatic environment, drinking water, wastewater, sewage and food. An emerging pathogen responsible for skin infections, gastroenteritis, peritonitis, bacteremia, meningitis, cholera-like illness, hemolytic uremic syndrome, and necrotizing fasciitis. Cause infections in both immunocompetent and immunocompromised humans.[67-69] Causes diarrhoeal disease in children.[70] Meningitis and fulminant sepsis in an Indian preterm male neonate. Bactreria isolated in both blood and CSF culture. Neonate died on 12th dasy of life despite targeted antimicrobial (meropenem).[71] Additional case reports of neonatal sepsis.[72-75] Case report of urinary tract infection in a neonate.[76]
Chryseobacterium indologenes *Likely pathogenic*	1 (BC) 0 d, survived	Ubiquitous in nature, mainly found in soil and water. Prevalent on wet or humid surfaces in hospitals and also in catheters containing fluids, e.g. feeding tubes and central venous catheters.[77 78] Emerging nosocomial pathogen associated with intrinsic antibiotic resistance. Causes bacteraemia, meningitis due to central nervous system shunt, pneumonia, urinary tract in fections, peritonitis due to peritoneal catheter dialysis, cellulitis, surgical wound infections, and occular infections.[78-83] Community-acquired cases in immunocompetent patients have also been reported.[84] Bacteraemia in neonates.[85-89] Meningitis in neonates[90 91] Ventilator-associated pneumonia in neonates with no underlying congenital malformations,[92] a neonate with congenital heart disease,[93] and a 3-month old infant with meningomyelocele and congenital diaphragmatic hernia.[94]
Comamonas kerstersii *Likely non-pathogenic*	1 (BC) 9 d, survived	Found in water, soil and plants.[95] Emerging cause of severe opportunistic infections mostly in adults. About 30 case reports published in literature.[96] Causes bacteraemia and septic shock in patients with underlying predisposing factors such as peritonitis, appendicitis, perforated appendix, and perforated colon.[96 97] Reported to also cause localized intra-abdominal infection (posas abscess), pelvic peritonitis.[95] First case report urinary tract infection in a 5 year old female patient.[98] No cases reported in infants.

Table 2. Culture isolates of unclear clinical significance (continued)

Gram negative	No. (Sample)	Associated clinical syndromes
Cupriavidus gilardii *Likely non-pathogenic*	1 (BC) 1 d, survived	Environment bacteria isolated from several ecological niches including plants and soils contaminated with heavy metals.[99] Emerging opportunistic multi-drug resistant pathogen that can cause infection such as bacteraemia in both immonocompetent and immunocompromised patients.[100] Muscular abscess in a male adult patient with history of renal transplant.[101] Case report of central venous catheter-related sepsis in a 7-year old female patient with acute lymphoblastic leukemia.[102] Fatal case of sepsis in a 12-year old female patient with severe idiopathic aplastic anaemia.[103] No cases reported in infants
Klebsiella oxytoca *Likely non-pathogenic*	1 (BC) 1 d, survived	Normally acquired from environmental sources that has been isolated from clinical samples (mostly blood and respiratory secretions). Variable pathogenic significance across different studies with clinical significance in immunocompromised patints in intensive care units and increasing antimicrobial resistance.[104] Outbreak of nosocomial sepsis in 28 infants from contaminated disinfectant.[105] Outbreak of infection involving 6 neonates by *K. oxytoca* isolated from clincial specimens and water reservoirs of humidifiers and with demonstrated resistance to aztreonam and ceftriaxone.[106] Four (0.2%) of 1,859 neonatal meningitis cases documented between 2006 and 2016 on the French national registry for pediatric bacterial meningitis.[107] Case report of hospital-acquired sepsis in a preterm neonate secondary to *K. oxytoca* contamination of intravenous fluid.[108] Case report of septic arthritis in a neonate following femoral vein puncture.[109]
Leclercia adecarboxylata *Likely non-pathogenic*	1 (BC) 10 d, survived	Environmental bacterium found in food and water, and part of normal gut flora in animals and humans.[110] Rare human pathogen that causes opportunistic infection in immunocompetent individuals or as part of polymicrobial infection inimmunocompetent patients.[110 111] Case reports include urinary tract infections,[110] folliculitis,[112] endocarditis[113] bacteraemia, and wound infections.[111 114] Late-onset bacteraemia and sepsis in premature neonates.[115 116]

Table 2. Culture isolates of unclear clinical significance (continued)

Gram negative	No. (Sample)	Associated clinical syndromes
Pantoea species, Pantoea septica *Likely non-pathogenic*	2 (BC) 0 d, survived 2 d, died 1 (BC) 47 d, died	Ubiquitous bacteria that are rarely pathogenic to humans.[117] Mostly implicated in nosocomial outbreaks among neonates and immunocompromised patients.[118] Case reports of neonatal bacteraemia and sepsis secondary to *Panotea agglomerans*[119-121] which is the most commonly isolated *Pantoea* spp.[118] No case reports in infants due to *Pantoea septica*.
Pseudomonas oryzihabitans *Likely non-pathogenic*	2 (BC) 1 d (n=2), survived	Found in damp environments.[122] Opportunistic human pathogen in patients with indwelling catheters and immunosuppression. Rarely isolated from clinical specimens.[123] Case reports of bacteraemia,[123] meningitis,[124] peritonitis,[125] and endophthalmitis in adults.[126] Sepsis in a 1-year old child with multiple pustular skin rashes.[127]
Pseudomonas stutzeri *Likely non-pathogenic*	5 (BC) 0 d (n=3), survived 4 d, survived 35 d, survived	An environmental bacterium and opportunistic human pathogen in immunosuppressed patients and patients with a recent history of invasive procedures.[128 129] Case reports of bacteraemia,[130] meningitis,[131] endocarditis,[129] septic arthritis,[128] endophthalmitis,[132] and community-acquired vertebral osteomyelitis.[133] No cases reported in infants.
Stenotrophomonas maltophilia *Presumed pathogen*	2 (BC) 0 d, died 6 d, died	An environmental bacterium found in aqueous habitats, including plant rhizospheres, animals, foods, and water sources. Emerging multidrug resistant opportunistic pathogen causing nosocomial and community-acquired infections (including respiratory tract infections, bacteraemia and meningitis) with significant case fatality ratios in children and adults.[134] Reported cases in preterms and term neonates, and young infants include early onset sepsis,[135 136] bacteraemia,[137] meningitis,[138-140] pneumonia,[135]and conjuctivitis.[138] Nosocomial outbreaks in NICUs of colonization and infection associated with contaminated tap water, sinks, humidifiers, ventilators and disinfectants.[141 142]
Unidentified gram-negative fastidious rod *Likely non-pathogenic*	2 (BC) 1 d, survived 2 d, survived	

Abbreviations: BC, blood culture; CSF, cerebrospinal fluid culture.

Maternal admission record used in the Kilifi Perinatal and Maternal (KIPMAT) surveillance study

Admission Record – Kilifi District Hospital Maternity Unit

☐ New Admission ☐ Readmission from home ☐ Readmission from shelter

Mother's admission details (midwife/clinician)

Mother's Name				Date of birth (Age)	
Date of admission	___/___/___	Time of admission	_____ AM / PM		
Hosp No.		Preg ID		PID	
Location		Village		District	
Marital status	Mar☐ Sing ☐ Div ☐ Wid☐	Educational level	None ☐ Prim ☐ Sec ☐ Higher☐	Mobile phone	

			Y	N
TRIAGE ASSESSMENT	Does the patient meet any of the following criteria?	Airway not patent?	Y	N
		Respiratory rate >30 or <10?	Y	N
		Systolic BP >160 or <90?	Y	N
		Diastolic BP >90?	Y	N
		HR <40 or >120	Y	N
		Unconscious or alert only to pain?	Y	N
		Another obstetric emergency (including imminent delivery (<1hr) requiring immediate intervention.	Y	N
IF ANY OF THE ABOVE ARE ANSWERED "Y" PATIENT NEEDS EMERGENCY CARE				

Reason for admission (midwife/clinician)

Admitted by				Initials	
LMP	Known☐ Not Known☐ Month only known ☐			LMP Date	/ /
EDD by dates	/ /			GBD (Gestation by dates)	weeks
EDD by ultrasound (If dating scan was done at <24weeks)					/ /
Presenting complaints:	Abdominal pain – labour	Y	N	Vaginal discharge (offensive)	Y N
	Abdominal pain – other	Y	N	Dysuria	Y N
	Drainage of liquor	Y	N	Cough (<2 weeks)	Y N
	PV bleeding	Y	N	Cough (>2 weeks)	Y N
	Convulsions	Y	N	Fever	Y N
	Visual changes	Y	N	Vomiting	Y N
	Headache	Y	N	Weight loss	Y N
	Decreased foetal movements	Y	N		
	Difficulty in breathing	Y	N	Other	Y N
	Oedema	Y	N	If other, specify :	_____

Obstetric History (midwife/clinician)

Is this the woman's first ever pregnancy (she is nulliparous)?		Y	N	If NO; complete table below.			
Total number of pregnancies (incl.current)		No. of pregs >28 weeks		Born Alive		No. Of pregs <28 weeks	Miscarriage
				Stillborn			Termination
Place of delivery: Hospital (H) Clinic (C) Home/dwelling (D), Other (O)				Complications: None (None), PET/Eclampsia (PET), Ante-partum haemorrhage (APH), Post-partum haemorrhage (PPH), uterine rupture (UR), post-partum sepsis (PPS), obstructed labour (OL), dystocia (Dys), retained placenta (RP), multiple pregnancy (MP), Abortion (ABO) or if other, specify			
Mode of delivery: V=vaginal, C=caesarean, I= vacuum							

