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Neonatal Infections; a hospital-based study in The Gambia examining aetiology and associated maternal colonisation

UDUAK ADIAKOT OKOMO

Thesis submitted in accordance with the requirements for the degree

of Doctor of Philosophy of the University of London

October 2017

Department of Infectious Disease Epidemiology,

Faculty of Epidemiology and Population Health

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

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Declaration by candidate

I, Uduak Adiakot Okomo, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature date: 9th October 2017

Uduak Adiakot Okomo

Abstract

An estimated 2.6 million newborns die each year, mostly from largely preventable causes – prematurity, intrapartum-related complications and infections. Data on neonatal infection aetiology and pathways of acquisition of infection are lacking particularly in sub-Saharan Africa (sSA), yet are essential to inform prevention. In a systematic review of neonatal infection aetiology studies in sSA, *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus*, Group B *Streptococci*, and *Enterococcus* were the top five reported bacterial pathogens across all regions. Application of the Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) checklist highlighted wide variation in clarity and completeness of reporting, impeding comparability and utility.

A four-year audit of neonatal admissions and quality of care at The Gambia's largest referral hospital showed that possible serious bacterial infection (pSBI) accounted for 44% (2166/4944) of admissions. There was a striking mismatch of high antibiotic use (95%) and low microbiological investigation for infection (1% blood culture and 2% lumbar puncture) was evident.

A hospital-based matched case-control study was undertaken in the three main referral health facilities in The Gambia to describe neonatal infection aetiology, and evaluate the role of maternal bacterial colonisation. Sick newborn-mother pairs (n=203) and healthy newborn-mother pairs (n=203) were recruited. Pathogenic bacteria were isolated from blood cultures of 45% (91/202) of the sick newborns, and the most frequently identified isolates were S. *aureus, Klebsiella,* and *Burkholderia cepacia*. There was notable lack of GBS detected by culture but in the molecular sub-study, GBS was identified in 5% (2/42) of cases. *Klebsiella* demonstrated near universal resistance to WHO-recommended first- and second-line antibiotics. For 14 mother-infant pairs, the isolates from infant blood and maternal rectovaginal cultures matched suggesting possible vertical transmission

This PhD shows that infections are a major problem among hospitalised newborns in The Gambia. Programmatic implications and priorities for research are outlined.

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Contributions by the candidate and others to the research presented in this thesis

Unless otherwise noted, I, Uduak Adiakot Okomo, designed the studies, trained field staff, supervised field work and took lead responsibility for ensuring the quality of the data collected. I performed all analyses and wrote up all sections included in this thesis with input from my supervisors and PhD advisory committee members. Although I spent time in the laboratories to familiarise myself with processing samples for culture and molecular diagnostic assays, and carried out minimal laboratory work, the laboratory work reported in this thesis was mostly carried out by others. Specific contributions of others to the work in this thesis are listed below.

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Abbreviations

AMR	Antimicrobial Resistance
ANISA	Aetiology of Neonatal Infection in South Asia
BCG	Bacillus Calmette-Guerin
CAI	Community-Acquired Infection
CDC	Centers for Disease Control and Prevention
CHERG	Child Health Epidemiology Reference Group
CoNS	Coagulase-negative Staphylococci
CSF	Cerebrospinal Fluid
EFSTH	Edward Francis Small Teaching Hospital
EPI	Expanded Programme on Immunization
ESBL	Extended Spectrum β-lactamase
GBS	Group B Streptococcus
HAI	Hospital-Acquired Infection
Hep B	Hepatitis B vaccine
HSV	Herpes Simplex Virus
IAP	Intrapartum Antibiotic Prophylaxis
IMCI	Integrated Management of Childhood Illness
K_2EDTA	Dipotassium Ethylene diamine tetra-acetic acid
KMC	Kangaroo Mother Care
LBW	Low Birth Weight
LSHTM	London School of Hygiene and Tropical Medicine
MDG	Millennium Development Goal
MIC	Minimum Inhibitory Concentration
MRCG	Medical Research Council Unit The Gambia
NHDU	Neonatal High Dependency Unit
NICU	Neonatal Intensive Care Unit
NMR	Neonatal Mortality Rate
NPS	Nasopharyngeal Swab
OPV	Oral Polio Vaccine
PCR	Polymerase chain reaction
PHDU	Paediatric High Dependency Unit
PICU	Paediatric Intensive Care Unit
PMTCT	Prevention of Mother-to-Child Transmission
pSBI	Possible Severe Bacterial Infection
RSV	Respiratory Syncytial Virus
RVS	Recto-vaginal swab
SGA	Small-for-Gestational Age
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
STROBE-NI	Strengthening the Reporting of Observational Studies in Epidemiology-Neonatal Infection Extension
sSA	sub-Saharan Africa

TAC	Taqman Array Card
TBA	Traditional Birth Attendant
VLBW	Very low birth weight
WGS	Whole genome sequencing
WHO	World Health Organization
WISN	Workload Indicators of Staffing Need
YICSSG	Young Infant Clinical Signs Study Group

CHAPTER 1. INTRODUCTION TO GLOBAL BURDEN AND CASE DEFINITIONS FOR NEONATAL INFECTIONS

Picture on the wall at the entrance to the neonatal ward, Edward Francis Small Teaching Hospital, Banjul, The Gambia *(used with permission)*

Overview

This chapter presents the global burden and case definitions for neonatal infection, as well as a description of the different diagnostic methods and the challenges associated with each diagnostic method. It also summarises the pathogens associated with neonatal infection in developed countries, known risk factors for infection, and the potential sources of transmission of infection in the newborn in these settings.

1.1 Global burden for neonatal infections

In 2016, 2.6 million newborns died from largely preventable causes; an estimated 1 million neonatal deaths occurred on the day of birth, and nearly 2 million died in the first week of life.[1] During the 25-year era of the Millennium Development Goals (1990 – 2015) with global targets set for health priorities, a significant overall reduction in under-five mortality was achieved. However, the decline in mortality during the neonatal period (the first 27 days after birth) was much slower than that of post-neonatal under-five mortality resulting in a shift in the concentration of deaths.[1] Consequently, the proportion of child deaths which occur in the neonatal period has increased in all World Health Organisation (WHO) regions over the last 25 years from 40% in 1990 to 46% in 2016, and is projected to increase to 52% in 2030 if current trends continue.[1] Reducing neonatal mortality is therefore increasingly important for ongoing progress for child survival, and also because the health interventions needed to address the major causes of neonatal deaths generally differ from those needed to address other under-five deaths.[2]

Infections are the third most common and preventable cause of global neonatal deaths after prematurity and intrapartum–related conditions (previously termed "birth asphyxia"), making up 23% of neonatal deaths.[3] In high neonatal mortality settings (30

or more neonatal deaths per 1000 live births) infections cause up to 50% of neonatal deaths.[4] The burden of neonatal infections is not limited to mortality and for those neonates who are treated and survive, there is substantial long-term morbidity in the form of neurodevelopmental impairment and disability after meningitis.[5] Reducing newborn morbidity and mortality will therefore require better prevention and case management of severe infections, preterm births, and inpatient supportive care of ill and small newborn babies including the promotion of kangaroo mother care (KMC). To develop research priorities and appropriate strategies for prevention and case management of infection, there is a need to better understand the aetiology of these infections and acquisition pathways.

1.2 Case definitions

Infectious syndromes

Neonatal infection encompasses several infectious syndromes during the neonatal period specifically; Septicaemia (overwhelming infection without much localization), meningitis (predominantly localised to the meninges) and pneumonia (predominantly localized to the lung).[6] Given the "invasive" nature of these infections within normally sterile body sites and their systemic manifestation, they are considered "serious" as opposed to superficial infections of non-sterile sites such as the skin and umbilicus.

Providing standardised definitions of neonatal infections is relevant for global efforts to address neonatal mortality, and a variety of definitions have been proposed and applied in both community and hospital studies.[6] Standardised definitions for global use must be relevant to all populations and settings, specifically developing countries where diagnostic services are limited and where the majority of newborn deaths occur. However, the lack of a standardised clinical or laboratory diagnosis for neonatal

infections, even in high-income settings where laboratory services are readily available,[7] makes it difficult to tailor a one-size-fits-all approach.

The definition proposed by the WHO Young Infant Clinical Signs Study Group (YICSSG) is the most widely applied in developing country settings.[8] It comprises seven clinical "danger" signs, which when used alone or in combination with the others reliably predicts the need for hospitalisation in young infants presenting to health facilities particularly in the first week of life (sensitivity 85%, specificity 75%). These signs formed the basis for the WHO Integrated Management of Childhood Illness (IMCI) referral decision algorithm used at the primary-care level in low-income settings.[9] The presence of any one of these signs and symptoms signals the presence of a serious illness or possible serious bacterial infection (pSBI) thereby prompting health workers to initiate empiric broad-spectrum antibiotic treatment before referral to the next level of care. Although this approach prioritises sensitivity at the expense of specificity, it is justified in developing-country settings where neonatal mortality rates are high and the majority of those caring for newborns even at hospital levels are health workers with limited training and skills.[10] The limited specificity of these signs however, makes it difficult to disaggregate pSBI from overlapping disorders including viral respiratory and other infections as well as non-infectious syndromes which do not require antibiotic treatment (Figure 1.1 below).[11]

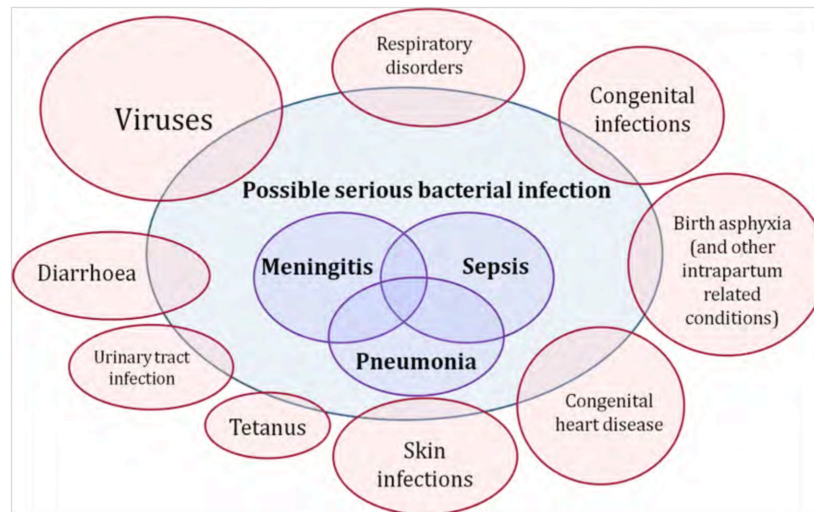


Figure 1.1 Possible serious bacterial infection (pSBI) and overlap with other clinical syndromes in the newborn.

Source: Seale et al 2014 *(Adapted to be consistent with the terminology of "serious")*

The proportion of neonatal respiratory distress attributable to pneumonia for example, depends on the source population (tertiary hospital, district hospital, or community), the stage in the perinatal period, the gestational age of the babies and the availability of intensive care, and the definition of pneumonia.[12] Fast breathing has low specificity as a single sign[13] and the environment may influence temperature.[14] In clinical trial settings, low specificity may introduce misclassification of disease.[15] In order to increase the specificity of diagnosis of clinically diagnosed severe infection, recent clinical trials for simplified antibiotic treatment in Africa and South Asia modified the YICSSG algorithm; excluding fast breathing as a single cause for pSBI, increasing the temperature cut-off for fever from 37.5°C to ≥38°C, and requiring that poor feeding be confirmed by observation.[16] One shortcoming of the YICSSG definition is that the presence of any one of the clinical signs does not provide a distinct diagnosis for sepsis, meningitis, or pneumonia but only gives a reasonable basis for initiating empiric treatment. Furthermore, different pathogens such as bacteria, viruses, fungi or parasites often

present in a clinically indistinguishable pattern in neonates, and localised infections may present with systemic signs making the clinical diagnosis difficult and often impossible without imaging confirmation and/or laboratory support.[6]

In developed or high-income countries, several case definitions for neonatal infections particularly focused on preterm infants have been produced and applied, due to advances in neonatal intensive care.[17-20] More recently, case definitions for neonatal infections have been driven by the need to establish safety and efficacy standards for vaccine trials to protect newborns against specific infections.[6] The Brighton Collaboration Neonatal Infections Working Group recently proposed separate definitions for the three different infection syndromes during the neonatal period (invasive bloodstream infections; meningitis and respiratory tract infections) each with three or more diagnostic levels.[6] Primarily intended to improve data comparability in clinical trials and epidemiological surveillance studies of vaccines in pregnancy, these definitions are also applicable in clinical trials and interventions aimed at reducing neonatal morbidity and mortality. The diagnostic levels do not reflect the different grades of clinical severity but diagnostic certainty within the definition context, with level one being highly specific for the condition. This is to enable capture of all possible cases of neonatal infections regardless of the setting or population in which they are assessed.

Time of onset

Traditionally neonatal infections are classified as early-onset and late-onset infections, according to time and mode of onset. Opinions differ as to what is the appropriate age for differentiating between them and the range is between 2 – 7 days.[21-23] In developed-country settings, early-onset infections are often caused by pathogens acquired by

vertical transmission from the mother, while late-onset infections are caused by pathogens acquired vertically or by horizontal transmission from the home or hospital environment.[21] The distinction in terms of acquisition pathway is blurred in developing-country settings where inadequate hygiene and aseptic measures during delivery and initial care of the baby in health facilities contribute to early-onset infections as well as transmission of pathogens from one mother in labour to another[21] In these settings therefore, early-onset infections may be "maternally-acquired" or "hospital-acquired". Making the distinction between the possible sources of acquisition is often difficult and has led some to classify infections in newborns in developing countries according to the place of birth irrespective of the time of onset; hence, any infection in hospital-born (in-born) baby is regarded as a hospital-acquired infection and any in a home-born (out-born) baby is community-acquired.[21] The advantage of this approach is that it enables the identification of neonates exposed to the health-care environment and facilitates the assessment of the adequacy of hospital-based infection control practices during labour and delivery. The disadvantage is that it fails to take account of the inherent differences in aetiology between infections acquired from the maternal genital tract and those from the hospital environment, which is important as the means of prevention are potentially different.