	Delivery date (MM/YYYY)	Place of delivery	Mode of delivery	Born Alive	Birth Weight	Gestation	Sex	Alive Now	Pregnancy Complication
1	/	H C D O	V C I	Y N	kg	/40	M F	Y N	
2	/	H C D O	V C I	Y N	kg	/40	M F	Y N	

Antenatal History (midwife/clinician)

No. ANC attendances this pregnancy?	0	1	2	3	4	5	>5	Date first ANC visit	/ /		
Which clinic?											
Number of doses **Malaria** prophylaxis:	0		1		2		3		4		
Number of doses of **TT** immunisation	0		1		2						
VDRL test result this pregnancy	R		NR		ND			Date of result	/ /		
First **Hb** result this pregnancy	____ • __ g/dl				ND			Date of result	/ /		
Blood group	ND	A	B	O	+ve	-ve					
Any transfusion this pregnancy?	Y	N									
De-worming this pregnancy?	Y	N									
Supplements in this pregnancy?	None☐	Iron☐	Folic acid☐	Calcium ☐	Vitamins☐						
Antibiotics in the last 4 weeks?	None☐ for PROM☐ not for PROM☐ Specify Antibiotic _____										
Other medication this pregnancy? (excluding ART)	None☐ TB treatment☐ NSAIDS☐ Steroids☐ Aspirin ☐ Insulin☐ ☐ Other Specify _____										

Examination on admission (clinician/midwife)

Observations	HR	/min	RR	/min	BP	/	Temp	__•__ °C		
					Second BP (at least > 6 hours after first BP)	/				
Measurements	Weight	___•___ kg	Height	___•___ cm	MUAC	___•___ cm	SFH	___cm		
Pres./Lie	Cephalic OA ☐	Cephalic OP ☐	Breech ☐	Transverse/oblique ☐						
	Descent	/5	Cervix	___ cm	Previous abdo scar?	Y N	FHR	☐ Not Heard ___/min		
Were the membranes already ruptured at time of admission?	Y N	IF YES:	Duration of ROM before admission	___days ___ hrs	Liquor	Clear☐ Offensive☐ Mec☐ Not seen☐				
Admitted	Before labour ☐	In labour ☐	Post-delivery ☐							
Examination										

Maternal admission record used in the Kilifi Perinatal and Maternal (KIPMAT) surveillance study (continued)

Delivery	Y	N	BBA	If No, go to section 14
1st Stage	Onset of labour?		No Labour☐ Spontaneous☐ Induced ☐ Induced ☐ Induced ☐	
	PROM (>=18hrs?)			Y
	Antibiotics before delivery?			☐
2nd Stage	Fetal bradycardia? (<110 for 5 mins)			Y
	Fetal tachycardia? (>180 for 5 mins)			Y
	Thick meconium?			Y
	Offensive amniotic fluid?			Y
	Mode of delivery		Vaginal- ☐ C/S – ☐	
	Indication If induction of labour/ CS		Induction of Labour Post term ☐ PROM ☐ PET ☐ Multiple pg ☐ Other mat ☐	
	Duration 2nd stage		H	M
3rd Stage	Placenta Complete	Y	N	
	Perineal Tear	Y	N	
	Episiotomy	Y	N	
	Duration 3rd stage		H	M
Delivery Complication	None☐ APH☐ PPH☐ Uterine ruptu			
	Dystocia☐ Cord prolapse☐ Retained tw			
	Blood loss		_____ mls	
	Other delivery notes:			

12. Newborn details (midwife) – complete all deta

Multiple deliveries?	Y	N
Baby	A	
Born alive	Y	N
PID if born alive		
SBN if born dead		
Date of birth	/ /	
Time of birth	___:___ AM / PM	
Place of Birth	Hospital☐	BBA ☐
Born alive but died in maternity	Y	N
	If Y: ____hrs ___mins from birt	
FSB fresh/MSB skin broken	FSB	MSB
Resuscitation	None☐ O2 ☐ BVM☐ CPR	
Sex	M	F
Gestation		/
Weight		grar
Length	____•____	c
OFC	____•____	c
MUAC	____•____	c
Admitted NNU <1 hr	Y	N
Apgar Score	/1 /5	/10
Placental weight	____g	
	Shared with ☐None	
	☐A ☐B ☐C	
TEO given	Y	N
Vit K given	Y	N
Nevirapine stat given	Y	N

Newborn check-stillbirths and those who die <1 hour of delivery (midwife) If multiple, attach additional sheets

Baby	☐A ☐B ☐C	Baby PID (if born alive)	PID:
Skin	Skin broken	Y N	If Yes:
Head	Head abnormal	Y N	If Yes: abnormal shape☐ other:
	Eyes abnormal	Y N	If Yes:
Chest	Chest abnormal	Y N	If Yes: abnormal chest shape☐ Other:
Abdom	Abdomen abnormal	Y N	If Yes :
	Genitalia abnormal	Y N	If Yes: Ambiguous ☐ Other :
Limbs/ Back	Hands/Feet/Arms/Legs Spine/Back abnormal?	Y N	If Yes: >10 digits ☐ <10 digits☐ feet abnormal shape☐ Other :

Newborn Check- live babies (clinician) Complete within 24 hrs of delivery. If multiple birth, attach additional sheets

Date of Delivery	/ /	Time of delivery	
Date of Newborn check	/ /	Time of newborn check	
		Age of baby in hours	hrs

Location	With mother ☐	Admitted neonatal care ☐	Admitted KEMRI/ HDU☐
Time to first feed after delivery	_____Hrs	_____ mins	Pre-lacteal feed given? Y N
Current feed	Breast milk ☐	Formula☐ IV fluids☐	Feeding well? Y N

	Observations	HR	bpm	RR	/min	O_2 Sat	%	☐On Air ☐On oxygen	Temp	___•__ °C

Baby outcome (fieldworker)

PID/SBN	Baby A:	Baby B:	Baby C:
Baby name (if known)			
Baby outcome (S= stillborn, BD = born alive died in maternity, T= transferred, D=discharged alive, A = absconded)	S BD T D A	S BD T D A	S BD T D A
Completed by (Initials)			
Date	/ /		

Ethical approvals (renewed annually)

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **July 30, 2021**

TO: **DR. CHRISTINA OBIERO**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DEPUTY DIRECTOR, CGMR-C**
KILIFI

Dear Madam,

RE: **SSC PROTOCOL NO. 3007 *(REQUEST FOR ANNUAL RENEWAL)*: AETIOLOGY, CLINICAL PRESENTATION AND OUTCOME OF SEPSIS AND MENINGITIS IN YOUNG INFANTS <60 DAYS OLD ADMITTED TO THE KILIFI COUNTY HOSPITAL: A PILOT STUDY**

Thank you for the continuing review report for the period **July 9, 2020 to July 8, 2021**

This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval** for continuation.

This approval is valid from **August 24, 2021** through to **August 23, 2022**. Please note that authorization to conduct this study will automatically expire on **August 23, 2022**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the **SERU** by **July 12, 2022.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SERU for review prior to initiation.

You may continue with the study.

Yours faithfully,

PROF. CHARLES OBONYO,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **May 25, 2021**

TO: **DR. CHRISTINA OBIERO,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DEPUTY DIRECTOR, CGMR-C,**
KILIFI

Dear Madam,

RE: **PROTOCOL NO. SERU 3853 *(REQUEST FOR ANNUAL RENEWAL)*: CLINICAL INDICATORS OF CONFIRMED BACTERAEMIA OR MENINGITIS IN INFANTS AGED <60 DAYS HOSPITALIZED AT THE KILIFI COUNTY HOSPITAL.**

Thank you for the continuing review report for the period **May 12, 2020** to **April 20, 2021.**

This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval**.

This approval is valid from **June 06, 2021** for a period of **one (1) year**. Please note that authorization to conduct this study will automatically expire on **June 05, 2022**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the SERU by **April 24, 2022.**

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation. You may continue with the study.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

Ethical approvals (renewed annually) (continued)

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **August 19, 2020**

TO: **PROF. JAMES BERKLEY,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DEPUTY DIRECTOR, CGMR-C,**
KILIFI.

Dear Sir,

RE: **PROTOCOL NO. SERU 3513 *(REQUEST FOR ANNUAL RENEWAL)*: INTRAVENOUS AND ORAL FOSFOMYCIN IN HOSPITALIZED NEONATES WITH CLINICAL SEPSIS: AN OPEN-LABEL SAFETY AND PHARMACOKINETIC STUDY (NEOFOSFO).**

Thank you for the continuing review report for the period **July 08, 2019** to **July 29, 2020.**

This is to inform that during the 302ND Committee B meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **August 19, 2020**, the Committee **conducted the annual review and approved** the above referenced application for another year.

This approval is valid from **September 12, 2020** through to **September 11, 2021.** Please note that authorization to conduct this study will automatically expire on **September 11, 2021**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to **SERU** by **July 31, 2021.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to SERU for review prior to initiation.

You may continue with the study.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

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KEMRI/RES/7/3/1 **April 23, 2021**

TO: **DR. CHRISTINA OBIERO**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DEPUTY DIRECTOR, CGMR-C**
KILIFI

Dear Madam,

RE: **SERU PROTOCOL NO. 3001 *(REQUEST STUDY CLOSURE)*: CLINICAL INDICATORS OF ACUTE BACTERIAL MENINGITIS IN CHILDREN IN RURAL KENYA FOLLOWING INTRODUCTION OF *HAEMOPHILUS INFLUENZA* AND *STREPTOCOCCUS PNEUMONIAE* CONJUGATE VACCINES.**

This is to inform you that at the 310th Committee B meeting of the KEMRI Scientific and Ethics Review Unit (SERU) held on **April 21, 2021,** the above study closure report was reviewed.

SERU acknowledges the receipt of the following documents:

1. Cover letter dated 22nd March 2021 and KEMRI SERU Close-out Form
2. Two manuscripts

The Committee noted the following:

1. Study began on **15th March 2015** and was completed on **21st March 2021.**
2. The study aimed to determine the clinical symptoms and signs that can currently be used as indicators for lumbar puncture and/or presumptive treatment of acute bacterial meningitis following introduction of conjugate vaccines.
3. Results and publication
   a. Of 4809 infants aged <60 days included, 81 (1.7%) had definite meningitis. WHO-recommended signs had sensitivity of 58% (95% CI 47% to 69%) and specificity of 57% (95% CI 56% to 59%) for definite meningitis. Addition of history of fever improved sensitivity to 89% (95% CI 80% to 95%) but reduced specificity to 26% (95% CI 25% to 27%). Presence of ≥1 of 5 previously identified signs had sensitivity of 79% (95% CI 69% to 87%) and specificity of 51% (95% CI 50% to 53%).
   b. Among 12,837 admissions, 98 (0.8%) had meningitis. Presence of KCH-2002 signs had sensitivity 86% (95%CI: 77-92) and specificity 38% (95%CI: 37-38). Exclusion of 'fever without malaria parasitaemia' reduced sensitivity to 58% (95%CI: 48-68) and increased specificity to 80% (95%CI: 79-80). IMCI signs had sensitivity 80% (95%CI: 70-87) and specificity 62% (95%CI: 61-63
4. Manuscripts
   a) Obiero, C. W., et al. (2020). "Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital." Arch Dis Child.
   b) Obiero, C. W. et al. Clinical features of meningitis among hospitalized children in Kenya. Undergoing final stages of peer review by BMC Medicine.
5. No specimens collected.
6. Study results will be communicated to the local community once safe to do so given the current Covid-19 pandemic. We plan to feedback results to KEMRI and hospital staff and relevant stakeholders through online platforms.

The Committee approved the close out of the above referenced study and wishes you success in future endeavors.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

References

1. Lishman J, Smit L, Redfern A. Infants 21-90 days presenting with a possible serious bacterial infection - are evaluation algorithms from high income countries applicable in the South African public health sector? *Afr J Emerg Med* 2021;11(1):158-64. doi: 10.1016/j.afjem.2020.09.015 [published Online First: 2021/03/09]
2. WHO IMCI. Integrated Management of Childhood Illness (IMCI) Chart Booklet. Distance Learn Course. 2014;(March):1–76. .
3. Dagan R, Powell KR, Hall CB, et al. Identification of infants unlikely to have serious bacterial infection although hospitalized for suspected sepsis. *The Journal of pediatrics* 1985;107(6):855-60. doi: 10.1016/s0022-3476(85)80175-x [published Online First: 1985/12/01]
4. Baskin MN, O'Rourke EJ, Fleisher GR. Outpatient treatment of febrile infants 28 to 89 days of age with intramuscular administration of ceftriaxone. *The Journal of pediatrics* 1992;120(1):22-7. doi: 10.1016/s0022-3476(05)80591-8 [published Online First: 1992/01/01]
5. Jaskiewicz JA, McCarthy CA, Richardson AC, et al. Febrile infants at low risk for serious bacterial infection--an appraisal of the Rochester criteria and implications for management. Febrile Infant Collaborative Study Group. *Pediatrics* 1994;94(3):390-6. [published Online First: 1994/09/01]
6. Baker MD, Bell LM, Avner JR. Outpatient management without antibiotics of fever in selected infants. *The New England journal of medicine* 1993;329(20):1437-41. doi: 10.1056/NEJM199311113292001 [published Online First: 1993/11/11]
7. McCarthy PL, Sharpe MR, Spiesel SZ, et al. Observation scales to identify serious illness in febrile children. *Pediatrics* 1982;70(5):802-9. [published Online First: 1982/11/01]
8. Lyons TW, Garro AC, Cruz AT, et al. Performance of the Modified Boston and Philadelphia Criteria for Invasive Bacterial Infections. *Pediatrics* 2020;145(4):e20193538. doi: 10.1542/peds.2019-3538 [published Online First: 2020/03/25]
9. Bonadio WA, Hagen E, Rucka J, et al. Efficacy of a protocol to distinguish risk of serious bacterial infection in the outpatient evaluation of febrile young infants. *Clin Pediatr (Phila)* 1993;32(7):401-4. doi: 10.1177/000992289303200703 [published Online First: 1993/07/01]
10. Lacour AG, Zamora SA, Gervaix A. A score identifying serious bacterial infections in children with fever without source. *The Pediatric infectious disease journal* 2008;27(7):654-6. doi: 10.1097/INF.0b013e318168d2b4 [published Online First: 2008/06/10]
11. Gomez B, Mintegi S, Bressan S, et al. Validation of the "Step-by-Step" Approach in the Management of Young Febrile Infants. *Pediatrics* 2016;138(2):e20154381. doi: 10.1542/peds.2015-4381 [published Online First: 2016/07/07]
12. Nigrovic LE, Mahajan PV, Blumberg SM, et al. The Yale Observation Scale Score and the Risk of Serious Bacterial Infections in Febrile Infants. *Pediatrics* 2017;140(1):e20170695. doi: 10.1542/peds.2017-0695 [published Online First: 2017/08/02]
13. Kuppermann N, Dayan PS, Levine DA, et al. A Clinical Prediction Rule to Identify Febrile Infants 60 Days and Younger at Low Risk for Serious Bacterial Infections. *JAMA pediatrics* 2019;173(4):342-51. doi: 10.1001/jamapediatrics.2018.5501 [published Online First: 2019/02/19]
14. Velasco R, Gomez B, Benito J, et al. Accuracy of PECARN rule for predicting serious bacterial infection in infants with fever without a source. *Archives of disease in*

childhood 2021;106(2):143-48. doi: 10.1136/archdischild-2020-318882 [published Online First: 2020/08/21]
15. Nigrovic LE, Malley R, Kuppermann N. Meta-analysis of bacterial meningitis score validation studies. *Archives of disease in childhood* 2012;97(9):799-805. doi: 10.1136/archdischild-2012-301798 [published Online First: 2012/07/06]
16. Weber MW, Carlin JB, Gatchalian S, et al. Predictors of neonatal sepsis in developing countries. *The Pediatric infectious disease journal* 2003;22(8):711-7. doi: 10.1097/01.inf.0000078163.80807.88 [published Online First: 2003/08/13]
17. Young Infants Clinical Signs Study G. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. *Lancet* 2008;371(9607):135-42. doi: 10.1016/S0140-6736(08)60106-3 [published Online First: 2008/01/15]
18. Singh P, Arora A, Strand TA, et al. Predictors of death in infants with probable serious bacterial infection. *Pediatric research* 2018;83(4):784-90. doi: 10.1038/pr.2017.299 [published Online First: 2017/11/23]
19. Wynn JL, Polin RA. A neonatal sequential organ failure assessment score predicts mortality to late-onset sepsis in preterm very low birth weight infants. *Pediatric research* 2020;88(1):85-90. doi: 10.1038/s41390-019-0517-2 [published Online First: 2019/08/09]
20. Fleiss N, Coggins SA, Lewis AN, et al. Evaluation of the Neonatal Sequential Organ Failure Assessment and Mortality Risk in Preterm Infants With Late-Onset Infection. *JAMA Netw Open* 2021;4(2):e2036518. doi: 10.1001/jamanetworkopen.2020.36518 [published Online First: 2021/02/05]
21. Wynn JL, Mayampurath A, Carey K, et al. Multicenter Validation of the Neonatal Sequential Organ Failure Assessment Score for Prognosis in the Neonatal Intensive Care Unit. *The Journal of pediatrics* 2021;236:297-300 e1. doi: 10.1016/j.jpeds.2021.05.037 [published Online First: 2021/05/23]
22. Zeigler AC, Ainsworth JE, Fairchild KD, et al. Sepsis and Mortality Prediction in Very Low Birth Weight Infants: Analysis of HeRO and nSOFA. *Am J Perinatol* 2021 doi: 10.1055/s-0041-1728829 [published Online First: 2021/05/11]
23. Bodaghi B, Dauga C, Cassoux N, et al. Whipple's syndrome (uveitis, B27-negative spondylarthropathy, meningitis, and lymphadenopathy) associated with Arthrobacter sp. infection. *Ophthalmology* 1998;105(10):1891-6. doi: 10.1016/S0161-6420(98)91036-3 [published Online First: 1998/10/27]
24. Bottone EJ. Bacillus cereus, a volatile human pathogen. *Clinical microbiology reviews* 2010;23(2):382-98. doi: 10.1128/CMR.00073-09 [published Online First: 2010/04/09]
25. Manickam N, Knorr A, Muldrew KL. Neonatal meningoencephalitis caused by Bacillus cereus. *The Pediatric infectious disease journal* 2008;27(9):843-6. doi: 10.1097/INF.0b013e31816feec4 [published Online First: 2008/08/06]
26. Lequin MH, Vermeulen JR, van Elburg RM, et al. Bacillus cereus meningoencephalitis in preterm infants: neuroimaging characteristics. *AJNR Am J Neuroradiol* 2005;26(8):2137-43. [published Online First: 2005/09/13]
27. Lebessi E, Dellagrammaticas HD, Antonaki G, et al. Bacillus cereus meningitis in a term neonate. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* 2009;22(5):458-61. doi: 10.1080/14767050802610336 [published Online First: 2009/06/17]
28. Hilliard NJ, Schelonka RL, Waites KB. Bacillus cereus bacteremia in a preterm neonate. *Journal of clinical microbiology* 2003;41(7):3441-4. doi: 10.1128/jcm.41.7.3441-3444.2003 [published Online First: 2003/07/05]