1.3 Diagnosis

Traditional microbiological methods

Bloodstream infections

Blood culture is often considered to be the "gold standard" for the detection of microorganisms in the blood. The isolation of an organism confers many advantages, including pathogen identification and antibiotic susceptibility testing, which facilitate

correct diagnosis and adjustment of empiric antibiotic therapy as well as the optimal choice and duration of antibiotic treatment.[24, 25] Negative results enable discontinuation of antibiotics thereby reducing the selection of resistant bacteria.

Blood cultures have a higher specificity but lower sensitivity compared to clinical signs.[26, 27] In some reports, only 5-10% of cases of suspected serious infections were positive for any bacterial aetiology by blood culture.[28] The aetiology of more than 90% of suspected serious neonatal infections therefore remains unknown, and many of these infections may possibly be due to bacteria or viruses that are currently unrecognised as causes of infection.[29] The positivity rate of blood cultures is affected by laboratory capabilities, volume of blood inoculated, and administration of antibiotics prior to sampling.[26, 30] False negative results may also arise from administration of intrapartum antibiotics to the mother. The use of automated blood culture systems has improved the sensitivity of blood cultures and has decreased time to detection of positive cultures compared with conventional methods by the use of enriched culture media containing antibiotic-binding resins that facilitate growth and recovery of organisms.[31] Sensitivity is however still less than optimal particularly with blood volumes less than the recommended 1.0mL (common from critically ill premature infants); when there is low-level bacteraemia (≤10 Colony-forming units (CFU)/mL), as many as 60% of cultures will be falsely negative with 0.5mL sample volumes. [24, 25, 32, 33]

Meningitis

Culture of the cerebrospinal fluid (CSF) is critical to establishing the diagnosis of meningitis. Some estimated 15-30% of infants with CSF culture-proven meningitis will have negative blood cultures in the presence of normal CSF parameters (white blood

cells, glucose, and protein).[34, 35] No single CSF parameter value can therefore reliably exclude meningitis.[36, 37]

Pneumonia

Where laboratory facilities are available to identify causative organisms, blood culture has been the primary means of aetiological diagnosis. The diagnostic yield from blood cultures is however low and will therefore underestimate the proportion of pneumonia that is bacterial.[38, 39] Lung aspiration with culture or molecular diagnostics provides a higher diagnostic yield and is more specific for causative pathogen than blood culture but is rarely performed in neonates.[40-43] Chest radiographic imaging is a useful adjunct despite the potentially limited predictive value of radiographic findings.

Molecular diagnostic methods

An ideal diagnostic test for neonatal sepsis should be rapid, sensitive, and specific, while providing detection of all organisms relevant in neonatal sepsis and limiting the effects of maternal antibiotics. The use of molecular methods to identify pathogens causing serious neonatal infections offers higher detection and rapid results and is a valuable adjunct to blood cultures.[44, 45] The sensitivity of molecular methods used to diagnose neonatal sepsis ranges from 41% to 100%, and specificity from 77% to 100% in comparison with blood culture.[46, 47] These variations have been attributed to differences in methodology and study design, including DNA extraction methods, as well as the characteristics of the population studied (term vs preterm infants). State-of-the-art molecular diagnostics are mainly available in well-resourced settings and not in the regions where the burden of neonatal infections is greatest. The positive and negative predictive value of a diagnostic test also depends on the prevalence of sepsis in the

population studied and will therefore differ between resource-limited and resource-rich settings.

An appropriate molecular method is important for simultaneous detection of diverse bacterial and viruses in multiple specimen types to determine the aetiology of infection.[48] Different molecular diagnostic platforms/methods have been developed based on real-time multiplex or multiple singleplex polymerase chain reactions (PCR) for simultaneous identification of multiple aetiological agents. Nucleic acid amplification techniques such as pan-bacterial (16S rRNA) or pan-fungal (18 rRNA) PCR assays are useful when followed by sequencing or microarray/probe hybridization.[49, 50] More advanced multiplex PCR approaches that utilize multiple primer pairs for multiple targets in a single PCR reaction allow for the simultaneous identification of more than one pathogen, with rapid detection times less than 5 hours and up to 90% concordance rates with blood cultures.[51] In addition to the requirement of small sample volumes and rapid diagnosis, molecular diagnostic techniques offer the ability to evaluate virulence and antibiotic resistance markers that may inform antibiotic therapy.[47] However, in a recent systematic review assessing the diagnostic accuracy of various molecular methods for the diagnosis of culture-positive bacterial and fungal sepsis in neonates, molecular tests did not have sufficient sensitivity (>0.98) to replace microbial cultures but were useful add-on tests in the diagnostic work-up.[52]

One technology that has emerged as a useful method for multiple pathogen detection is the TaqMan® Array Card (TAC), (Life Technologies, Foster City, CA, USA). TAC is a 384-well microfluidic card pre-loaded with dried-down individual singleplex real-time PCR (rtPCR) reactions for simultaneous detection of multiple pathogen targets from a single specimen therefore conserving samples, and significantly shortening testing and

response times.[53] One major advantage of the TAC is that total nucleic acids from one clinical specimen can be combined with PCR enzyme-mix, loaded once into a TAC port, and separated by microfluidics into 48 separate reactions capable of amplifying either DNA or RNA. This format allows for 1 – 8 samples (clinical specimen or control material) to be run in parallel against multiple pathogen targets.[53]

TAC has been successful in the detection of respiratory,[54] and enteropathogens,[55-57] and was therefore selected for use in the Aetiology of Neonatal Infection in South Asia (ANISA) study which aims to identify the leading causes of neonatal infections in 5 population-based sites (rural and suburban) in Bangladesh, India, and Pakistan.[58] ANISA is the first study to employ the use of advanced molecular diagnostics for the diagnosis of neonatal sepsis in a high neonatal mortality setting.[58] Two cards – a respiratory card and a blood card - were customised to include common invasive pathogens associated with neonatal infections in the region based on organisms identified as the highest priority for testing in respiratory samples (nasopharyngeal and oropharyngeal swabs), and blood samples. In addition to common respiratory pathogens, specific genomic signatures for *K. pneumoniae* and *E. coli* were also put on the respiratory card as they are common colonisers as well as common invasive pathogens among the neonates of South Asia. One challenge in the use of the TAC by ANISA was that its utility for pathogen detection in human blood samples had not previously been investigated. Studies involving blood from murine infection models had demonstrated low sensitivity of TAC for detection of several bacterial agents.[59] This necessitated modification and optimization of the TAC methodology to improve its sensitivity for pathogen detection particularly in blood samples.[53] The highly-anticipated results from the ANISA study

will provide insights as to the suitability of expanding TAC technology to the clinical setting.[48, 58]

Haematological indices and biomarkers

Haematological indices such as total and differential leucocyte count, neutrophil count and immature neutrophil to total neutrophil ratio, have been investigated as adjunct markers of infection in newborns.[60] In spite of the advantage of not requiring aseptic collection of blood samples, these indices are unreliable especially in the septic newborn where they may be falsely low.[45]

Biological biomarkers (e.g. acute phase reactants, cytokines, genes, proteins) which reflect the host's inflammatory response to infection are useful adjuncts to good clinical acumen and improved molecular diagnostic techniques in confirming serious bacterial infection in young infants and children.[61-69] This is particularly useful in cases of persisting clinical symptoms and in the absence of a confirmatory positive blood culture result. One of the major drawbacks with biomarkers is the difficulty in differentiating sepsis-associated inflammation from non-infectious inflammation. In the newborn, cord and postnatal blood cytokines, for example, can be depressed in the presence of maternal pregnancy-induced hypertension and can rise after induced vaginal or urgent caesarean delivery, delivery room intubation and muscular damage.[70] Another difficulty is defining the "zero hour" for measurement in order to characterise the biomarker as a detector of early or late-onset infection.[66] In a systematic review of the potential of biomarkers as a point-of-care diagnostic for neonatal infection, most studies based the definition on the time of blood collection rather than the first onset of illness or time of presentation.[66] This is important given the differences in time course of release, time to

measurable levels following 'infection,' the half-life, and time to normalise after eradication of the infectious agent.

1.4 Aetiology

Historical reviews from high-income countries have shown an evolution in the bacteriologic profile of pathogens responsible for neonatal infections with time.[71-74] In the USA, *Escherichia coli* and *Staphylococcus aureus* were the principal causes of sepsis in early infancy until the 1970s when Group B streptococci (GBS) emerged as the leading cause of neonatal sepsis.[74] Following the recommendations for risk-based screening for GBS with introduction of intrapartum antibiotic prophylaxis (IAP) to reduce GBS in 1996 and universal screening in 2002, rates of GBS early-onset serious bacterial infection have declined but reflect a continued burden of disease as GBS remains the most frequent pathogen in term infants.[75, 76] Increased administration of IAP has also led to a change in the epidemiology of early-onset infection among very low birth-weight (<1500 g birth-weight) infants; *E. coli* is now the most common cause of early-onset serious bacterial infection in this population,[77, 78] and coagulase-negative staphylococci (CoNS) the most common cause of late-onset infection.[79] The epidemiology of neonatal infection in the UK is similar: GBS and E. coli are the most common organisms causing early-onset serious bacterial infection while CoNS, Enterobacteriaceae and *S. aureus* are the most common organisms associated with late-onset infections.[73] Table 1.1 shows the range of pathogens associated with neonatal infections in developed countries.

Table 1.1 Pathogens associated with neonatal infections in developed countries

Recognised bacterial pathogens [a]			Opportunistic bacterial pathogens [b,c]
Achromobacter spp *A. xylosoxidans* ***Acinetobacter spp:*** *Acinetobacter baumannii* *Acinetobacter lwoffii* Aeromonas species Alcaligenes species *Bacillus cereus* ***Bordetella spp*** *Bordetella bronchiseptica* *Bordetella parapertussis* *Bordetella pertussis* *Burkholderia cepacia* ***Campylobacter spp*** *Campylobacter fetus* *Campylobacter jejuni* Chryseobacterium species ***Citrobacter spp.*** *Citrobacter koseri* *Citrobacter diversus* *Citrobacter freundii* ***Clostridium spp.*** *Clostridium difficile* *Clostridium perfringens* *Eikenella corrodens* ***Enterococcus spp.*** *Enterococcus faecalis* *Enterococcus faecium* *Enterococcus gallinarum*	***Enterobacter spp*** *Enterobacter aerogenes* *Enterobacter agglomerans* *Enterobacter cloacae* *Escherichia coli* *Flavobacterium spp* ***Haemophilus spp.*** *Haemophilus influenzae* *Haemophilus parainfluenzae* ***Klebsiella spp.*** *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumonia* *Lactobacillus spp.* *Listeria monocytogenes* ***Moraxella spp*** *Moraxella. catarrhalis* ***Mycobacterium spp.*** *Mycobacterium tuberculosis* *Morganella morganii* ***Neisseria spp.*** *Neisseria meningitidis* *Neisseria gonorrhoeae* ***Nocardia spp*** *Nocardia asteroides* *Pantoea spp* *Pasteurella species* *Plesiomonas shigelloides*	***Prevotella spp*** ***Proteus spp.*** *Proteus mirabilis* *Proteus vulgaris* ***Providencia spp*** *Providencia rettgeri* *Providencia stuartii* ***Pseudomonas spp*** *Pseudomonas aeruginosa* *Pseudomonas cepacia* *Pseudomonas stutzeri* *Ralstonia spp* *Salmonella spp.* ***Shigella spp.*** *Shigella dysenteriae* *Shigella flexneri* *Shigella sonnei* ***Serratia spp.*** *Serratia liquefaciens* *Serratia marcescens* *Staphylococcus aureus (MSSA or MRSA)* *Streptococcus agalactiae or group B streptococcus* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Stenotrophomonas maltophilia* *Ureaplasma spp*	*Bacillus spp. (other than B. cereus)* ***Bacteroides spp.*** *Bacteroides fragilis* *Coagulase-negative staphylococci* *Corynebacterium spp.* *Diphtheroids* *Micrococcus spp.* ***Propionibacterium spp.*** *Propionibacterium acnae* ***Peptococcus spp.*** *Peptostreptococcus spp.* *Peptostreptococcus magnus* *Peptostreptococcus micros* ***Streptococcus spp.*** *Streptococcus acidominimus* *Streptococcus anginosus* *Streptococcus bovis* *Staphylococcus capitis* *Streptococcus constellatus* *Staphylococcus epidermidis* *Streptococcus equinus* *Staphylococcus haemolyticus* *Staphylococcus hominis* *Streptococcus mitis* *Streptococcus mutans* *Streptococcus oralis*
Fungi		***Viruses***	***Protozoa***
Aspergillus spp *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus glaucus* *Aspergillus niger* *Aspergillus terreus* *Aspergillus versicolor*	***Candida spp.*** *Candida albicans* *Candida dubliniensis* *Candida glabrata* *Candida guilliermondii* *Candida kefyr* *Candida krusei* *Candida parapsilosis* *Candida tropicalis* ***Cryptococcus spp.*** *Cryptococcus neoformans*	Adenovirus Bocavirus Coronavirus Enteroviruses Herpes simplex viruses Human metapneumovirus Influenza Parainfluenza Parechoviruses Parvovirus Respiratory Syncytial Virus Rhinovirus Varicella zoster virus	Plasmodium falciparum Plasmodium knowlesi Plasmodium malariae Plasmodium ovale Plasmodium vivax Toxoplasma gondii Trypanosoma cruzii

spp = species

[a, b] Source: Vergnano et al 2016;[6] Vermont-Oxford Network 2017[80]
[C] Considered opportunistic in the neonatal period

1.5 Risk factors

Maternal, host and environmental factors determine which infant exposed to a potentially pathogenic organism will develop sepsis. An estimated 30-40% of infections resulting in neonatal sepsis deaths are transmitted at the time of childbirth and have

early-onset of symptoms, emphasizing the need to address maternal and environmental sources of infection.[28, 81] Several pre-partum and intra-partum obstetric complications have been associated with an increased risk of infection in the newborn; the most significant of which are premature onset of labour, prolonged rupture of membranes, chorioamnionitis and maternal fever. The immature immune system of the newborn infant is less equipped to provide a robust defence against virulent organisms, particularly the premature and low birth-weight infant due to lack of protective maternal antibodies, underdeveloped innate immunity, and fragile, easily damaged skin.[82]

In developing countries, the likelihood of infection is increased due to other additional risk factors (Table 1.2).