29. John AB, Razak EA, Razak EE, et al. Intractable Bacillus cereus bacteremia in a preterm neonate. *Journal of tropical pediatrics* 2007;53(2):131-2. doi: 10.1093/tropej/fml069 [published Online First: 2006/12/15]
30. Ramarao N, Belotti L, Deboscker S, et al. Two unrelated episodes of Bacillus cereus bacteremia in a neonatal intensive care unit. *American journal of infection control* 2014;42(6):694-5. doi: 10.1016/j.ajic.2014.01.025 [published Online First: 2014/04/15]
31. Lotte R, Herisse AL, Berrouane Y, et al. Virulence Analysis of Bacillus cereus Isolated after Death of Preterm Neonates, Nice, France, 2013. *Emerg Infect Dis* 2017;23(5):845-48. doi: 10.3201/eid2305.161788 [published Online First: 2017/04/19]
32. Gray J, George RH, Durbin GM, et al. An outbreak of Bacillus cereus respiratory tract infections on a neonatal unit due to contaminated ventilator circuits. *The Journal of hospital infection* 1999;41(1):19-22. doi: 10.1016/s0195-6701(99)90032-4 [published Online First: 1999/02/09]
33. Van Der Zwet WC, Parlevliet GA, Savelkoul PH, et al. Outbreak of Bacillus cereus infections in a neonatal intensive care unit traced to balloons used in manual ventilation. *Journal of clinical microbiology* 2000;38(11):4131-6. doi: 10.1128/JCM.38.11.4131-4136.2000 [published Online First: 2000/11/04]
34. Crisafulli E, Aredano I, Valzano I, et al. Pleuritis with pleural effusion due to a Bacillus megaterium infection. *Respirol Case Rep* 2019;7(1):e00381. doi: 10.1002/rcr2.381 [published Online First: 2018/12/05]
35. Ramos-Esteban JC, Servat JJ, Tauber S, et al. Bacillus megaterium delayed onset lamellar keratitis after LASIK. *J Refract Surg* 2006;22(3):309-12. [published Online First: 2006/04/11]
36. Duncan KO, Smith TL. Primary cutaneous infection with Bacillus megaterium mimicking cutaneous anthrax. *J Am Acad Dermatol* 2011;65(2):e60-e61. doi: 10.1016/j.jaad.2011.02.024 [published Online First: 2011/07/19]
37. Guo FP, Fan HW, Liu ZY, et al. Brain Abscess Caused by Bacillus megaterium in an Adult Patient. *Chin Med J (Engl)* 2015;128(11):1552-4. doi: 10.4103/0366-6999.157697 [published Online First: 2015/05/30]
38. Shivamurthy VM, Gantt S, Reilly C, et al. Bacillus pumilus Septic Arthritis in a Healthy Child. *Can J Infect Dis Med Microbiol* 2016;2016:3265037. doi: 10.1155/2016/3265037 [published Online First: 2016/07/02]
39. Kimouli M, Vrioni G, Papadopoulou M, et al. Two cases of severe sepsis caused by Bacillus pumilus in neonatal infants. *Journal of medical microbiology* 2012;61(Pt 4):596-99. doi: 10.1099/jmm.0.033175-0 [published Online First: 2011/12/17]
40. Bentur HN, Dalzell AM, Riordan FA. Central venous catheter infection with Bacillus pumilus in an immunocompetent child: a case report. *Ann Clin Microbiol Antimicrob* 2007;6:12. doi: 10.1186/1476-0711-6-12 [published Online First: 2007/10/31]
41. Asai N, Suematsu H, Yamada A, et al. Brevibacterium paucivorans bacteremia: case report and review of the literature. *BMC infectious diseases* 2019;19(1):344. doi: 10.1186/s12879-019-3962-y [published Online First: 2019/04/27]
42. Janda WM, Tipirneni P, Novak RM. Brevibacterium casei bacteremia and line sepsis in a patient with AIDS. *J Infect* 2003;46(1):61-4. doi: 10.1053/jinf.2002.1076 [published Online First: 2002/12/31]
43. Castagnola E, Conte M, Venzano P, et al. Broviac catheter-related bacteraemias due to unusual pathogens in children with cancer: Case reports with literature review. *Journal of Infection* 1997;34(3):215-18. doi: 10.1016/s0163-4453(97)94199-2
44. Seale AC, Obiero CW, Jones KD, et al. Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya?

The Pediatric infectious disease journal 2017;36(11):1073-78. doi: 10.1097/INF.0000000000001699 [published Online First: 2017/07/22]
45. Oteo J, Aracil B, Ignacio Alos J, et al. [Significant bacteremias by Corynebacterium amycolatum: an emergent pathogen]. *Enferm Infecc Microbiol Clin* 2001;19(3):103-6. doi: 10.1016/s0213-005x(01)72578-5 [published Online First: 2001/05/03]
46. Berner R, Pelz K, Wilhelm C, et al. Fatal sepsis caused by Corynebacterium amycolatum in a premature infant. *Journal of clinical microbiology* 1997;35(4):1011-2. doi: 10.1128/JCM.35.4.1011-1012.1997 [published Online First: 1997/04/01]
47. Djossou F, Bezian MC, Moynet D, et al. Corynebacterium mucifaciens in an immunocompetent patient with cavitary pneumonia. *BMC infectious diseases* 2010;10:355. doi: 10.1186/1471-2334-10-355 [published Online First: 2010/12/18]
48. Cantarelli VV, Brodt TC, Secchi C, et al. Fatal case of bacteremia caused by an atypical strain of Corynebacterium mucifaciens. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases* 2006;10(6):416-8. doi: 10.1590/s1413-86702006000600013 [published Online First: 2007/04/11]
49. Senaobar Tahaei SA, Mencser Z, Reisz Z, et al. An Unusual Case (Metastatic-Like Lesion) of Septic Cerebral Embolus Caused by Corynebacterium mucifaciens in a Diabetic Patient. *J Investig Med High Impact Case Rep* 2020;8:2324709620930930. doi: 10.1177/2324709620930930 [published Online First: 2020/06/05]
50. Sanz-Rodriguez N, Almagro-Molto M, Vozmediano-Serrano MT, et al. [First case of a corneal ulcer associated with Corynebacterium mucifaciens]. *Enferm Infecc Microbiol Clin* 2014;32(8):543-4. doi: 10.1016/j.eimc.2013.11.012 [published Online First: 2014/02/15]
51. Kaya A, Tekkesin AI, Kalenderoglu K, et al. An unusual etiological agent of implantable cardioverter device endocarditis: Corynebacterium mucifaciens. *Indian Heart J* 2016;68(2):190-1. doi: 10.1016/j.ihj.2015.11.022 [published Online First: 2016/05/03]
52. Ogasawara M, Matsuhisa T, Kondo T, et al. Pyogenic spondylitis with acute course caused by Corynebacterium simulans. *J Infect Chemother* 2020;26(3):294-97. doi: 10.1016/j.jiac.2019.10.012 [published Online First: 2019/11/19]
53. Whitworth JM. Lancefield group F and related streptococci. *Journal of medical microbiology* 1990;33(3):135-51. doi: 10.1099/00222615-33-3-135 [published Online First: 1990/11/01]
54. Libertin CR, Hermans PE, Washington JA, 2nd. Beta-hemolytic group F streptococcal bacteremia: a study and review of the literature. *Rev Infect Dis* 1985;7(4):498-503. doi: 10.1093/clinids/7.4.498 [published Online First: 1985/07/01]
55. DeAngelo AJ, Dooley DP, Skidmore PJ, et al. Group F streptococcal bacteremia complicating a Bartholin's abscess. *Infect Dis Obstet Gynecol* 2001;9(1):55-7. doi: 10.1155/S1064744901000102 [published Online First: 2001/05/23]
56. Maki-Ikola O, Peltonen R, Hanninen P. Group F beta-hemolytic streptococcus, tonsillitis and myocarditis. *Scandinavian journal of infectious diseases* 1994;26(6):753-4. doi: 10.3109/00365549409008646 [published Online First: 1994/01/01]
57. Snyder RW, Braun TI. Purulent pericarditis with tamponade in a postpartum patient due to group F streptococcus. *Chest* 1999;115(6):1746-7. doi: 10.1378/chest.115.6.1746 [published Online First: 1999/06/23]

58. Pedro-Aguilar L, Ramirez-Miranda A, Bautista-de Lucio VM, et al. Epidemiology and Outcomes of Kocuria Keratitis. *Eye Contact Lens* 2016;42(5):e20-4. doi: 10.1097/ICL.0000000000000173 [published Online First: 2015/07/30]
59. Kandi V, Palange P, Vaish R, et al. Emerging Bacterial Infection: Identification and Clinical Significance of Kocuria Species. *Cureus* 2016;8(8):e731. doi: 10.7759/cureus.731 [published Online First: 2016/09/16]
60. Gallois N, Piette L, Ortet P, et al. Proteomics data for characterizing Microbacterium oleivorans A9, an uranium-tolerant actinobacterium isolated near the Chernobyl nuclear power plant. *Data Brief* 2018;21:1125-29. doi: 10.1016/j.dib.2018.10.136 [published Online First: 2018/11/21]
61. Fujita A, Kawashima A, Ota H, et al. A cyclic tetrasaccharide, cycloisomaltotetraose, was enzymatically produced from dextran and its crystal structure was determined. *Carbohydr Res* 2020;496:108104. doi: 10.1016/j.carres.2020.108104 [published Online First: 2020/08/17]
62. Grady EN, MacDonald J, Liu L, et al. Current knowledge and perspectives of Paenibacillus: a review. *Microb Cell Fact* 2016;15(1):203. doi: 10.1186/s12934-016-0603-7 [published Online First: 2016/12/03]
63. Gao Z, Tseng CH, Pei Z, et al. Molecular analysis of human forearm superficial skin bacterial biota. *Proc Natl Acad Sci U S A* 2007;104(8):2927-32. doi: 10.1073/pnas.0607077104 [published Online First: 2007/02/13]
64. Ouyang J, Pei Z, Lutwick L, et al. Case report: Paenibacillus thiaminolyticus: a new cause of human infection, inducing bacteremia in a patient on hemodialysis. *Ann Clin Lab Sci* 2008;38(4):393-400. [published Online First: 2008/11/08]
65. Hehnly C, Zhang L, Paulson JN, et al. Complete Genome Sequences of the Human Pathogen Paenibacillus thiaminolyticus Mbale and Type Strain P. thiaminolyticus NRRL B-4156. *Microbiol Resour Announc* 2020;9(15):e00181-20. doi: 10.1128/MRA.00181-20 [published Online First: 2020/04/11]
66. Hunt B, Rogers C, Blais RM, et al. Paenibacillus Sepsis and Meningitis in a Premature Infant: A Case Report. *The American Journal of Forensic Medicine and Pathology* 9000;Publish Ahead of Print
67. Altwegg M, Geiss HK. Aeromonas as a human pathogen. *Crit Rev Microbiol* 1989;16(4):253-86. doi: 10.3109/10408418909105478 [published Online First: 1989/01/01]
68. Citterio B, Francesca B. Aeromonas hydrophila virulence. *Virulence* 2015;6(5):417-8. doi: 10.1080/21505594.2015.1058479 [published Online First: 2015/06/10]
69. Bhowmick UD, Bhattacharjee S. Bacteriological, Clinical and Virulence Aspects of Aeromonas-associated Diseases in Humans. *Pol J Microbiol* 2018;67(2):137-49. doi: 10.21307/pjm-2018-020 [published Online First: 2018/07/18]
70. Diaz A, Velasco AC, Hawkins F, et al. Aeromonas hydrophila-associated diarrhea in a neonate. *Pediatr Infect Dis* 1986;5(6):704. doi: 10.1097/00006454-198611000-00022 [published Online First: 1986/11/01]
71. Kali A, Kalaivani R, Charles P, et al. Aeromonas hydrophila meningitis and fulminant sepsis in preterm newborn: A case report and review of literature. *Indian J Med Microbiol* 2016;34(4):544-47. doi: 10.4103/0255-0857.195383 [published Online First: 2016/12/10]
72. Chaudhari T, Todd DA. Aeromonas hydrophila sepsis in a preterm neonate. *Indian pediatrics* 2009;46(10):913-4. [published Online First: 2009/11/06]
73. Verghese SL, Raju BB, Ramanath A, et al. Septicaemia caused by Aeromonas hydrophilia in a neonate. *J Assoc Physicians India* 1994;42(11):909-10. [published Online First: 1994/11/01]
74. Gupta P, Ramachandran VG, Seth A. Early onset neonatal septicemia caused by Aeromonas hydrophilia. *Indian pediatrics* 1996;33(8):703-4. [published Online First: 1996/08/01]

75. Sirinavin S, Likitnukul S, Lolekha S. Aeromonas septicemia in infants and children. *Pediatr Infect Dis* 1984;3(2):122-5. doi: 10.1097/00006454-198403000-00008 [published Online First: 1984/03/01]
76. Bartolome RM, Andreu A, Xercavins M, et al. Urinary tract infection by Aeromonas hydrophila in a neonate. *Infection* 1989;17(3):172-3. doi: 10.1007/BF01644023 [published Online First: 1989/05/01]
77. Hsueh PR, Teng LJ, Ho SW, et al. Clinical and microbiological characteristics of Flavobacterium indologenes infections associated with indwelling devices. *Journal of clinical microbiology* 1996;34(8):1908-13. doi: 10.1128/JCM.34.8.1908-1913.1996 [published Online First: 1996/08/01]
78. Mukerji R, Kakarala R, Smith SJ, et al. Chryseobacterium indologenes: an emerging infection in the USA. *BMJ Case Rep* 2016;2016:bcr2016214486. doi: 10.1136/bcr-2016-214486 [published Online First: 2016/04/08]
79. Asaad AM, Al-Ayed MS, Qureshi MA. Emergence of unusual nonfermenting Gram-negative nosocomial pathogens in a Saudi hospital. *Japanese journal of infectious diseases* 2013;66(6):507-11. doi: 10.7883/yoken.66.507 [published Online First: 2013/11/26]
80. Chen FL, Wang GC, Teng SO, et al. Clinical and epidemiological features of Chryseobacterium indologenes infections: analysis of 215 cases. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2013;46(6):425-32. doi: 10.1016/j.jmii.2012.08.007 [published Online First: 2012/10/02]
81. Das P, Karade S, Kaur K, et al. Chryseobacterium Indologenes Pneumonitis in an Infant: A Case Report. *J Clin Diagn Res* 2017;11(6):DD07-DD08. doi: 10.7860/JCDR/2017/27237.10047 [published Online First: 2017/08/03]
82. Ozcan N, Dal T, Tekin A, et al. Is Chryseobacterium indologenes a shunt-lover bacterium? A case report and review of the literature. *Infez Med* 2013;21(4):312-6. [published Online First: 2013/12/18]
83. Olbrich P, Rivero-Garvia M, Falcon-Neyra MD, et al. Chryseobacterium indologenes central nervous system infection in infancy: an emergent pathogen? *Infection* 2014;42(1):179-83. doi: 10.1007/s15010-013-0479-y [published Online First: 2013/05/28]
84. Cunha V, Ferreira M, Fonseca AG, et al. Community-acquired Chryseobacterium indologenes in an immunocompetent patient. *JMM Case Reports* 2014;1(1) doi: 10.1099/jmmcr.0.000588
85. Mirza IA, Khalid A, Hameed F, et al. Chryseobacterium Indologenes as a Novel Cause of Bacteremia in a Neonate. *J Coll Physicians Surg Pak* 2019;29(4):375-78. doi: 10.29271/jcpsp.2019.04.375 [published Online First: 2019/03/31]
86. Douvoyiannis M, Kalyoussef S, Philip G, et al. Chryseobacterium indologenes bacteremia in an infant. *Int J Infect Dis* 2010;14(6):e531-2. doi: 10.1016/j.ijid.2009.06.015 [published Online First: 2009/09/05]
87. Mehta R, Pathak A. Emerging Chryseobacterium indologenes Infection in Indian Neonatal Intensive Care Units: A Case Report. *Antibiotics (Basel)* 2018;7(4) doi: 10.3390/antibiotics7040109 [published Online First: 2018/12/19]
88. Ceylan A, Guducuoglu H, Akbayram S, et al. [Sepsis caused by Chryseobacterium indologenes in a patient with hydrocephalus]. *Mikrobiyol Bul* 2011;45(4):735-40. [published Online First: 2011/11/18]
89. Aydin Teke T, Oz FN, Metin O, et al. Chryseobacterium indologenes Septicemia in an Infant. *Case Rep Infect Dis* 2014;2014:270521. doi: 10.1155/2014/270521 [published Online First: 2014/08/13]
90. Alford EL, Shelton CM. Ciprofloxacin and imipenem for Chryseobacterium indologenes presumed meningitis in a preterm neonate. *Pediatric Infectious Disease* 2014;6(3):94-96. doi: 10.1016/j.pid.2014.05.003