Table 1.2 Risk factors for neonatal infections in developing countries

Maternal Factors [a]	Environmental [a]	Neonatal factors [a]
• Poor antenatal care - Maternal infections - Bacterial vaginosis - Urinary tract infection - Genital tract colonisation • Maternal malnutrition (especially micronutrient deficiency) • Premature or prolonged rupture of membranes • Poor obstetric care and hygiene • Intrapartum related events (including birth asphyxia)	• Environmental contamination • Poor cord care • Poor hand washing • Artificial feeding • Overcrowding • Poor newborn care practices (e.g. early bathing, removal of vernix caseosa, prelacteal feeds, discarding colostrum)	• Complications of prematurity and low birth-weight

[a] Adapted from Bhutta ZA.[83]

Unsafe birthing practices are common; whereas in the other WHO regions over 70% to 99% of all births are attended by skilled health personnel, only 50% of births in sub-Saharan African (sSA) region are attended by a skilled birth attendant,[84] often resulting in unhygienic practices such as delivery onto an unsterile floor, unsterile cord cutting and potentially unsafe cultural customs such as spreading dung on the newborn's umbilicus.[85] Although these risk factors have been linked to poor neonatal outcomes in

sSA,[86] they have not studied in relation to early-onset neonatal bacterial sepsis in the context of high rates of HIV, maternal undernutrition, foetal anaemia, and placental malaria.

1.6 Acquisition pathways

Maternal transmission

The close relationship between mothers and their newborns leads to common risk factors and aetiologies of infectious diseases. Endogenous bacteria (or flora) that commonly colonise the maternal genital tract, and which may or may not cause disease in the mother, can cause "vertical" early-onset infection in the newborn.[87, 88] The maternal genital tract flora normally includes several bacterial species including *Bacteriodes*, *Lactobacillus* species, *Bifidobacterium* species, *E. coli*, GBS, Streptococcus spp, Staphylococci, *Klebsiella* and *Enterobacter* species.[89] Newborns come into direct contact with the bacterial flora in the vaginal canal and perineum during labour and delivery, in which case newborns may acquire infection through the eyes (e.g. gonococcal conjunctivitis), mouth, the umbilicus, or a break in the skin.[90] Ascending infections from the mother to the foetus may occur during labour, when colonised organisms from the maternal perineum spread through the vaginal canal to the placenta, and into the once sterile amniotic fluid.[87] The amniotic fluid, which bathes the foetus, also circulates through its lungs and intestinal tract, which are potential hot spots for bacterial translocation.[91] Figure 1.2 summarises sources of maternal microbial transmission to the foetus and newborn.

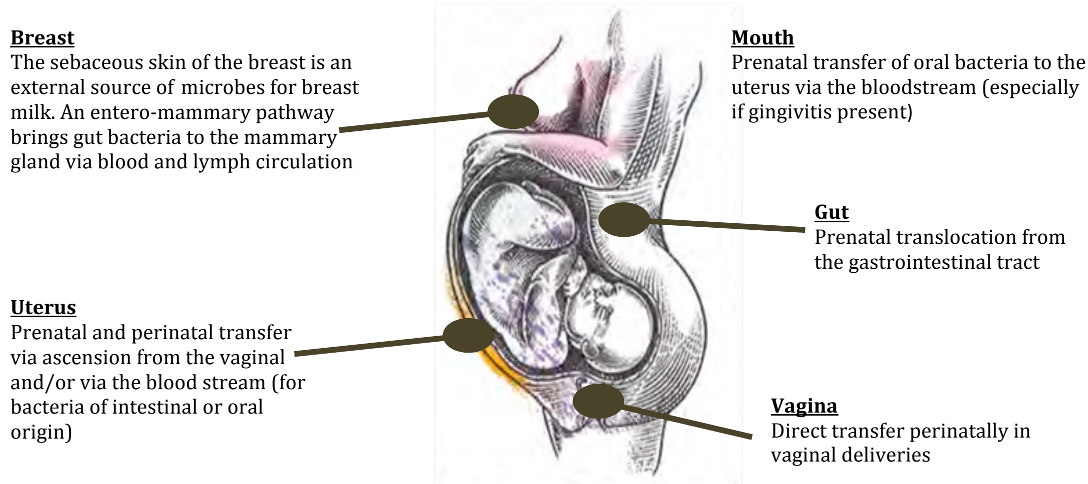


Figure 1.2 Sources of maternal microbial transmission to the newborn.

Source: Adapted from - Funkhouser LJ. Bordenstein SR, (2013) Mom knows best: The universality of maternal microbial transmission. PLoS Biol 11(8): e1001631)

In high-income countries, maternal colonisation with GBS is the most extensively studied in relation to infection in the newborn.[74, 92] Approximately 10%–35% of women are asymptomatic carriers of GBS in the genital and gastrointestinal tracts.[93] At birth, 1 in 2 infants who are born to colonised mothers will themselves be colonised on the skin or mucosal surfaces.[94] Approximately 98% of colonised newborns are without symptoms, but 1%–2% develop early-onset disease, in which sepsis, pneumonia, or meningitis occur during the first week after birth. The odds of laboratory-confirmed GBS infection among newborns of mothers with GBS colonisation are more than nine times higher than newborns of non-colonised mothers.[88]

Environmental transmission and nosocomial outbreaks

In developed countries, late onset sepsis is considered environmental in origin mostly due to invasive infection by community- or hospital-acquired organisms that colonize the infant's skin, respiratory tract, conjunctivae, gastrointestinal tract and umbilicus. In these settings, advances in medical technology that have occurred over the last few

decades have improved the survival and quality of life for neonates, particularly those infants born with extreme prematurity or with congenital defects. These infants are usually hospitalized in the neonatal intensive care unit (NICU) where they can be exposed to and acquire infections from both human and inanimate sources.[95] The most common source of infection in health care facilities are the colonized hands of health care workers, contaminated intravenous solutions, tubing, and supplies such as suction bottles and catheters, feeding tubes, and various environmental surfaces (incubators, mattresses, wash-basins),[21]

In addition to sepsis, pneumonia, infections such as tetanus, and diarrhoea make up a substantial proportion of newborn deaths and are directly related to water, sanitation and hygiene (WASH) conditions during childbirth and the immediate postpartum period through practices such as birth attendant handwashing, cleanliness of the perineum and delivery surface, hygienic cord cutting/care, bathing and feeding practices.[96] Aetiological data on sepsis in developing countries implicate lack of appropriate hygiene during labour, delivery, and post-natal care as major contributors in the development of overwhelming neonatal infections and death both in the hospital and in the community.[97, 98] Unclean practices during delivery and initial care of the baby, at home or in the hospital can also lead to very early onset infections, as well as transmission of pathogens from one mother in labour to another.[21] This makes it difficult to ascribe early-onset infection as “maternally-acquired” or “hospital-acquired”, which has implications for prevention strategies as these differ according to the source of infection.

CHAPTER 2. AIM AND OBJECTIVES

2.1 Aim

The aim of this PhD is to describe the aetiology of neonatal infections and the potential role of maternal bacterial colonisation in acquisition of neonatal infections in The Gambia from the perspective of inpatient care by focusing on sick neonates admitted with pSBI.

2.2 Objectives

1. To systematically review the available data on the aetiology of serious neonatal infections and antimicrobial resistance within sSA.
2. To describe The Gambian context for serious neonatal infections reviewing historical data on neonatal infection aetiology.
3. To retrospectively audit neonatal inpatient care between 2009 and 2013 at The Gambia's largest hospital with a focus on newborns admitted with pSBI, examining outcomes and risk factors for infection.
4. To prospectively recruit cases with pSBI in order to describe the pathogens associated with serious neonatal infections utilising standard microbiological techniques, and explore novel molecular diagnostic methods in a pilot study.
5. To compare the epidemiological profiles of cases with neighbourhood-matched controls.
6. To describe maternal colonisation in the infant cases with pSBI and;

a) To determine what proportion of symptomatic bacterial infections in neonates are maternally acquired, comparing bacterial isolates from mother-newborn case pairs in order to study the extent to which the same isolates are found in both mother and newborn for the following comparisons:

i. Baby blood culture vs mother recto-vaginal flora

ii. Baby nasopharyngeal flora vs mother recto-vaginal flora

iii. Baby nasopharyngeal flora vs mother nasopharyngeal flora

iv. Baby blood culture vs baby rectal flora

b) Compare recto-vaginal colonisation with GBS, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella* species between mothers of neonates with culture-confirmed bacterial infection and mothers of healthy newborns.

7. To describe implications of these results for practice and research in the Gambia and beyond.

2.3 Outline of Thesis

This is a "Book style" thesis with the objectives presented as chapters. There is no overall methods chapter because the methods used to address each objective differ. Detailed methods for each objective are presented in the respective chapter to avoid repetition. By necessity, each chapter also includes a discussion of the results/conclusions presented therein.

Chapter three presents a systematic review of neonatal infection aetiology studies in sSA. It proposes reasons for the 'invisibility' of African biomedical research in large electronic databases. Through a comprehensive search of major medical databases including African databases, the review describes the overall and regional difference in reported aetiology of serious neonatal infections and antimicrobial resistance within sSA. It also presents an assessment of the quality of reporting across identified studies by applying the Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) checklist.

Chapter four describes The Gambian context for neonatal mortality in the millennium development goal era (1990 and 2016) as well as the paucity of local neonatal infection aetiology data, and presents a background to the research questions addressed in the case-control study.

Chapter five describes the audit of neonatal inpatient care that was carried out at the neonatal ward of the national tertiary referral hospital in The Gambia. The clinical care audit contextualises the efforts of the case-control study to describe the aetiology of serious infection among hospitalised newborns. audit has been published in Paediatrics and International Child Health[99] and the printed version is presented in Appendix 3.

Chapter six addresses objectives 4 – 6 and presents the results of a hospital-based case-control study carried out in three major urban/periurban health facilities in The Gambia to describe neonatal infection aetiology, and evaluate the role of maternal bacterial colonisation. This chapter contains only a brief discussion of some of the study findings which are presented in detail in the subsequent chapter.

Chapter seven synthesizes the main findings from the case-control study in relation to the results and conclusions from earlier chapters.

Finally, **chapter eight** discusses the combined implications of this PhD work for researchers and policy makers in The Gambia, and provides recommendations for The Gambia to improve neonatal infection outcomes, and for future research on this topic.

CHAPTER 3. SYSTEMATIC REVIEW OF NEONATAL INFECTION AETIOLOGY IN SUB-SAHARAN AFRICA

Overview

Nearly a quarter of all neonatal deaths sSA are due to serious infections.[100] There is also a significant need-to-treat population with an estimated 2.6 million cases in need of treatment based on a clinical diagnosis of pSBI.[101] The impact of infection-related mortality and the long-term morbidity from neurodevelopmental impairment among survivors on regional health systems already strained by limited resources and shortage of skilled personnel is considerable as neonatal sepsis and infections account for 2.5% disability-adjusted life years in the WHO Africa region.[102] Knowledge of neonatal infection aetiology is critical for the development of appropriate interventions and programme priorities at national and regional level.

3.1 Sub-Saharan African burden for serious neonatal infections – the data gap

There is a significant gap in aetiology-specific data for neonatal infections in sSA, including historical data on the evolution and causative organisms. Pathogens associated with neonatal infection are likely to differ between countries in sSA and from other regions of the world, particularly in the context of cultural and geographic diversity, high rates of maternal HIV infection, and different neonatal care setting and practices; these differences may be of programmatic relevance.

One reason for this paucity of data is the apparent 'invisibility' of African biomedical research in large electronic databases. Primarily due to difficulties accessing them, African research papers have been under-utilised, under-valued and under-cited in the international and African research arenas.[103, 104] The main information resources, published journals and journal articles available to and used by African researchers are the same as those used in Europe and America, and these do not adequately reflect the research output of Africa. Information from the developed world is usually more readily

available than that of developing countries simply because major biomedical databases do not sufficiently include peer-reviewed journals published from developing countries.

Although access to global information resources is essential; equally important and essential is access to the local research output from the continent. African regional research databases such as Africa Wide Information[105] and African Index Medicus[103, 106-108] were launched to improve and facilitate access to African health information, and give greater local, regional and international visibility to African health and biomedical research. Africa Wide Information is an aggregation of 50 databases sourced from Africa, Europe and North America on research and publications by Africans about Africa. The WHO-hosted African Index Medicus consists of grey literature, technical reports, theses and dissertations as well as medical journals (some with full-text articles). African Journals Online (AJOL) provides an online system for the aggregation of African-published scholarly journals and offers global access to, and visibility of, the research output of the continent. The mission of AJOL is to support African research and counter the "North-South" and "West-East" inequality of information flow by facilitating awareness of, and access to, research published in Africa.[109, 110] AJOL hosts over 350 African-published, peer-reviewed scholarly journals from 27 African countries covering a variety of disciplines including health, science and technology, for free – and includes both open access and subscription-based journals. Searches of these African databases are scarcely included in the highly cited reviews on neonatal infections in developing countries,[21, 22, 111-113] and as a result of this limitation in the search strategy, relatively few studies from sSA were included in those reviews.

Widespread health system challenges in providing even the most basic microbiological support in the form of laboratory equipment (e.g. blood culture bottles and culture

media) as well as appropriately trained personnel (e.g. microbiologists and laboratory technicians), even in tertiary referral facilities, have further contributed to the underestimation of the true burden of neonatal infections in the sSA.[99] In the absence of microbiological confirmation, treatment of neonatal infection remains empirical and in such settings, the majority of cases do not receive treatment with the appropriate antibiotic or for the necessary duration.[114] This is further compounded by high uncontrolled rates of antimicrobial prescribing and limited antimicrobial availability because of the essential medicines list.[99] The treatment of neonatal infection is therefore threatened by the steady increase in the prevalence of antimicrobial resistance (AMR) and its potential for excess morbidity and mortality.[115] Estimates from South Asia indicate that up to 60,000 neonates die each year from resistance-attributable neonatal sepsis caused by bacteria resistant to first-line antibiotics.[116]

Where data is available, variation in the clarity and quality of reporting hampers interpretation of burden, distribution, and risk factors. The Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) checklist (Appendix 1) was developed in 2016 to improve scientific reporting of neonatal infection studies and therefore to facilitate reliable comparison of newborn infection data across settings.[117] STROBE-NI is an extension of the 22-item STROBE (The Strengthening the Reporting of Observational Studies in Epidemiology)[118] checklist with 28 additional elements relating to neonatal infection. Implementation of STROBE-NI recommendations and the linked checklist will also increase data utility, allowing meta-analyses and much-needed pathogen-specific burden estimates to inform policy and targeted interventions.

Detailed consideration of neonatal infection aetiology in sSA is needed to guide policy decisions regarding appropriate treatment and prevention. The objective of this review is to synthesize the available data on the aetiology of serious neonatal infections within sSA, antimicrobial resistance, and the quality of data applying the STROBE-NI criteria, in order to inform policy, programme and research priorities in the region.

3.2 Methods

3.2.1 Search strategy and selection criteria

Studies for this review were identified through searches of the following six databases: Medline, Embase, Global Health, PubMed, African Index Medicus (accessed through WHO) and Africa Wide Information. Search terms, identified from Medical Subject Headings (MeSH) related to age, clinical syndromes, and geographical descriptors, as well as terms used for systematic reviews on similar topics, included the following in various combinations: "neonatal", "newborn", "infant", "sepsis", "infection", "pathogen", "bacteria", "virus", "aetiology", "Africa", "sub-Saharan". A compound search strategy was developed for Medline, Embase, and Global Health and these were searched through Ovid. Separate search strategies were developed respectively for PubMed, Africa Wide Information, and African Index Medicus. The search strategy for each database is presented in Appendix 2.

Abstracts and titles from the search and in all languages, were compiled into Endnote (Thomson Reuters) and reviewed individually by myself and a second reviewer (Dr. Edem Akpalu) with a goal to locate articles that seemed to report on laboratory-confirmed serious neonatal infection. All articles flagged by either reviewer as possibilities for inclusion were retrieved in full text (where available) and their reference lists were again independently assessed by both reviewers by use of PubMed

and African Journals Online (AOL) to obtain abstracts as needed. Each article identified by this process as eligible for inclusion was retrieved as full text. I developed a checklist of predetermined inclusion and exclusion criteria which was used for independent assessment of each of the full-text articles by both reviewers. We both made a decision on inclusion versus exclusion, and disagreements were resolved by consensus. Studies were included if they met the following criteria: (1) reported data originating from sSA; and (2) reported microbiological data on infections (bacteraemia/sepsis/septicaemia, meningitis or pneumonia) in neonates. Studies of infections in children were also included where separate neonatal data were presented. Infection was categorized as sepsis/bacteraemia, meningitis, or pneumonia as reported by the authors.

During the abstract review, we excluded obvious case reports/series and studies investigating a single pathogen (as these would lead to a biased estimate of the significance of such a pathogen); studies presenting data for only very high-risk neonatal populations (e.g. very low birth-weight, extremely premature, encephalopathy); and studies focusing on fungal infection, malaria, tetanus, syphilis, tuberculosis, HIV, or other congenital infection. During the full-text review, we further excluded studies with erroneous, incomplete, or internally inconsistent data, and studies that assessed the diagnostic accuracy of any particular test using only positive samples and not in the clinical context of suspected neonatal infection were also excluded. For full-texts not available in the public domain, we made every attempt to contact the authors for copies of the articles. Where the full-text of the article could not be retrieved, but sufficient detail was presented in the abstract, we used data from the abstract. Grey literature (theses and dissertations) were not excluded. The review was restricted to studies published after 1980 in keeping with other published reviews.

3.2.2 Data extraction and synthesis

Descriptive and quantitative data from each paper were extracted individually by both reviewers and entered into a Microsoft Excel 2007 spreadsheet; this included country, hospital setting and location, region of sSA according to regional groupings detailed by the African Union,[119] year of data collection and study time frame, year of publication, specific inclusion or exclusion criteria, and culture techniques. Quantitative data collected included number of neonates, pathogens and contaminants isolated, antimicrobial susceptibilities. To account for variation across studies, the neonatal period was defined as the day of birth up to 30 days of age. Inconsistencies between reviewers after data extraction were resolved by return to the original papers.

3.2.3 Statistical analysis

Data on the number of blood and cerebrospinal fluid (CSF) cultures performed and the number of sample-specific positive cultures from individual studies were compiled to compare the prevalence of bacteraemia and meningitis across regions. The numbers of specific pathogens reported by each study and their antimicrobial resistance (AMR) patterns were also compiled to compare the prevalence of pathogens and AMR across regions. Meta-analysis was not carried out as it was made inappropriate by the existence of marked heterogeneity across studies.

3.3 Results

The online database search done on June 17, 2016, yielded 9052 articles located in at least one of the five databases (Figure 3.1).

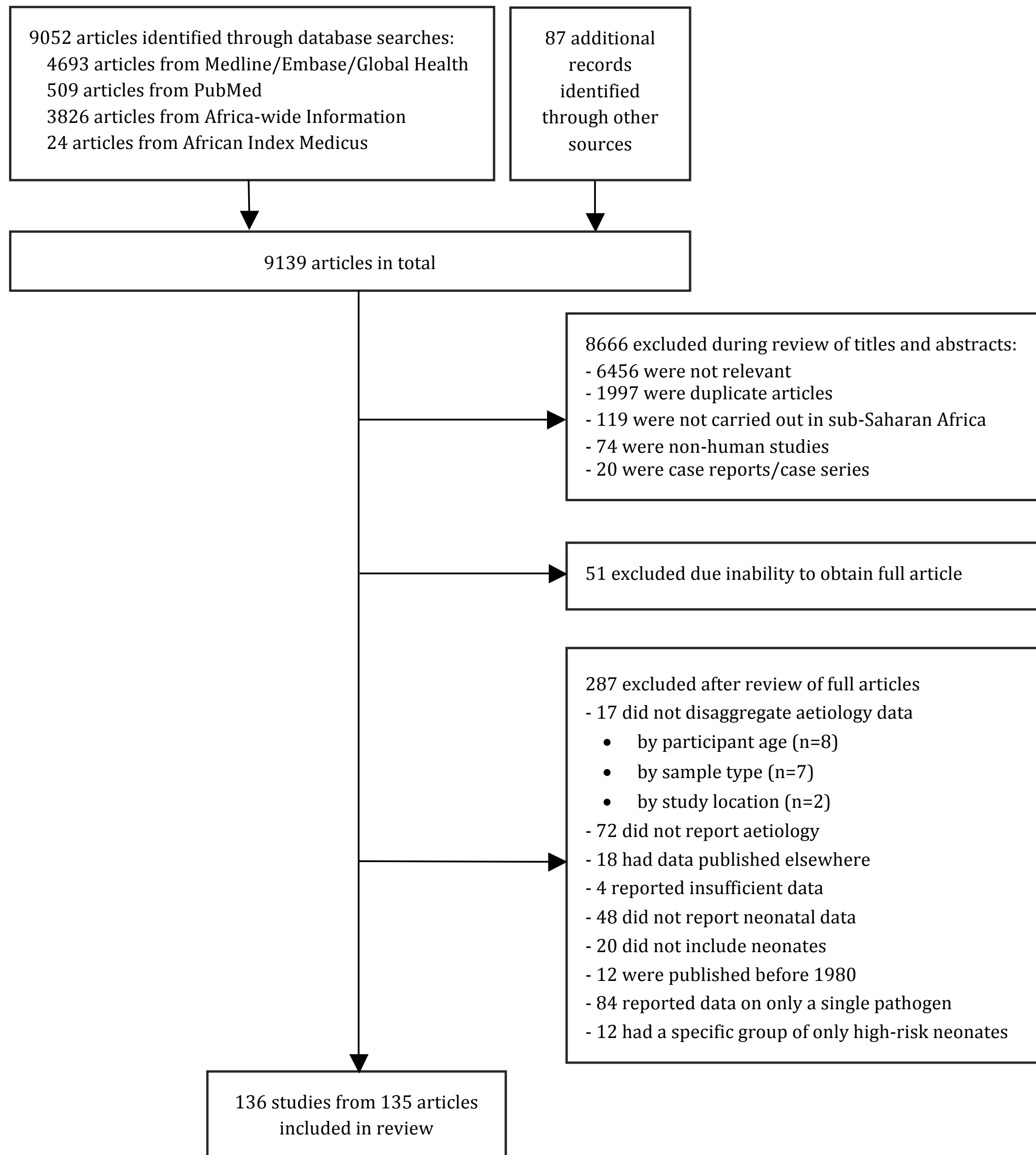


Figure 3.1 Selection of eligible articles

During screening of titles and abstracts, most of these articles were excluded on the basis of irrelevance. Fifty-one articles were further excluded due to inability to locate the full-text as well as not having sufficient data in the abstract. Four hundred and twenty-two full text articles (75 in French, the rest in English) were obtained for detailed evaluation, 87 of these (mostly French articles) had been identified through a

review of references. During detailed screening, 287 articles were excluded. Among these, 72 were excluded because they did not report aetiology data, 48 because they did not report neonatal data, and 17 because they did not disaggregate data by age, sample type or study location (for multicountry studies). Eighteen studies were excluded based on the same data having been published in another journal. In all, 135 studies, including 9 theses/dissertations carried out between 1984 and 2016 were eligible for inclusion; however, one multi-country study with two West African country sites was counted as two separate studies bringing the total number of included studies to 136 (121 in English and 15 in French). Seven studies were from Central Africa, 32 from East Africa, 33 from Southern Africa, and 64 from West Africa. (Figure 3.2).

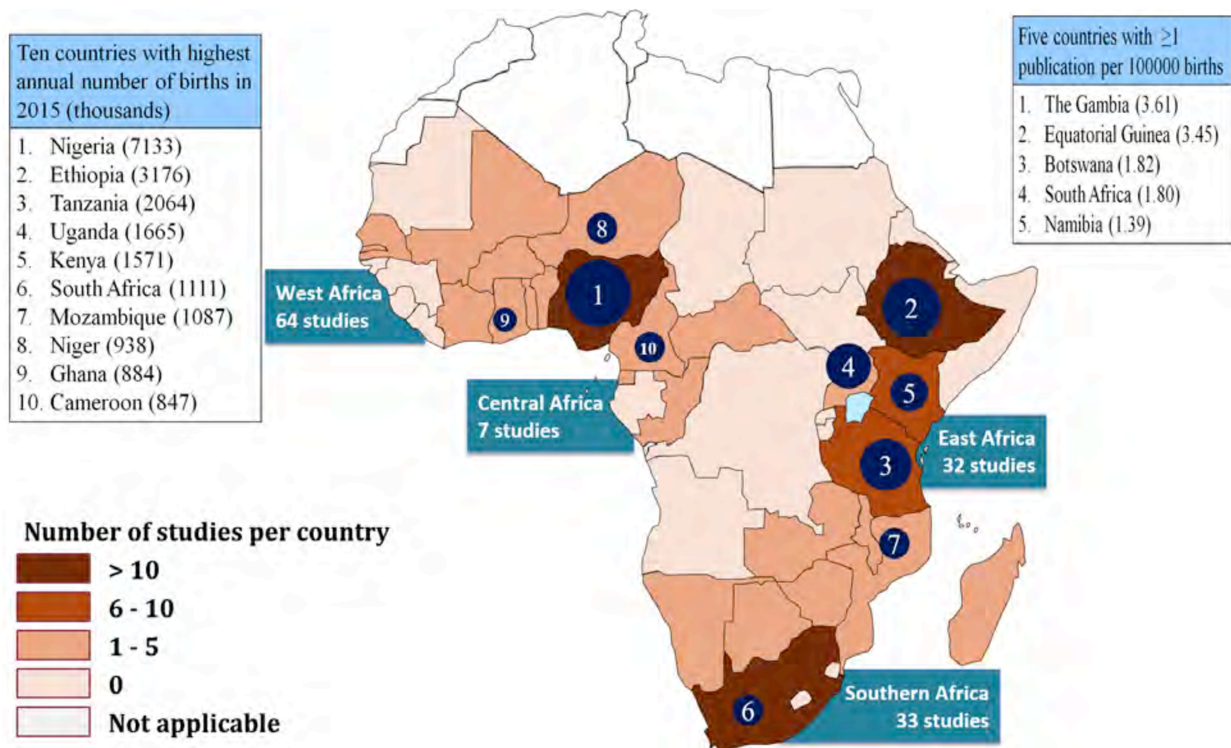


Figure 3.2 Variation between countries within sub-Saharan Africa in the number of studies reporting microbiological data on serious neonatal infections (bacteraemia/sepsis, meningitis or pneumonia

Source of data on annual births: State of The World's Children 2016 Report

The publication ratio (number of studies per 100 000 births) was calculated for each country as an indicator of the reporting of neonatal infection aetiology. Although Nigeria, South Africa and Kenya had the most studies, the publication ratio was highest in The Gambia with 3.61 publications per 100,000 births.