91. Hendaus MA, Zahraldin K. Chryseobacterium indologenes Meningitis in a Healthy Newborn: A Case Report. *Oman Med J* 2013;28(2):133-4. doi: 10.5001/omj.2013.35 [published Online First: 2013/04/20]
92. Radera S, Tripathi S, Agarwal J, et al. Chryseobacterium Indologenes-Associated Pneumonia in 2 Neonates. *The Pediatric infectious disease journal* 2017;36(3):337-39. doi: 10.1097/INF.0000000000001426 [published Online First: 2016/11/24]
93. Calderon G, Garcia E, Rojas P, et al. Chryseobacterium indologenes infection in a newborn: a case report. *J Med Case Rep* 2011;5:10. doi: 10.1186/1752-1947-5-10 [published Online First: 2011/01/18]
94. Atici S, Unkar ZA, Erdem K, et al. Ventilator-associated pneumonia caused by Chryseobacterium indologenes: a rare infant case and review of the literature. *Springerplus* 2016;5(1):1741. doi: 10.1186/s40064-016-3449-x [published Online First: 2016/11/01]
95. Almuzara M, Barberis C, Veiga F, et al. Unusual presentations of Comamonas kerstersii infection. *New Microbes New Infect* 2017;19:91-95. doi: 10.1016/j.nmni.2017.07.003 [published Online First: 2017/08/11]
96. Liu XJ, Qiao XW, Huang TM, et al. Comamonas kerstersii bacteremia. *Med Mal Infect* 2020;50(3):288-90. doi: 10.1016/j.medmal.2019.12.005 [published Online First: 2020/03/15]
97. Zhou YH, Ma HX, Dong ZY, et al. Comamonas kerstersii bacteremia in a patient with acute perforated appendicitis: A rare case report. *Medicine (Baltimore)* 2018;97(13):e9296. doi: 10.1097/MD.0000000000009296 [published Online First: 2018/03/30]
98. Almuzara M, Cittadini R, Estraviz ML, et al. First report of Comamonas kerstersii causing urinary tract infection. *New Microbes New Infect* 2018;24:4-7. doi: 10.1016/j.nmni.2018.03.003 [published Online First: 2018/06/21]
99. Coenye T, Goris J, De Vos P, et al. Classification of Ralstonia pickettii-like isolates from the environment and clinical samples as Ralstonia insidiosa sp. nov. *Journal of medical microbiology* 2003;53(4):1075-80.
100. Zhang Z, Deng W, Wang S, et al. First case report of infection caused by Cupriavidus gilardii in a non-immunocompromised Chinese patient. *IDCases* 2017;10:127-29. doi: 10.1016/j.idcr.2017.10.009 [published Online First: 2017/11/22]
101. Tena D, Losa C, Medina MJ, et al. Muscular abscess caused by Cupriavidus gilardii in a renal transplant recipient. *Diagn Microbiol Infect Dis* 2014;79(1):108-10. doi: 10.1016/j.diagmicrobio.2014.01.023 [published Online First: 2014/03/04]
102. Wauters G, Claeys G, Verschraegen G, et al. Case of catheter sepsis with Ralstonia gilardii in a child with acute lymphoblastic leukemia. *Journal of clinical microbiology* 2001;39(12):4583-4. doi: 10.1128/JCM.39.12.4583-4584.2001 [published Online First: 2001/11/29]
103. Karafin M, Romagnoli M, Fink DL, et al. Fatal infection caused by Cupriavidus gilardii in a child with aplastic anemia. *Journal of clinical microbiology* 2010;48(3):1005-7. doi: 10.1128/JCM.01482-09 [published Online First: 2010/01/15]
104. Singh L, Cariappa MP, Kaur M. Klebsiella oxytoca: An emerging pathogen? *Med J Armed Forces India* 2016;72(Suppl 1):S59-S61. doi: 10.1016/j.mjafi.2016.05.002 [published Online First: 2017/01/05]
105. Reiss I, Borkhardt A, Füssle R, et al. Disinfectant contaminated with Klebsiella oxytoca as a source of sepsis in babies. *The Lancet* 2000;356(9226):310. doi: 10.1016/s0140-6736(00)02509-5
106. Jeong SH, Kim WM, Chang CL, et al. Neonatal intensive care unit outbreak caused by a strain of Klebsiella oxytoca resistant to aztreonam due to overproduction of chromosomal beta-lactamase. *The Journal of hospital infection* 2001;48(4):281-8. doi: 10.1053/jhin.2001.1018 [published Online First: 2001/07/20]

107. Carrie C, Walewski V, Levy C, et al. Klebsiella pneumoniae and Klebsiella oxytoca meningitis in infants. Epidemiological and clinical features. *Archives de pediatrie : organe officiel de la Societe francaise de pediatrie* 2019;26(1):12-15. doi: 10.1016/j.arcped.2018.09.013 [published Online First: 2018/12/19]
108. Eshetu B, Gashaw M, Berhane M, et al. Intravenous fluid contaminated with Klebsiella oxytoca as a source of sepsis in a preterm newborn: Case report. *American journal of infection control* 2019;47(7):840-42. doi: 10.1016/j.ajic.2018.12.025 [published Online First: 2019/02/07]
109. Hertting O, Gremark O, Kallman O, et al. An infant with Klebsiella oxytoca septic arthritis. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2018;51(1):153-54. doi: 10.1016/j.jmii.2016.12.011 [published Online First: 2017/07/18]
110. Li J, Park A, Fulmer BR, et al. Leclercia adecarboxylata urinary tract infection in a patient with bladder cancer and recurrent hematuria. *Urology Case Reports* 2021:101579. doi: 10.1016/j.eucr.2021.101579
111. Spiegelhauer MR, Andersen PF, Frandsen TH, et al. Leclercia adecarboxylata: a case report and literature review of 74 cases demonstrating its pathogenicity in immunocompromised patients. *Infect Dis (Lond)* 2019;51(3):179-88. doi: 10.1080/23744235.2018.1536830 [published Online First: 2018/11/30]
112. Broderick A, Lowe E, Xiao A, et al. Leclercia adecarboxylata folliculitis in a healthy swimmer-An emerging aquatic pathogen? *JAAD Case Rep* 2019;5(8):706-08. doi: 10.1016/j.jdcr.2019.06.007 [published Online First: 2019/08/24]
113. Lee B, Sir JJ, Park SW, et al. A case of Leclercia adecarboxylata endocarditis in a woman with endometrial cancer. *Am J Med Sci* 2009;337(2):146-7. doi: 10.1097/MAJ.0b013e31817bf997 [published Online First: 2009/02/14]
114. Jean SS, Lee WS, Bai KJ, et al. Leclercia adecarboxylata bacteremia in a patient with long-term use of nonsteroidal anti-inflammatory drugs. *Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi* 2016;49(3):452-4. doi: 10.1016/j.jmii.2013.09.004 [published Online First: 2013/11/05]
115. Myers KA, Jeffery RM, Lodha A. Late-onset Leclercia adecarboxylata bacteraemia in a premature infant in the NICU. *Acta paediatrica* 2012;101(1):e37-9. doi: 10.1111/j.1651-2227.2011.02431.x [published Online First: 2011/07/29]
116. Nelson MU, Maksimova Y, Schulz V, et al. Late-onset Leclercia adecarboxylata sepsis in a premature neonate. *Journal of perinatology : official journal of the California Perinatal Association* 2013;33(9):740-2. doi: 10.1038/jp.2013.34 [published Online First: 2013/08/30]
117. Kletke SN, Brissette AR, Gale J. Endogenous bacterial endophthalmitis caused by Pantoea species: a case report. *Can J Ophthalmol* 2014;49(1):e1-2. doi: 10.1016/j.jcjo.2013.09.004 [published Online First: 2014/02/12]
118. Gajdács M. Epidemiology and antibiotic resistance trends of Pantoea species in a tertiary-care teaching hospital: A 12-year retrospective study. *Developments in Health Sciences* 2019;2(3):72-75.
119. Tiwari S, Beriha SS. Pantoea species causing early onset neonatal sepsis: a case report. *J Med Case Rep* 2015;9:188. doi: 10.1186/s13256-015-0670-0 [published Online First: 2015/09/06]
120. Bergman KA, Arends JP, Scholvinck EH. Pantoea agglomerans septicemia in three newborn infants. *The Pediatric infectious disease journal* 2007;26(5):453-4. doi: 10.1097/01.inf.0000261200.83869.92 [published Online First: 2007/05/01]
121. Van Rostenberghe H, Noraida R, Wan Pauzi WI, et al. The clinical picture of neonatal infection with Pantoea species. *Japanese journal of infectious diseases* 2006;59(2):120-1. [published Online First: 2006/04/25]
122. Gilardi GL. Pseudomonas and related genera. 1991

123. Nei T, Sonobe K, Onodera A, et al. Two cases with bacteremia suspected to be due to relatively rare Pseudomonas (Flavimonas) oryzihabitans. *J Infect Chemother* 2015;21(10):751-5. doi: 10.1016/j.jiac.2015.06.005 [published Online First: 2015/07/18]
124. Castellanos-Alcarria AJ, Menasalvas-Ruiz AI, Alfayate-Miguelez S, et al. [Pseudomonas oryzihabitans meningitis]. *An Pediatr (Barc)* 2013;79(5):336-7. doi: 10.1016/j.anpedi.2013.02.001 [published Online First: 2013/03/27]
125. Esteban J, Martin J, Ortiz A, et al. Pseudomonas oryzihabitans peritonitis in a patient on continuous ambulatory peritoneal dialysis. *Clin Microbiol Infect* 2002;8(9):607-8. doi: 10.1046/j.1469-0691.2002.00447.x [published Online First: 2002/11/13]
126. Yu EN, Foster CS. Chronic postoperative endophthalmitis due to pseudomonas oryzihabitans. *American Journal of Ophthalmology* 2002;134(4):613-14. doi: 10.1016/s0002-9394(02)01586-6
127. Owusu M, Owusu-Dabo E, Acheampong G, et al. Pseudomonas oryzihabitans sepsis in a 1-year-old child with multiple skin rashes: a case report. *J Med Case Rep* 2017;11(1):77. doi: 10.1186/s13256-017-1230-6 [published Online First: 2017/03/24]
128. Ranieri L, López-Salguero S, González Galván Y, et al. A Case Report: Pseudomonas Stutzeri, An Unusual Cause of Septic Arthritis. *Revista Colombiana de Reumatología (English Edition)* 2020;27(3):215-17. doi: 10.1016/j.rcreue.2019.09.002
129. Halabi Z, Mocadie M, El Zein S, et al. Pseudomonas stutzeri prosthetic valve endocarditis: A case report and review of the literature. *J Infect Public Health* 2019;12(3):434-37. doi: 10.1016/j.jiph.2018.07.004 [published Online First: 2018/07/28]
130. Goetz A, Yu VL, Hanchett JE, et al. Pseudomonas stutzeri bacteremia associated with hemodialysis. *Arch Intern Med* 1983;143(10):1909-12. [published Online First: 1983/10/01]
131. Ma BT, Anuta M, Ferrer P. Pseudomonas stutzeri infection presenting as delayed-onset adult bacterial meningitis in two post-neurosurgery patients: A case report. *Journal of the Neurological Sciences* 2019;405:112. doi: 10.1016/j.jns.2019.10.1781
132. Shah A, Senger D, Garg B, et al. Post cataract Pseudomonas stutzeri endophthalmitis: Report of a case and review of literature. *Indian J Ophthalmol* 2020;68(1):232-33. doi: 10.4103/ijo.IJO_334_19 [published Online First: 2019/12/21]
133. Reisler RB, Blumberg H. Community-acquired Pseudomonas stutzeri vertebral osteomyelitis in a previously healthy patient: case report and review. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1999;29(3):667-9. doi: 10.1086/598650 [published Online First: 1999/10/26]
134. Brooke JS. Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. *Clinical microbiology reviews* 2012;25(1):2-41. doi: 10.1128/CMR.00019-11 [published Online First: 2012/01/11]
135. Basany L, Aepala R. Early onset sepsis with pneumonia in a full term neonate due to Stenotrophomonas maltophilia. *Int J Contemp Pediatr* 2015;2:148-50.
136. Viswanathan R, Singh AK, Ghosh C, et al. Stenotrophomonas maltophilia causing early onset neonatal sepsis. *Indian pediatrics* 2011;48(5):397-9. doi: 10.1007/s13312-011-0063-4 [published Online First: 2011/06/10]
137. Sah R, Siwakoti S, Baral R, et al. Stenotrophomonas maltophilia causing blood stream infection in neonates and infants: a cause for concern. *Trop Doct*