All studies were facility-based; however, 10 studies - five from Kenya,[120-124] two from Mozambique,[125, 126] two from Nigeria,[127, 128] and one from Cameroon[129] - reported data from predominantly rural populations. Seventy-six studies were prospective studies of newborns admitted with clinically suspected invasive bacterial disease, sepsis, septicaemia, and/or meningitis, and one South African study involved a neonatal cohort recruited in during a randomised controlled trial investigating the efficacy of maternal and newborn chlorhexidine washes in preventing neonatal sepsis.[130] The remaining studies were retrospective reviews of laboratory and clinical records of neonatal inpatients. The characteristics of included studies are summarised in Table3.1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 - 2016

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Central Africa	Chiabi (2011)[131]	2008-2009	Retrospective	Yaounde Gynaeco-Obstetric and Pediatric Hospital, Cameroon	Admissions to urban tertiary referral hospital	218 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood & CSF	**Blood:** Klebsiella spp 9, *E. coli* 5, GBS 2, Enterobacter spp 2, Citrobacter spp 1, *P. mirabilis* 1 *A. baumanii* 2, , *Chryseomonas luteola* 1, Non-Group A or B Streptococcus 1, GDS 1, GAS 1, **CSF:** Enterobacter spp 1, GBS 1
	Chiabi (2005)[129]	1998-2000	Prospective	Rural Hospital, Yaounde, Cameroon	Admissions to rural hospital	154 neonatal sepsis admissions	Blood	*E. coli* 10, Others 2
	Kago (1990)[132]	1985-1986	Retrospective	Hopital central de Yaounde, Cameroon	Admissions to urban tertiary referral hospital	56 inborn and outborn neonatal bacterial meningitis admissions (0-30 days)	CSF	GBS 15, *S. pneumoniae* 9, *E. coli* 7, Acinetobacter spp 5, *E. cloacae* 1, Flavobacterium 1, *P. mirabilis* 2, GGS 2, GDS 1, *L. monocytogenes* 1, *H. influenzae* 1, Pseudomonas 1, Serratia 1, *Pasturella pneumotropica* 1
	Kemeze (2016)[133]	2015	Prospective	Laquitinie Hospital, Douala, Cameroon	Admissions to district referral hospital	300 neonates with suspected bacterial infection (0-28 days)	Blood & CSF	**Sepsis :** *S. aureus* 10, *E. coli* 6 **Meningitis:** *E. coli* 1, Klebsiella spp 1,
	Bercion (2008)[134]	2004-2005	Prospective	Complexe Pediatrique de Bangui, Central African Republic	Admissions to urban tertiary referral hospital	417 paediatric CSF samples collected (1 day-16 years)	CSF	*S. pneumoniae* 3, *H. influenzae* 2, Salmonella spp 2, GBS 2, Others 1
	Ekouya Bowassa (2015)[135]	2014	Prospective	CHU de Brazaville, Congo	Admissions to neonatal unit of an urban tertiary referral hospital	130 early neonatal admissions (0-4 days)	Blood	*K. pneumoniae* 7, K. terrigena 4, *Klebsiella* spp 1, *S. aureus* 3, *Staphylococcus haemolyticus* 3, *Staphylococcus hominis* 1, *Staphylococcus lentus* 1, *Staphylococcus cohnii* 1, *E. cloacae* 2, *E. coli* 2, *Streptococcus equinus* 2, *GAS* 1, Streptococcus spp 1
	Shatalov (2015)[136]	2013-2015	Prospective	La Paz Medical Center, Malabo, Equatorial Guinea	Admissions to urban tertiary referral hospital	293 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*K. pneumoniae* 16, Acinetobacter spp 4, *E. coli* 4, CoNS 4

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
East Africa	Ghiorghis (1997)[137]	1993-1993	Retrospective	Ethio-Swedish Childrens' Hospital, Addis Ababa, Ethiopia	Admissions to urban tertiary referral hospital	542 inborn and outborn neonatal sepsis admissions	Blood	Klebsiella spp 34, *E. coli* 9, Pseudomonas spp 5, *S. epidermidis* 13
	Dagnew (2013)[138]	2006-2012	Retrospective	University of Gondar Hospital, Gondar, Ethiopia	Laboratory data from admissions to urban tertiary referral hospital	34 neonatal admissions with suspected septicaemia (< 28 days)	Blood	CoNS 7, *S. aureus* 2, *E. coli* 1, Klebsiella spp 1, Salmonella spp 1
	Gebrehiwot (2012)[139]	2011-2012	Prospective	Gondar University Hospital, Gondar, Ethiopia	Admissions to neonatal unit of an urban tertiary referral hospital	181 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 17, *K. pneumoniae* 5, *Klebsiella ozaenae* 10, Klebsiella spp 1, *E. coli* 6, CoNS 4, Salmonella spp 2, Shigella spp 2, *Serratia* spp 1, Enterobacter spp 1, α-haemolytic Streptococci 1, Citrobacter spp 1, other Gram negatives 7,
	Gebremariam (1998)[140]	1987-1996	Retrospective	Tertiary Hospital, Addis Ababa, Ethiopia	Admissions to urban tertiary referral hospital	55 neonatal meningitis admissions (0-28 days)	CSF	*K. pneumoniae* 9, *E. coli* 7, Enterobacter spp 4, *S. aureus* 2, Salmonella spp 2, *S. pneumoniae* 2, *S. epidermidis* 2, GAS 1, Haemophilus spp 1
	Muhe (1999)[141]	1991-1993	Prospective	Ethio-Swedish Childrens' Hospital, Addis Ababa, Ethiopia	Admissions to neonatal unit of an urban tertiary referral hospital	405 sick and 411 well young infants enrolled (<91 days); 440 infants investigated	Blood & CSF	**Blood:** GAS 9, *E. coli* 9, *S. pneumoniae* 4, Salmonella spp 3, *H. influenzae* 1, *K. pneumoniae* 1, *S. aureus* 1 **CSF:** *S. pneumoniae* 1, GAS 1, *E. coli* 1.
	Mulu (2005)[142]	2002-2003	Retrospective	Gondar University Hospital, Gondar, Ethiopia	Laboratory data from admissions to urban tertiary referral hospital	35 neonates with suspected meningitis investigated by lumbar puncture	CSF	*N. meningitidis* 2
	Mulu (2014)[143]	2013-2014	Prospective	Tikur Anbessa and Yekatit 12 specialized hospitals, Addis Ababa, Ethiopia	Admissions to a paediatric ward of an urban tertiary referral hospital	154 inborn and outborn neonatal admissions with suspected meningitis investigated by lumbar puncture (1 - 30 days)	CSF	*S. pneumoniae* 4, *N. meningitidis* 1, Citrobacter spp1, Proteus spp 1, Acinetobacter spp 1, *P. aeruginosa* 1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
East Africa (continued)	Negussie (2015)[144]	2011-2012	Prospective	Tikur Anbessa Specialized University Hospital and Yekatit-12 Hospital, Addis Ababa, Ethiopia	Admissions to paediatric ward of urban tertiary referral hospital	147 neonatal outpatients and inpatients (0-28 days)	Blood	*S. marcescens* 12, S aureus 9, *K. pneumoniae* 5, *Klebsiella terrigena* 4, CoNS 8, *Salmonella Paratyhi C* 3, *E. cloacae* 2, *A. baumanii* 1, *Pseudomonas luteola* 1, Enterococcus spp 1.
	Shitaye (2010)[145]	2006-2007	Prospective	Tikur Anbessa Specialized University Hospital, Addis Ababa, Ethiopia	Admissions to neonatal unit of an urban tertiary referral hospital	302 neonates with suspected sepsis (0-28 days)	Blood	*K. pneumoniae* 50, *Klebsiella terrigena* 3, *S. aureus* 30, *E. coli* 10, CoNS 10, Enterococcus spp 7, *GAS* 6, *S. pneumoniae* 6, *S. marcescens* 5, *P. mirabilis* 4, *Serratia ficaria* 4
	Zewdie (2014)[146]	2013-2014	Prospective	Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia	Admissions to neonatal unit of an urban tertiary referral hospital	115 inborn and outborn neonatal admissions with suspected meningitis investigated by lumbar puncture (0 - 28 days)	CSF	*P. aeruginosa* 1, *E. coli* 1. *S. pneumoniae* 2, *K. pneumoniae* 1, Acinetobacter 1.
	Berkley (2005) [120]	1998-2002	Prospective	Kilifi District Hospital, Kenya	Admissions to paediatric ward of rural district referral hospital	867 inborn and outborn neonates (0-6 days)	Blood	*E. coli* 25, Acinetobacter spp 16, Klebsiella spp 13, GBS 11. *S. aureus* 7, Pseudomonas spp 6, *S. pneumoniae* 5, GAS 3, NTS 1, *H. influenzae* 1, other Gram negative 30, Other Gram positive 11.
	English (2003) [121]	1999-2001	Prospective	Kilifi District Hospital, Kenya	Admissions to paediatric ward of rural district referral hospital	692 inborn and outborn neonates (0-30 days)	Blood & CSF	GBS 12, Klebsiella spp 10, *E. coli* 9, *S. pneumoniae* 7, Pseudomonas spp 6, GAS 5, *S. aureus* 4, Enterococcus spp 4, *P. mirabilis* 4, *H. influenzae* 1, Others 3
	Kasiyere-Banda (1983)[147]	1982-1983	Prospective	Kenyatta National Hospital, Nairobi, Kenya	Admissions to neonatal unit of an urban tertiary referral hospital	939 inborn and outborn neonatal admissions	Blood/CSF	Klebsiella species 28, Staphylococcus albus 22, *E. coli 8*, Streptococcus fecalis 8, *S. aureus* 5, Citrobacter species 5, *P. aeruginosa* 3, GBS 3, Salmonella typhimurium 2, Acinetobacter species 3.
	Kumar (2010) [148]	2005	Prospective	Kenyatta National Hospital, Nairobi, Kenya	Admissions to neonatal unit of an urban tertiary referral hospital	310 inborn and outborn neonatal sepsis admissions	Blood & CSF	*Enterobacter agglomerans* 22, *K. pneumoniae* 11, CoNS 11, Citrobacter 10, Acinetobacter spp 7, E. *coli* 5, *S. aureus* 4, Enterococcus 4, *Salmonella typhi* 1,

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
East Africa (continued)	Kohli-Kochhar (2011) [149]	2000-2009	Retrospective	Aga Khan University Hospital, Nairobi, Kenya	Admissions to NICU of urban tertiary referral private hospital	665 inborn and outborn neonatal sepsis admissions	Blood	S. epidermidis 52, *S. aureus* 41, Klebsiella spp 14, Streptococcus spp 12, Enterococcus spp 12, Kluyvera spp 2, Aeromonas spp 2, *P. aeruginosa* 1, *E. coli* 2, *B. cepacia* 2, Pasturella spp 1, Acinetobacter spp 1, Enterobacter spp 2, Acrobacterium spp 1, Serratia spp 1, GBS 1. *S. pneumoniae* 1,
	Laving (2003) [150]	1999	Prospective	Kenyatta National Hospital, Nairobi, Kenya	Admissions to neonatal unit of an urban tertiary referral hospital	84 inborn and outborn neonatal sepsis admissions	Blood & CSF	**CSF culture positive -** *K. pneumoniae* 2, GBS 1, *E. cloacae* 1 ***(Latex particle agglutination & Gram stain:*** *E. coli 7, GBS 3, H. influenzae 1, S. pneumoniae 1)*
	Musoke (2000) [151]	1997-1998	Retrospective	Kenyatta National Hospital, Nairobi, Kenya	Admissions to neonatal unit of an urban tertiary referral hospital	192 inborn and outborn neonatal sepsis admissions	Blood	Klebsiella spp 38, Citrobacter spp 26, Enterobacter spp 19, *S. epidermidis* 19, *S. aureus* 9, *E. coli* 4, Enterococci 4, *Salmonella typhimurium* 2,
	Mwangi (2002) [122]	1994-2000	Prospective	Kilifi District Hospital, Kenya	Admissions to paediatric ward of rural district referral hospital	390 paediatric meningitis admissions (78 neonates with confirmed, probable or possible meningitis)	CSF	*S. pneumoniae* 13, *H. influenzae* 2, NTS 3, other Enterobacteriaceae 11, GAS/GBS 14
	Mwaniki (2011) [123]	2001-2005	Prospective	Kilifi District Hospital, Kenya	Admissions to paediatric ward of rural district referral hospital	1790 inborn and outborn neonates (0-6 days); 845 lumbar punctures performed	CSF	Enterobacter spp 3, GBS 2, GDS2, *S. viridans* 1, *S. aureus* 1, *E. coli* 1, *H. influenzae* 1, *P. aeruginosa* 1, *S. pneumoniae* 1, *K. pneumoniae* 1, Acinetobacter spp 1, Aeromonas spp 1, Kluyvera spp 1, *Vibrio Cholera* 1, other Gram positive 2.
	Simiyu (2003) [152]	2000	Retrospective	Kenyatta National Hospital, Nairobi, Kenya	Admissions to neonatal unit of an urban tertiary referral hospital	219 Inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	CoNS 8, Citrobacter 6, *S. aureus* 5, Klebsiella 3, *Alkaligenes fecalis* 1, Acinetobacter 1, Enterococci 1, *P. aeruginosa* 1, *E. coli* 1,
	Razafindralambo (2004)[153]	1988-1990	Prospective	Antatanarivo, Madagascar	Admissions to urban tertiary referral hospital	134 children with culture-confirmed meningitis (0-15 years)	CSF	*S. pneumoniae* 2, *N. meningitidis* 2

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
East Africa (continued)	Talbert (2010) [124]	2001-2009	Prospective	Kilifi District Hospital, Kenya	Admissions to paediatric ward of rural district referral hospital	4467 outborn infants with invasive bacterial infection (0-60 days)	Blood & CSF	**Bacteraemia:** Klebsiella spp 46, *S. aureus* 44, Acinetobacter spp 38, *E. coli* 36, Enterobacter spp 31, GAS 30, GBS 29, GDS 24, *S. pneumoniae* 22, Aeromonas spp 21, Salmonella spp 20, *S. viridans* 15, *P. aeruginosa* 9, Vibrio spp 8, Proteus spp 5, GGS 4, GCS 1, *H. influenzae* 2, *N. meningitidis* 1, Others 9 **Meningitis:** GBS 14, *S. pneumoniae* 12, Salmonella spp 8, Enterobacter spp 6, *E. coli* 6, GAS 4, GDS 1, Klebsiella spp 4, Vibrio spp 4, *P. mirabilis* 1, *Aeromonas sobria* 1, *N. meningitidis* 1, *S. viridans* 1, other Gram-negative rods 2.
	Andrianarivelo (2010) [154]	2009	Prospective	CHU d'Antatanarivo, Madagascar	Admissions to NICU of urban tertiary referral hospital	105 inborn and outborn neonates investigated by blood cultures	Blood	CoNS 51, *K. pneumoniae* 18, *Enterobacter aerogenes* 8, Enterobacter gergoviae 8, *Enterobacter sakazaki* 1, Enterococcus 9 *K. oxytoca* 7, *E. coli* 6, *S. aureus* 5, Serratia 2, *Escherichia vulneris* 1, Pseudomonas sp 1, Corynebacteriae 1, other Streptococci 2
	Blomberg (2007)[155]	2001-2002	Prospective	Muhimbili National Hospital, Dar es Salaam, Tanzania	Admissions to neonatal unit of an urban tertiary referral hospital	535 inborn and outborn neonates (0-28 days)	Blood	Klebsiella spp 24, *S aureus* 11, *E coli* 9, Enterococci 7, Enterobacter spp 5, GBS 3, Acinetobacter spp 1, other non-Enterobacteriaceae 4, other Gram-negative 2, other Streptococcus 1, other Enterobacteriaceae 1.
	Kayange (2010)[156]	2009	Prospective	Bugando Medical Centre, Mwanza, Tanzania	Admissions to neonatal unit of an urban tertiary referral hospital	300 inborn and outborn neonatal sepsis admissions.	Blood	*K. pneumoniae* 50, *S. aureus* 32, *E. coli* 22, CoNS 14, GBS 2, Listeria spp 5, Enterococcus spp 9, Other Gram negatives 15 (Acinetobacter spp, Enterobacter spp)
	Klingenberg (2003)[157]	1998-1999	Prospective	Kilimanjaro Christian Medical Centre, Moshi, Tanzania	Admissions to SCBU of an urban tertiary referral hospital	246 inborn and outborn neonatal admissions; 148 investigated by blood culture)	Blood	*S. aureus* 6, CoNS 5, Gram negative rods 2, Enterococcus faecium 1, *K. pneumoniae* 1, Enterococcus faecalis 1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
East Africa (continued)	John (2015)[158]	2013	Prospective	Kidera Health Centre, Kidera County, Buyende district, Uganda	Admissions to urban tertiary referral hospital	174 inborn and outborn neonatal sepsis admissions (1-27 days)	Blood	*S. aureus* 12, *N. meningitidis 11*, *E. coli* 5, GAS 4, *H. influenzae* 2, Salmonella spp 2, *K. pneumoniae* 1, *S. pneumoniae* 1
	Mhada (2012)[159]	2009-2010	Prospective	Muhimbili National Hospital, Dar es Salaam, Tanzania	Admissions to neonatal unit of an urban tertiary referral hospital	330 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 27, Klebsiella spp 22, *E. coli* 14, *S. epidermidis* 6, GBS 1, Pseudomonas spp 2, Streptococcus spp 2.
	Mkoney (2014)[160]	2012-2013	Prospective	Muhimbili National Hospital, Dar es Salaam, Tanzania	Admissions to neonatal unit of an urban tertiary referral hospital	208 Inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	Klebsiella spp 14, *E. coli* 12, CoNS 9, *S. aureus* 4, *P. aeruginosa* 1
	Onken (2015)[161]	2012-2013	Prospective	Mnazi Mmoja Hospital, Zanzibar, Tanzania	Admissions to urban tertiary referral hospital	113 neonatal admissions with suspected bacterial infection (≤ 1 month)	Blood	*E. coli* 6, *K. pneumoniae* 8, *Enterococcus faecalis* 4, *S. aureus* 3, *Enterococcus faecium* 1, GBS 1, *Rhodococcus equi* 1, Other Enterobacteriaceae 5, non-Enterobacteriaceae 11, Unidentified Gram-negative rods 3,
	Kiwanuka (2013)[162]	2010	Prospective	Mbarara Hospital, Uganda	Admissions to paediatric ward of urban tertiary referral hospital	80 inborn and outborn neonatal sepsis admissions (< 1 month)	Blood & CSF	**Sepsis:** *S. aureus* 16, *E. coli* 4, Klebsiella spp 2, Unidentified coliforms 3, GBS 1 **Meningitis:** *S. pneumoniae* 1, Coliform 1
	Mugalu (2006)[163]	2002	Prospective	Mulago Hosptital, Kampala, Uganda	Admissions to SCBU of a urban tertiary referral hospital	293 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 69, *E. coli* 17, GBS 7, Salmonella spp 3, *P. mirabilis* 3, *Pseudomonas putida* 2, *Klebsiella planticola* 2, *K. pneumoniae* 1, *S. epidermidis* 1, *H. influenzae* 1, *E. agglomerans* 1, *S. pneumoniae* 1, Non-haemolytic streptococcus 1, E. faecalis 1,
Southern Africa	Mudzikati (2015)[164]	2012	Retrospective	Princess Marina Hospital, Gaborone, Botswana	Laboratory data from admissions to urban tertiary referral hospital	909 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*K. pneumoniae* 27, *K. oxytoca* 8, Klebsiella spp 8, GBS 15, *E. coli* 11, *S. aureus* 8, Enterococcus spp 4, Enterobacter spp 4, *S. pneumoniae* 2, GCS 1, Other Gram negative bacteria 4.
	Molyneux (1998)[165]	1996-1997	Prospective	Queen Elizabeth Central Hospital, Blantyre, Malawi	Admissions to district referral hospital	Paediatric meningitis admissions (1 day-14 years). 1120 lumbar punctures performed	CSF	GBS 14, *Salmonella typhimurium* 9, *S. pneumoniae* 7, *H. influenzae* 1, other Gram negative rods 7, others 23

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Southern Africa (continued)	Gwee (2012)[166]	2002-2007	Retrospective	Queen Elizabeth Central Hospital, Blantyre, Malawi	Laboratory data from admissions to urban tertiary referral hospital	6399 inborn and outborn neonates investigated by blood cultures (0-28 days)	Blood	*S. aureus* 123, GBS 118, *E. coli* 86, NTS 74, α-haemolytic Streptococci 55, Klebsiella spp 50, GDS 42, Enterobacter spp 40, *S. pneumoniae* 31, GAS 27, Citrobacter 22, Acinetobacter spp 19, *P. aeruginosa* 16, Group B haemolytic Streptococcus 15, other Gram negatives 33, other Gram positives 17.
	Milledge (2005) [167]	1996-2001	Retrospective	Queen Elizabeth Central Hospital, Blantyre, Malawi	Admissions to district referral hospital	784 inborn and outborn neonatal sepsis admissions (0-30 days)	Blood & CSF	**Bacteraemia:** *S. aureus* 85, GBS 76, NTS 77, *E. coli* 57, Klebsiella spp 54, GAS 49, *S. pneumoniae* 32, other Gram positive 61, other Gram negative 91. **Meningitis:** GBS 60, *S. pneumoniae* 47, NTS 33, *E. coli* 10, GAS 7, Klebsiella spp 6, *S. aureus* 2, other Gram positive 11, other Gram negative 26.
	Swann (2014)[168]	2002-2008	Retrospective	Queen Elizabeth Central Hospital, Blantyre, Malawi	Admissions to district referral hospital	259 inborn infants with suspected meningitis (≤ 60 days)	CSF	GBS 27, *S. pneumoniae* 13, *S. enterica serovar Typhimurium* 7, *E. coli* 3, *E. cloacae* 3, *K. pneumoniae* 3, *P. aeruginosa* 1, *H. influenzae* type b 1, GDS 1, Enterobacter spp 1.
	Walsh (2000)[169]	1996-1997	Prospective	Queen Elizabeth Central Hospital, Blantyre, Malawi	Admissions to district referral hospital	10508 children (1 day-15 years)	Blood	GBS 10, NTS 5, Enteric Gram-negative bacteria 21.
	Sigauque (2008)[126]	1998-2003	Prospective	Manhiça District Hospital, Mozambique	Admissions to paediatric ward of rural district referral hospital	48 neonatal CSF samples collected (<29 days)	CSF	*S. pneumoniae* 2, GBS 1, *S. aureus* 1
	Sigauque (2009)[125]	2001-2006	Prospective	Manhiça District Hospital, Mozambique	Admissions to paediatric ward of rural district referral hospital	952 blood cultures from neonates (<28 days)	Blood	*S. aureus* 60, GBS 31, NTS 9, *E. coli* 9, GDS 10, *S. pneumoniae* 7, GAS7, Enterobacter spp 6, *H. influenzae* 1, *N. meningitidis* 1, Klebsiella spp 1, Pseudomonas spp 1, other Gram negative 8, other Gram positive 3.

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Southern Africa (continued)	Mengistu (2013)[170]	2009-2012	Retrospective	33 State Hospitals in Namibia, Namibia	Laboratory data from admissions to urban tertiary referral hospital	503 positive bacterial CSF cultures	CSF	ESBL *K. pneumoniae* 4, *S. pneumoniae* 2, Neisseria spp 2, *E. coli* 1,
	Adhikari (1995)[171]	1988-1991	Retrospective	King Edward VIII Hospital, Durban, South Africa	Admissions to neonatal unit of an urban tertiary referral hospital	60 inborn and outborn neonatal meningitis admissions	CSF	GBS 21, *K. pneumoniae* 17, *E. coli* 10, *S. pneumoniae* 4, *P. aeruginosa* 2, Salmonella spp 2, *P. mirabilis* 2, E. cloacae 1, *Acinetobacter anitratus* 1
	Ballot (2012)[172]	2009-2010	Retrospective	Charlotte Maxeke Johannesburg Academic Hospital, South Africa	Admissions to neonatal unit of an urban tertiary referral hospital	181 inborn and outborn neonatal sepsis admissions (≤28 days)	Blood	CoNS 62, ESBL *K. pneumoniae* 34, *K. pneumoniae* 12, *K. oxytoca* 1, *A. baumanii* 27, Acinetobacter spp 2, *E. faecalis* 13, *E. faecium* 11, *E. coli* 23, MRSA 16, MSSA 7, *S. viridans* 5, *P. aeruginosa* 4, GBS 3, *Stenotrophomonas maltophilia* 1, *Aeromonas caviae* 1, *P. mirabilis* 1.
	Cutland (2009)[173]	2004-2007	Prospective	Chris Hani-Baragwanath Hospital, Soweto, South Africa	Admissions to neonatal unit of an urban tertiary referral hospital	8129 neonates enrolled (0-28 days). 289 neonates with suspected sepsis	Blood	*A. baumanii* & lwoffi 3, *Enterococcus faecalis & faecium* 3, S. viridans 2, *K. pneumoniae* 1, Enterobacter 1, *E. coli 1*, *S. aureus* 1,
	Coovadia (1989)[174]	1981-1987	Retrospective	King Edward VIII Hospital, Durban, South Africa	Admissions to neonatal unit of an urban tertiary referral hospital	97 inborn and outborn neonates with culture-proven meningitis (≤1 month)	CSF	*K. pneumoniae* 40, *E. coli* 17, GBS 15, *P. mirabilis* 5, Enterobacter spp 3*P. aeruginosa* 2, *Acinetobacter anitratus* 2, *S. pneumoniae* 2, *Enterococcus faecalis* 2, *L. monocytogenes* 2, *Streptococcus lactis* 1, *Alcaligenes faecalis* 1, Salmonella spp 1, *Flavobacterium meningosepticum* 1, other Gram-negative bacilli 3
	Donald (1996)[175]	1985- 1993	Prospective	Tyberg Hospital, Western Cape Province, South Africa	Admissions to urban tertiary referral hospital	117 culture -confirmed neonatal meningitis admissions	CSF	GBS 27, *E. coli* 21, Klebsiella spp 11, *S. marcescens* 9, *S. pneumoniae* 2, *H. influenzae* 2, *N. meningitidis* 4, Others 26