2018;48(3):227-29. doi: 10.1177/0049475517743360 [published Online First: 2017/11/23]
138. Ibrahim J, Hamwi N, Rabei H, et al. Stenotrophomonas maltophilia Meningitis in a Term Healthy Neonate: A Case Report and Literature Review. *Case Rep Pediatr* 2018;2018:1543934. doi: 10.1155/2018/1543934 [published Online First: 2018/07/17]
139. Lo WT, Wang CC, Lee CM, et al. Successful treatment of multi-resistant Stenotrophomonas maltophilia meningitis with ciprofloxacin in a pre-term infant. *European journal of pediatrics* 2002;161(12):680-2. doi: 10.1007/s00431-002-1095-5 [published Online First: 2003/01/23]
140. Rojas P, Garcia E, Calderon GM, et al. Successful treatment of Stenotrophomonas maltophilia meningitis in a preterm baby boy: a case report. *J Med Case Rep* 2009;3(1):7389. doi: 10.4076/1752-1947-3-7389 [published Online First: 2009/10/16]
141. Verweij PE, Meis JF, Christmann V, et al. Nosocomial outbreak of colonization and infection with Stenotrophomonas maltophilia in preterm infants associated with contaminated tap water. *Epidemiol Infect* 1998;120(3):251-6. doi: 10.1017/s0950268898008735 [published Online First: 1998/08/06]
142. Gulcan H, Kuzucu C, Durmaz R. Nosocomial Stenotrophomonas maltophilia cross-infection: three cases in newborns. *American journal of infection control* 2004;32(6):365-8. doi: 10.1016/j.ajic.2004.07.003 [published Online First: 2004/09/30]

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PhD portfolio

Name PhD student: Christina Obiero

PhD period: Sep 2018 - Jul 2022

Names of PhD supervisors: Prof. James A. Berkley & Prof. Michaël Boele van Hensbroek

1. PhD training

	Year	ECTS
General courses		
Amsterdam UMC Doctoral School		
- The Amsterdam UMC World of Science	Oct 2019	0.7
- Talents in PhD	Oct 2019	0.2
- Project Management	Jul 2020	0.6
Specific courses		
- Global Health Project Management, *University of Washington*	Jul-Sep 2019	4.0
- Human Immunodeficiency Virus (HIV) Testing Services, Division of National Acquired Immunodeficiency Syndrome (AIDS) and Sexually Transmitted Infections (STI) Control Program	Feb 2020	1.4
- Antimicrobial Stewardship: Improving Clinical Outcomes by Optimization of Antibiotic Practices, *Stanford School of Medicine*	Sep 2020	0.8
- Improving the Health of Women, Children and Adolescents: from Evidence to Action, *London School of Hygiene & Tropical Medicine*	Dec 2020	0.9
- Measuring Disease in Epidemiology, *Imperial College London*	Dec 2020	0.8
- Predictive Modelling and Transforming Clinical Practice, *University of Colorado*	Jan 2021	0.8
- Clinical Pharmacokinetics: Dosing and Monitoring, *Taipei Medical University*	Mar-Apr 2021	2.9
- Pharmacokinetics: Drug Dosing in Renal Disease, *Taipei Medical University*	Mar-Apr 2021	2.9
- Adaptive design training for CHAIN network, *Mediana*	Aug-Sep 2021	0.4

- Measuring and Modelling Impact in Evaluations, *Johns Hopkins University*	Oct 2021	1.4
- Introduction to Clinical Data Science, *University of Colorado*	Oct-Nov 2021	2.9
- Measuring and Modelling Impact in Evaluations, *Johns Hopkins University*	Oct 2021	1.4
- Insel Basic Pharmacokinetic Modelling Workshop, *University Hospital Bern*	Jan 2022	0.3
Seminars, workshops and master classes		
- 8th Infection Control Africa Network (ICAN) Congress. *Panellist at Workshop 2: Setting up point prevalence surveillance of HAIs – the basics.*	Nov 2021	0.6
- VII Scientific Week of the Graduate Programme on Applied Microbiology and Parasitology (PPGMPA) of the Universidade Federal Fluminense, Rio de Janeiro, Brazil *Participated in a round table discussion on "Antibiotic resistance in maternal and paediatric bacterial infections"*	Nov 2021	0.1
- International Society for Infectious Diseases (ISID) Knowledge Exchange and E-Learning Platform *Panellist: Defeating meningitis by 2030: The need for global invasive meningococcal disease surveillance and prevention*	Apr 2021	0.1
- Kenya Medical Research Institute Annual Scientific and Health (KASH KASH) Biotechnology Webinar	Jul2021	0.1
- Pre- European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) day on antimicrobial resistance	Jul 021	0.3
- World Congress of the World Society for Paediatric Infectious Diseases (WSPID) pre-congress workshop: The Sally Gatchalian Research Workshop	Feb 2022	0.3
Presentations		
- International Congress on Infectious Diseases (ICID) *Oral presentation: Clinical features to distinguish meningitis amongst infants aged <60 days admitted at a rural Kenyan hospital*	Dec 2020	0.6
- Global Antibiotic Research & Partnership Development/St. George's University of London NeoOBS study meeting *Oral presentation: NeoOBS study - Challenges and lessons learned*	Jul 2020	0.6

- 5th African Conference on Emergency Medicine *Oral presentation: Clinical features of meningitis in young infants in rural Kenya*	Nov 2020	0.3
- 8th Infection Control Africa Network (ICAN) Congress. *Oral presentation: HAI PPS for neonates in LMICs – the NeoOBS study*	Nov 2021	0.6
- VII Scientific Week of the Graduate Programme on Applied Microbiology and Parasitology (PPGMPA) of the Universidade Federal Fluminense, Rio de Janeiro, Brazil *Oral presentation: Antimicrobial resistance in paediatric invasive bacterial infection*	Nov 2021	0.1
- 12th Kenya Medical Research Institute Annual Scientific and Health (KEMRI KASH) Conference. Three oral presentations done: *Safety and pharmacokinetics of fosfomycin to treat neonatal sepsis: a randomized clinical trial* *Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital* *Clinical features of bacterial meningitis among hospitalised children in Kenya*	Feb 2022	0.04
- 12th World Congress of the World Society for Paediatric Infectious Diseases (WSPID). Three oral presentations done: *Safety and pharmacokinetics of fosfomycin to treat neonatal sepsis: a randomized clinical trial* *Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital* *Clinical features of bacterial meningitis among hospitalised children in Kenya*	Feb 2022	0.04
(Inter)national conferences		
- American Society of Tropical Medicine & Hygiene annual meeting	Nov 2020	1.4
- 5th African Conference on Emergency Medicine	Nov 2020	0.3
- Uppsala Health Summit: Managing Antimicrobial Resistance (AMR) through behaviour change	Mar 2021	1.1
- JPIAMR Therapeutics Workshop – Feeding the AMR Therapeutics Pipeline	Apr 2021	0.9
- World Sepsis Congress: Advancing prevention, survival and survivorship of sepsis and Covid-19	Apr 2021	0.6

- 31st European Congress of Clinical Microbiology & Infectious Diseases (ECCMID) *Co-chair of oral session: Interventions for improving Covid-19 outcome*	Jul 2021	1.2
- International Symposium on Streptococcus agalactiae disease (ISAAD) Global Conference on Group B Streptococcus	Nov 2021	0.9
- 8th Infection Control Africa Network conference (ICAN)	Nov 2021	1.1
- 12th Kenya Medical Research Institute Annual Scientific and Health (KEMRI KASH) Conference	Feb 2022	0.9
- 12th World Congress of the World Society for Paediatric Infectious Diseases (WSPID)	Feb 2022	0.9
- AMR – Genomes, Big Data and Emerging Technologies, Wellcome Connecting Science, The Wellcome Sanger Institute	Apr 2022	0.9
Other		
Assistant editor, International Journal of Infectious Diseases (IJID)	2020-2022	8
2. Teaching		
- Tutoring, mentoring, supervising of junior clinical research staff		
3. Parameters of esteem		
Grants		
- World Society for Paediatric Infectious Diseases Fellowship, USD 1,650	Nov 2019	
- European Commission, HORIZON-HLTH-2021-DISEASE-04-03, "Innovative approaches to enhance poverty-related diseases research in sub-Saharan Africa, "co-applicant, not funded	Aug 2021	
- World Society for Paediatric Infectious Diseases grant to attend online congress	Feb 2022	
Awards and prizes		
- International Society for Infectious Diseases {ISID), Emerging Leader in International Infectious Diseases	2020-2022	

List of publications

Part of this thesis

Obiero CW, Ngari M, Mwarumba S, Mturi N, Sharland M, van Hensbroek MB & Berkley JA. Validation of clinical predictors of mortality among hospitalised young infants with suspicion of serious bacterial infection. Manuscript in preparation.