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Southern Africa (continued)	Dhlamini (2013)[176]	2009-2010	Prospective	Rahima Moosa, Chris Hani Baragwanath & Charlotte Maxeke Academic Hospitals, Johannesburg, South Africa	Admissions to urban tertiary referral hospitals	76 neonates inborn and outborn neonatal sepsis admissions	Blood	EBSL *K. pneumoniae* (1)
	Dramowski (2015)[177]	2009-2013	Retrospective	Tygerberg Hospital, Western Cape Province, South Africa	Laboratory data from admissions to urban tertiary referral hospital neonatal unit	23,920 neonates with suspected nosocomial blood stream infection (6521 investigated)	Blood	*K. pneumoniae* 235, *S. aureus* 112, Enterococcus spp 88, *S. marcescens* 84, *A. baumanii* 69, *E. coli* 58, GBS 36, Enterobacter spp 15, *P. aeruginosa* 12, CoNS 5, other Gram positives 3, other Gram negatives 46.
	Friedland (1992)[178]	1988-1990	Retrospective & Prospective	Chris Hani-Baragwanath Hospital, Soweto, South Africa	Admissions to urban tertiary referral hospital	Neonatal ward admissions investigated by blood and/or CSF culture	Blood & CSF	Klebsiella spp 192, Pseudomonas spp 37, Serratia spp 30, Other Gram-negatives 136.
	Haffejee (1991)[179]	1986-1989	Retrospective	R.K. Khan Hospital, Durban, South Africa	Admissions to neonatal unit of an urban district referral hospital	2171 inborn and outborn neonatal sepsis admissions investigated by blood culture; 277 investigated by lumbar puncture	Blood & CSF	**Blood :** GBS 36, *Streptococcus sanguis* 26, *Streptococcus salavirius* 19, *S. faecalis* 16, *Streptococcus mitis* 9, *S. aureus* 32, *E. coli* 9, Others 20 **CSF:** GBS 8, Streptococcus faecalis 1.
	Kitambala (2012)[180]	2011	Prospective	Kalafong Hospital, Gauteng Province, South Africa	Admissions to neonatal unit of a district referral hospital	53 neonatal admission with nosocomial infection	Blood	CoNS 7, MRSA 1, Enterococcus faecalis 4, Enterococcus faecium 2, Klebsiella oxytoca 2, *P. aeruginosa* 1, Enterobacter cloacae 1, Proteus mirabilis 1, GBS 1,
	Lebea (2015)[181]	2012	Retrospective	Charlotte Maxeske Johannesburg Hospital, Johannesburg, South Africa	Laboratory data from admissions to a neonatal unit of an urban tertiary referral hospital	196 neonates inborn and outborn neonatal sepsis admissions	Blood	ESBL *K. pneumoniae* 76, *A. baumanii* 20, *E. coli* 14, Klebsiella. oxytoca 2, CoNS 56, GBS 3, MRSA 31, *E. faecalis* 7, *E. faecium* 2, *E. cloacae* 1, *L. monocytogenes* 1, *K. pneumoniae* 2,

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Southern Africa (continued)	Liebowitz (1984)[182]	1980-1982	Retrospective	JG Strijdom Hospital, Hillbrow, Baragwanath and Coronation Hospitals, Johannesburg, South Africa	Laboratory data from admissions to urban tertiary referral hospital	All hospital admissions due to confirmed acute bacterial meningitis	CSF	GBS 25, *E. coli* 20, *S. pneumoniae* 6, *N. meningitidis* 2, *H. influenzae* 1
	Morkel (2014)[183]	2008	Retrospective	Tygerberg Children's Hospital, Western Cape Province, South Africa	Laboratory data from admissions to urban tertiary referral hospital	503 Neonatal intensive care unit admissions, 354 investigated:	Blood	CoNS 22, MRSA 5, *S. pneumoniae* 1, *E. faecalis* 2, *Bacillus cereus* 1, *A. baumanii* 14, *K. pneumoniae* 17, *E. coli* 2, *P. aeruginosa* 1, *S. marcescens* 1, *E. cloacae* 2, *P. mirabilis* 1
	Motara (2005)[184]	2002-2003	Retrospective	Johannesburg General Hospital, South Africa	Admissions to neonatal unit of an urban tertiary referral hospital	1129 neonatal admissions	Blood	CoNS 65, *E. coli* 20, Klebsiella sp 12, Enterobacter spp 9, GBS 2, *E. faecalis* 4, *S. viridans* 4, *P. aeruginosa* 2, H. influenzae 1, Citrobacter spp1, *P. mirabilis* 1
	Nel (2000)[185]	1981-1992	Retrospective	Tygerberg Hospital, Western Cape Province, South Africa	Admissions to urban tertiary referral hospital	88 inborn and outborn neonatal meningitis admissions (0-28 days)	CSF	GBS 28, *E. coli* 20, *K. pneumoniae* 13, *N. meningitidis* 2, *H. influenzae* 1, *L. monocytogenes* 1, other Gram positives 8, other Gram negatives 4
	Potter (1984)[186]	1981	Prospective	Teaching Hospitals of Cape Peninsula, South Africa	Admissions to urban tertiary referral hospital	213 children less than 13 years of which 2 neonates with confirmed meningitis	CSF	*N. meningitidis* 1, Pneumococci 1
	Schrag (2012)[130]	2004-2007	Prospective	Chris Hani-Baragwanath Hospital, Soweto, South Africa	Admissions to urban tertiary referral hospital	323 neonatal sepsis admissions (0-28 days); 289 early-onset (0-2 days) and 34 late-onset (3-28 days)	Blood & CSF	GBS 21, E. faecalis 3, *E. coli* 10, *S. aureus* 4, Klebsiella spp 2, *K. pneumoniae* 1, *A. baumanii* 2, *Acinetobacter lwoffi* 1, *S. viridans* 2, *Enterococcus faecium* 2, Streptococcus spp 1
	White (2007)[187]	2004	Prospective	Johannesburg General Hospital, South Africa	Admissions to neonatal unit of an urban tertiary referral hospital	194 inborn and outborn neonatal early-onset sepsis admissions (0-6 days)	Blood	GBS 1 and *E. coli* 1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Southern Africa (continued)	Thomas KM (2013)[188]	2005 - 2010	Retrospective	Government Hospitals in The Eastern Cape, The Free State, Gauteng, Limpopo, Mpumalanga, The Northern Cape, North West and The Western Cape, South Africa	Laboratory data from admissions to government hospitals	1178 neonates with positive CSF bacterial culture (0 - 28 days)	CSF	GBS 359, *S. aureus* 47, *N. meningitidis* 6, MRSA 23, *H. influenzae* 5, *S. pneumoniae* 94, Viridans Streptococcus 11, other Gram-negative bacilli 378, other Streptococci 35, Enterococcus 59, CoNS 148, *L. monocytogenes* 2, others 11.
	Wolzak (2012)[189]	2007-2009	Retrospective	Tygerberg Children's Hospital, Western Cape Province, South Africa	Laboratory data from admissions to urban tertiary referral hospital	62 neonatal admissions with suspected meningitis investigated by lumbar puncture	CSF	*K. pneumoniae* 11, GBS 7, *E. coli* 4, *S. marcescens* 3, *S. pneumoniae* 2, *N. meningitidis* 1, Others 8
	Fubisha (2012)[190]	2002-2003	Prospective	University Teaching Hospital, Lusaka, Zambia	Admissions to a paediatric ward and NICU of an urban tertiary referral hospital	444 inborn and outborn neonatal sepsis/meningitis admissions (0 – 28 days)	CSF	*K. pneumoniae* 7, *S. pneumoniae* 5, *Streptococcus* spp 2, *Acinetobacter* 1, CoNS 1, *Pseudomonas* 1, *Salmonella* 1, Others 4
	Kabwe (2016)[191]	2013-2014	Prospective	University Teaching Hospital, Lusaka, Zambia	Admissions to NICU of urban tertiary referral hospital	313 inborn and outborn neonatal sepsis admissions	Blood	*Klebsiella* spp 77, CoNS 7, *S. aureus* 6, *E. coli* 5, *Acinetobacter* 1. *Bacillus* spp 1, Gram negative diplococcus 1
	Aiken (1992)[192]	1989-1990	Prospective	Mpilo Maternity Hospital, Zimbabwe	Admissions to neonatal unit of an urban tertiary referral hospital	3272 neonatal admissions (0-28 days)	Blood	GBS 39, *S. epidermidis* 37, *Klebsiella* spp 27, *S. aureus* 20, GDS 12, *E. coli* 10, Coliforms 7, GAS 4, *Proteus* spp 2, *S. pneumoniae* 1, *Pseudomonas* spp 1, *Salmonella* spp 1. *H. influenzae* 1
	Nathoo (1990)[193]	1987-1988	Prospective	Harare Central Hospital, Zimbabwe	Admissions to neonatal unit of an urban district referral hospital	94 inborn and outborn neonates with culture-positive meningitis	CSF	*S. aureus* 51, *S. epidermidis* 18, GBS 18, *Klebsiella* spp 9, E. coli 6, *Salmonella* sp 2, *Pseudomonas* spp 2, *Proteus* spp 1, *Listeria* 1, Coliforms 31, other streptococci 15, others 6.

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
Southern Africa (continued)	Nathoo (1991)[194]	1987	Retrospective	Harare Central Hospital, Zimbabwe	Admissions to neonatal unit of an urban district referral hospital	161 inborn and outborn neonatal sepsis admissions with positive blood cultures (0-28 days)	Blood	GBS 57, *S. pneumoniae* 8, other Streptococci 8, *Klebsiella* spp 6, *Salmonella* spp 4, *E. coli* 3, *Proteus* spp 1, Coliforms 7
West Africa	Agossou (2016)[195]	2013	Prospective	Borgou Regional University Teaching Hospital, Parakou, Benin	Admissions to neonatal unit of an urban tertiary referral hospital	203 neonatal admission with suspected bacterial infection (95 had blood culture & 85 had lumbar puncture)	Blood & CSF	*E. coli* 5, *S. aureus* 3, *K. pneumoniae* 2, GBS 1, *P. aeruginosa* 1, *S. saprophyticus* 1, *S. epidermidis* 1,
	Balaka (2004)[196]	1991-1996	Retrospective	CHU Souro Sanou, Bobo-Dioulasso, Burkina-Faso	Admissions to urban tertiary referral hospital	37 neonatal bacterial meningitis admissions (0-28 days)	CSF	*E. coli* 8, *Enterobacter* 5, GBS 4, *K. pneumoniae* 4, *S. pneumoniae* 3, *S. aureus* 2, *Salmonella enteritidis* 2
	Hein (2001)[197]	1995 - 1999	Retrospective	Centre Hospitalier Yalgado Ouedraogo, Ouagadougou, Burkina Faso	Admissions to urban tertiary referral hospital	10 neonatal meningitis admissions (<1 month)	CSF	*S. Pneumoniae* 3, Others 2
	Akoua-Koffi (2001)[198]	1995-1998	Retrospective	CHU de Yopougon, Abidjan, Cote d'Ivoire	Admissions to urban tertiary referral hospital	309 paediatric CSF samples (0-15 years)	CSF	GBS 15, *S. pneumoniae* 3, others 1
	Do Rego (1988)[199]	1985-1986	Retrospective	Multicentric, Cote d'Ivoire	Admissions to urban tertiary referral hospital	87 neonatal admissions with suspected meningitis investigated by lumbar puncture (0-30 days)	Blood & CSF	**Blood:** *K. pneumoniae* 1, *P. aeruginosa* 1, Citrobacter spp 1, *E. cloacae* 1. **CSF:** *H. influenzae* 2, *P. mirabilis* 1, *K. pneumoniae* 1, *E. coli* 1, *P. aeruginosa* 1,
	Orega (1997)[200]	1985-1989	Retrospective	CHU Trechville, Abidjan, Cote d'Ivoire	Admissions to urban tertiary referral hospital	521 children with bacterial meningitis (25 neonates)	CSF	*H. influenzae* 1, *S. pneumoniae* 1, Streptococcus spp 1, Staphylococcus spp 2, *K. pneumoniae* 3
	Acquah (2013)[201]	2011-2012	Prospective	Tamale Teaching Hospital, Ghana	Admissions to NICU of urban tertiary referral hospital	63 inborn and outborn neonates with blood cultures (< 30 days)	Blood	CoNS 10, Coagulase positive staphylococci 8, *K. pneumoniae* 2, *E. coli* 2, Klebsiella spp 2, Acinetobacter spp 2, NTS 1, *P. aeruginosa* 1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Adetunde (2014)[202]	2008-2011	Retrospective	Komofo Anokye Teaching Hospital, Kumasi, Ghana	Laboratory data from admissions to urban tertiary referral hospital	107 children with culture-positive meningitis (0-12 years)	CSF	*S. pneumoniae* 14, Pseudomonas spp 3, Coliforms 3, Salmonella spp 2, *N. meningitidis* 2, *E. coli* 2
	Anyebuno (1995)[203]	1991-1992	Retrospective	Korle Bu Teaching Hospital, Ghana	Admissions to neonatal unit of an urban tertiary referral hospital	2833 neonatal admissions	Blood	Enterobacter spp 131, *S. faecalis* 64, *S. aureus* 48, Klebsiella spp 40, Acinetobacter spp 42, *E. coli* 39, Salmonella spp 28, Pseudomonas spp 23, GBS 8, Proteus spp 6, Citrobacter spp 5, Non-group B streptococcus 9
	Enweronu-Laryea (2007)[204]	2001-2002	Retrospective	Korle Bu Teaching Hospital, Ghana	Admissions to neonatal unit of an urban tertiary referral hospital	4213 neonatal admissions	Blood	CoNS 80, Enterobacter spp 59, Klebsiella spp 31, *S. aureus* 27, Acinetobacter spp 16, *E. coli* 9, Pseudomonas spp 8, Citrobacter spp 8, *S. faecalis* 7, Salmonella spp 5, Non-group B streptococcus 1
	Campagne (1999)[205]	1981-1997	Retrospective	Hopital National de Niamey, Niger	Laboratory data from admissions to urban tertiary referral hospital	1481 children with bacterial meningitis (0-16 years)	CSF	*S. pneumoniae* 34, *N. meningitidis* 12, *H. influenzae* type b 10, Others 47
	Owusu (2012)[206]	2008-2010	Retrospective	Komofo Anokye Teaching Hospital, Kumasi, Ghana	Laboratory data from admissions to urban tertiary referral hospital	163 confirmed meningitis cases (all age groups)	CSF	*S. pneumoniae* 8, *P. aeruginosa* 2, Salmonella spp 1, *E. coli* 1, *N. meningitidis* 1, Enterobacter spp 1, Coliforms 1
	Choketu (2005)[207]	2002-2003	Retrospective	CHU Gabriel Touré, Bamako, Mali	Admissions to a NICU of an urban tertiary referral hospital	367 neonates (0 - 28 days)	Blood/CSF	**Blood:** *S. aureus* 21, *S. pneumoniae* 17, *E. coli* 12, Salmonella typhi 5, *H. influenzae* 3, Others 12. **CSF**: *S. aureus* 21, *S. pneumoniae* 4, *E. coli* 2, *H. influenzae* 3, *N. meningitidis* 2, Others 3.
	Adesiyun (2012)[208]	Not specified	Prospective	University of Ilorin Teaching Hospital, Ilorin, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	193 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 18, *S. faecalis* 10, *Klebsiella aeruginosa* 6, CoNS 6, *E. coli* 3, *S. pneumoniae* 2, Coliforms 20,