Obiero CW, Gumbi W, Mwakio S, Mwangudzah H, Seale AC, Taniuchi M, Liu J, Houpt E, & Berkley JA. (2022). Detection of pathogens associated with early-onset neonatal sepsis in cord blood at birth using quantitative PCR. *Wellcome Open Res, 7*, 3.

Obiero CW, Williams P, Murunga S, Thitiri J, Omollo R, Walker AS, Egondi T, Nyaoke B, Correia E, Kane Z, Gastine S, Kipper K, Standing JF, Ellis S, Sharland M, & Berkley JA (2022). Randomized controlled trial of fosfomycin in neonatal sepsis: pharmacokinetics and safety in relation to sodium overload. *Arch Dis Child*, archdischild-2021-322483.

Obiero CW, Mturi N, Mwarumba S, Ngari M, Newton CR, van Hensbroek MB, & Berkley JA (2021, 2021/06/04). Clinical features of bacterial meningitis among hospitalised children in Kenya. *BMC Med, 19*(1), 122.

Obiero CW, Mturi N, Mwarumba S, Ngari M, Newton C, Boele van Hensbroek M, & Berkley JA (2021). Clinical features to distinguish meningitis among young infants at a rural Kenyan hospital. *Arch Dis Child, 106*(2), 130-136.

Not part of this thesis

Talbert A, Ngari M, **Obiero CW**, Nyaguara A, Mwangome, Mturi N, Ouma N, Otiende M & Berkley JA. Inpatient and post-discharge mortality among young infants admitted to a rural Kenyan hospital. Submitted.

Russell N, Stöhr W, Plakkal N, Cook A, Berkley JA, Adhisivam B, et al. Patterns of antibiotic use, pathogens and clinical outcomes in hospitalised neonates and young infants with sepsis in the NeoAMR global neonatal sepsis sepsis observational cohort study (NeoOBS). Submitted.

Russell N, Stöhr W, Cook A, Berkley JA, Adhisivam B, Agarwal R, et al. The NeoSep Severity and Recovery scores to predict mortality in hospitalized neonates and young infants with sepsis: the global NeoOBS observational cohort. Submitted.

Riddell A, Cook A, Khavessian N, Ellis S, Bilardi D, Correia E, et al. Challenges in the implementation of a global pragmatic observational cohort study to investigate the aetiology and management of neonatal sepsis in the hospital setting. Submitted.

Antimicrobial Resistance, C. (2022, Feb 12). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet, 399*(10325), 629-655.

Gastine S, **Obiero C**, Kane Z, Williams P, Readman J, Murunga S, Thitiri J, Ellis S, Correia E, Nyaoke B, Kipper K, van den Anker J, Sharland M, Berkley JA, & Standing JF (2022, Feb 2). Simultaneous pharmacokinetic/pharmacodynamic (PKPD) assessment of ampicillin and gentamicin in the treatment of neonatal sepsis. *J Antimicrob Chemother, 77*(2), 448-456.

Kane Z, Gastine S, **Obiero C**, Williams P, Murunga S, Thitiri J, Ellis S, Correia E, Nyaoke B, Kipper K, van den Anker J, Sharland M, Berkley JA, & Standing JF (2021, Jun 18). IV and oral fosfomycin pharmacokinetics in neonates with suspected clinical sepsis. *J Antimicrob Chemother, 76*(7), 1855-1864.

Ngari MM, **Obiero C**, Mwangome MK, Nyaguara A, Mturi N, Murunga S, Otiende M, Iversen PO, Fegan GW, Walson JL, & Berkley JA (2020). Mortality during and following hospital admission among school-aged children: a cohort study. *Wellcome Open Res, 5*, 234.

Obiero CW, Ndiaye AGW, Scire AS, Kaunyangi BM, Marchetti E, Gone A M, Schutte LD, Riccucci D, Auerbach J, Saul A, Martin LB, Bejon P, Njuguna P, & Podda A (2017). A Phase 2a Randomized Study to Evaluate the Safety and Immunogenicity of the 1790GAHB Generalized Modules for Membrane Antigen Vaccine against Shigella sonnei Administered Intramuscularly to Adults from a Shigellosis-Endemic Country. *Front Immunol, 8*, 1884.

Obiero CW, Seale AC, Jones K, Ngari M, Bendon CL, Morpeth S, Mohammed S, Mturi N, Fegan G, & Berkley JA (2017). Should first-line empiric treatment strategies cover coagulase-negative staphylococcal infections in severely malnourished or HIV-infected children in Kenya? *PLoS ONE, 12*(8), e0182354.

Seale AC, **Obiero CW**, Jones KD, Barsosio HC, Thitiri J, Ngari M, Morpeth S, Mohammed S, Fegan G, Mturi N, & Berkley JA (2017, Nov). Should First-line Empiric Treatment Strategies for Neonates Cover Coagulase-negative Staphylococcal Infections in Kenya? *Pediatr Infect Dis J, 36*(11), 1073-1078.

Fitchett, Elizabeth JA, et al. "Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI): an extension of the STROBE statement for neonatal infection research." *The Lancet infectious diseases* 16.10 (2016): e202-e213

Seale AC, **Obiero CW**, & Berkley JA (2015, Jun). Rational development of guidelines for management of neonatal sepsis in developing countries. *Curr Opin Infect Dis, 28*(3), 225-230.

Obiero CW, Seale AC, & Berkley JA (2015). Empiric treatment of neonatal sepsis in developing countries. *Pediatr Infect Dis J, 34*(6), 659-661.

About the author

Growing up in a large middle-class Nairobi household, Christina never realized how sheltered she was from the high disease burden and mortality risk faced by majority of Kenya's rural population, until she moved to work in Siaya County. Prior to this, she had completed her medical training and internship at the University of Nairobi and Machakos County Hospital respectively and worked for about two years as a Senior House Officer at the Aga Khan University Hospital (Nairobi).

Christina worked with a great team of research staff in the RTS,S ASO1(Mosquirix™) Phase 3 malaria vaccine trial at the Siaya County Hospital and peripheral health centres. It was while working here that she was inspired to pursue work aimed at improving health outcomes among vulnerable and poorly resourced communities after observing the impact of research capacity on government healthcare facilities' access to medication, diagnostic tests, and medical expertise.

In 2012, Christina received a Joint Japan/World Bank Graduate scholarship that supported her Master of Public Health studies at the Johns Hopkins University, Bloomberg School of Public Health (USA). Her coursework included epidemiology and biostatistics, and her Capstone project was on the prevalence and association of Vitamin D deficiency with pneumonia among hospitalised children. Christina was an exceptional student at Bloomberg and was inducted into the Alpha Chapter of the Delta Omega, the Honorary Society in Public Health. Upon completion of her studies, she moved back to Kenya to work as a clinical investigator at the Kenya Medical Research Institute – Wellcome Trust Research Programme (KWTRP) where she is currently based. This was her second time in Kilifi, having spent about two weeks at the KWTRP while working on her Capstone project.

Christina has led/co-led several research studies in Kilifi since she joined the programme in 2014. Initially, she supported clinical care and several ongoing projects within the Kilifi County Hospital, while working towards identifying an area of research interest. Christina quickly observed the high burden of neonatal morbidity and mortality at KCH, and through extensive literature review, identified key research gaps contributing to this. Her first study as a principal investigator was funded by the GSK Vaccines Institute for Global

Health and investigated the safety and immunogenicity profile of a candidate vaccine (1790GHAB) against *Shigella sonnei*. Following this clinical trial, Christina was funded by the World Health Organization Special Programme for Research and Training in Tropical Diseases (WHO TDR) to conduct a study investigating the causes of early-onset neonatal sepsis in rural Kenya. This is the first study investigating the diagnostic utility of cord blood following delivery in a sub-Saharan Africa setting. Christina plans to carry this work forward by conducting further prospective research on cord blood testing using advanced diagnostics.

Working in Kilifi has solidified Christina's resolve to contribute to reduction of disease burden in vulnerable population. She is particularly passionate about neonates and young children who are known to be at high risk of poor outcomes. Over the past years, her research work converged under a common theme of serious infections in neonates and young children, leading to her registration for a PhD at the University of Amsterdam (the Netherlands). Upon completion of her PhD, she plans to advance her career in further research of cost-effective interventions, and development and implementation of evidence-based guidelines and policies for improved health outcomes.

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