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Airede (1993)[209]	1988-1992	Prospective	Jos University Teaching Hospital, Jos, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	36 inborn and outborn neonatal meningitis admissions	CSF	*S. aureus* 11, Klebsiella spp 4, *S. pneumoniae* 3, *E. coli* 3, *Staphylococcus albus* 2, Citrobacter spp 1, Pseudomonas spp 1, Non-haemolytic Streptococcus 1
	Airede (1992)[210]	1987-1989	Prospective	Jos University Teaching Hospital, Jos, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	99 Inborn and outborn neonatal septicaemia admissions. (0-28 days)	Blood	Klebsiella spp 27. *S. aureus* 27, Others 72
	Airede (2008)[211]	1992-1995	Prospective	University of Maiduguri Teaching Hospital, Maiduguri, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	69 inborn and outborn neonatal meningitis admissions	CSF	*S. aureus* 13, *E. coli* 11, *K. oxytoca* 7, *S. pneumoniae* 4, *S. epidermidis* 4, *N. meningitidis* 2, Citrobacter spp 2, *P. aeruginosa* 1. *H. influenzae* type b 1, *S. faecalis* 1, *H. influenzae* untyped 1, Coliform spp 3,
	Ajayi (1997)[212]	1998-1990	Retrospective & Prospective	University of Ilorin Teaching Hospital, Ilorin, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	713 inborn and outborn neonatal meningitis admissions	CSF	*S. pneumoniae* 10, Klebsiella spp 8, Pseudomonas spp 7, *E. coli* 5, *N. meningitidis* 1, *S. faecalis* 1, Coliforms 3.
	Ambe (2007)[213]	1995-1999	Retrospective	University of Maiduguri Teaching Hospital, Maiduguri, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	813 Inborn and outborn neonatal sepsis admissions. (358 blood cultures and 14 lumbar punctures performed)	Blood & CSF	**Sepsis:** *S. aureus 54, Klebsiella spp 29, Proteus spp 14, E. coli 8, Coliform 7, Salmonella 3, Pseudomonas spp 1, S. pneumoniae 1.* **Meningitis:** *Klebsiella spp 2, H. influenzae 3, S. pneumoniae 1*
	Ako-Nai (1999)[214]	1994-1995	Prospective	Obafemi Awolowo University Teaching Hospital, Ile-Ife, Nigeria	Admissions to urban tertiary referral hospital	107 inborn and outborn neonates with suspected sepsis (0-28 days)	Blood & CSF	**Blood:** *S. aureus* 21, *P. aeruginosa* 11, CoNS 11, *L. monocytogenes* 5, *K. pneumoniae* 6, *E. coli* 3, *Citrobacter freundii* 1, *Enterobacter aerogenes* 2, Salmonella spp 1, Proteus spp 1. **CSF:** *P. aeruginosa* 7, *K. pneumoniae* 5, *Citrobacter freundii* 5, *E. coli* 3, *S. aureus* 2, *Enterobacter aerogenes* 1, Salmonella spp 1.
	Anah (2008)[215]	2002-2004	Retrospective	University of Calabar Teaching Hospital, Calabar, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	717 inborn and outborn neonates with blood cultures	Blood	*S. aureus* 197, Coliforms 72, Streptococcus spp 30, Enterobacteriaceae 20, C. violaceum 18, *K. pneumoniae* 12, *P. aeruginosa* 9, *Salmonella typhi* 7, *P. mirabilis* 2

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Antia-Obong (1992)[216]	1989	Retrospective	University of Calabar Teaching Hospital, Calabar, Nigeria	Admissions to SCBU of a urban tertiary referral hospital	132 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood & CSF	**Sepsis:** *S. aureus* 15, Streptococcus spp 6, Pseudomonas spp 3, *P. aeruginosa* 1, Klebsiella spp 1, Proteus spp 1, Coliforms 52, **Meningitis:** Coliforms 3, Klebsiella spp 1
	Antia-Obong (1991)[217]	1985-1987	Prospective	University of Calabar Teaching Hospital, Calabar, Nigeria	Admissions to an urban tertiary referral hospital	275 Inborn and outborn neonatal sepsis admissions	Blood	*S. aureus* 46, GDS 5, Klebsiella spp 7, *E. coli* 3, Streptococcus spp 3, Salmonella spp 2, Proteus spp 1, Pseudomonas spp 1, *S. pyogenes* 1, *Enterobacter aerogenes* 1, Coliforms 30,
	Egbule (2016)[218]	2015	Prospective	Delta State University, Abraka, Nigeria	Admissions to NICU of urban tertiary referral hospital	98 neonatal blood cultures	Blood	*E. coli* 30, *K. pneumoniae* 20, *S. aureus* 18
	Egri-Okwaji (1996)[219]	1996	Prospective	Lagos University Teaching Hospital, Lagos, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	250 outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 24, *K. pneumoniae* 17, *Burkholderia cepacia* 12, *E. cloacae* 7, *E. coli* 6, *Flavimonas oryzihabitans* 5, *E .faecalis* 4, , *Chryseomonas luteola* 4, Acinetobacter calcoaceticus 4, CoNS 1, *P. mirabilis* 3, Salmonella spp 3, *P. aeruginosa* 2, *Citrobacter freundii* 2, *Stenotrophomonas maltophilia* 2, *Enterobacter agglomerans* 1, *Morganella marganii* 1, *Salmonella arizonae* 1, Shigella spp 1, Acinetobacter spp 1, Enterococcus spp 1, Pasturella spp 1, *Sphingobacterium multivorum* 1, *Flavobacterium meningosepticum* 1,
	Emele (2000)[220]	1987- 1992	Prospective	Usman Danfodio University Teaching Hospital, Sokoto, Nigeria	Admissions to urban tertiary referral hospital	11 neonates with CSF samples	CSF	*S. pneumoniae* 3, *S. aureus* 3, Coliform bacilli 5
	Fadero (2007)[221]	2004-2005	Prospective	Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	63 inborn and outborn neonates investigated by blood culture (< 30 days)	Blood	*S. aureus* 18, Proteus spp 6, Klebsiella spp 3, *E. coli* 2, CoNS 2

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Iregbu (2013)[222]	2010-2012	Retrospective	National Hospital, Abuja, Nigeria	Laboratory data from admissions to urban tertiary referral hospital special care baby unit	245 neonatal blood culture samples, 127 CSF samples	Blood & CSF	**Blood :** S. *aureus* 62, Klebsiella spp 8, Enterococcus spp 6, *P. aeruginosa* 5, CoNS 4, *S. marcescens* 2, *E. coli* 1, *Citrobacter freundii* 1, *Morganella marganii* 1, *L. monocytogenes* 1, Proteus spp 1, **CSF:** *S. aureus* 4, Enterococcus spp 1, Klebsiella spp 1, Neisseria spp 1
	Iregbu (2006)[223]	2002-2004	Retrospective	National Hospital, Abuja, Nigeria	Laboratory data from admissions to urban tertiary referral hospital	390 neonatal blood cultures (0-28 days)	Blood	*K. pneumoniae* 37, *S. aureus* 34, CoNS 2, Acinetobacter spp 1, *P. aeruginosa* 4. Enterococcus spp 1, *S. pneumoniae* 1, *E. coli* 1, α-haemolytic streptococci 1, *E. faecalis* 1
	Longe (1984)[224]	1974-1982	Prospective	University of Benin Teaching Hospital, Benin City, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	53 inborn and outborn neonatal sepsis admissions	CSF	*S. aureus* 13, *E. coli* 9, *S. pneumoniae* 4, Klebsiella spp 4, Pseudomonas spp 3, Coliforms 3, Salmonella spp 2, Proteus spp 2, Non-haemolytic streptococcus 2, *Staphylococcus albus* 2 Achromobacter 1
	Meremikwu (2005)[225]	1996-2002	Prospective	University of Calabar Teaching Hospital, Calabar, Nigeria	Laboratory data from admissions to urban tertiary referral hospital	533 inborn and outborn neonatal sepsis admissions (≤1 month)	Blood	*S. aureus* 138, Pseudomonas spp 16, Chromobacterium spp 15, β-haemolytic Streptococci 5, Other Streptococci 8, Salmonella spp 5, CoNS 1, Proteus spp 1, other Gram negatives 18, Coliforms 63
	Mokuolu (2002)[226]	1995-1996	Retrospective	University of Ilorin Teaching Hospital, Ilorin, Nigeria	Admissions to NICU of urban tertiary referral hospital	198 Inborn and outborn neonates (0-28 days)	Blood	*S. aureus* 18, CoNS 15, *K. pneumoniae* 10, *E. coli* 4, Acinetobacter spp 3, *E. faecalis* 2, *P. aeruginosa* 1, Coliforms 16
	Mordi (2010) [227]	2008-2009	Prospective	University of Benin Teaching Hospital, Benin City, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	700 inborn and outborn neonatal admissions	Blood	*S. aureus* 54, *K. pneumoniae* 20, *E. coli* 5, *P. mirabilis* 4, *Providencia stuartii* 4, *Acinetobacter calcoaceticus* 3, *Enterobacter aerogenes* 3, *Citrobacter freundii* 3, *P. aeruginosa* 2, *P. vulgaris* 1
	Nottidge (1985) [228]	1976-1980	Retrospective & Prospective	University College Hospital, Ibadan, Nigeria	Admissions to urban tertiary referral hospital	463 children with culture-confirmed meningitis (0-15 years)	CSF	*S. pneumoniae* 10, *H. influenzae* 2

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Nwadioha (2015) [229]	2012-2015	Retrospective	Ekiti State University Teaching Hospital, Nigeria	Laboratory data from admissions to urban tertiary referral hospital	51 neonatal blood cultures (≤ 28 days)	Blood	*S. aureus* 6, Klebsiella spp 6, Enterococci 3, Proteus spp 2, *E. coli* 1
	Nwadioha (2013) [230]	2006-2009	Retrospective	Amino Kano Teaching Hospital, Kano, Nigeria	Laboratory data from admissions to urban tertiary referral hospital special care baby unit	250 neonatal CSF samples (0-28 days)	CSF	*E. coli* 7, GBS 4, Enterococcus spp 3, S. epidermidis 1, Klebsiella spp 1, Salmonella spp 1,
	Nwadioha (2010) [231]	2006-2008	Retrospective	Amino Kano Teaching Hospital, Kano, Nigeria	Laboratory data from admissions to urban tertiary referral hospital special care baby unit	1270 neonatal blood culture samples (0-28 days)	Blood	*E. coli* 180, *S. aureus* 90, Klebsiella spp 40, Proteus spp 14, *S. pneumonia* 1, *Enterococcus* spp 1
	Nwankwo (2011) [232]	2007-2008	Prospective	Aminu Kano Teaching Hospital, Kano, Nigeria	Admissions to SCBU of a urban tertiary referral hospital	547 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 38, *K. pneumoniae* 32, *E. coli* 24, Streptococcus spp 18, Salmonella spp 15, *E. faecalis* 7, *P. aeruginosa* 7, *P. mirabilis* 4, *S. epidermidis* 4, *P. vulgaris* 2, *Citrobacter freundii* 1,
	Ogundare (2016) [127]	2008-2009	Prospective	Wesley Guild Hospital, Ilesa, Nigeria	Admissions to SCBU of a rural referral hospital	360 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 53, Klebsiella spp 8, α-haemolytic Streptococci 6, Pseudomonas 5, *E. coli* 2, Proteus spp 1 *(extrapolated from data)*
	Ogunlesi (2010) [233]	2006-2008	Retrospective & Prospective	Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	527 Inborn and outborn neonatal sepsis admissions	Blood	*S. aureus* 54, Klebsiella 40, CoNS 22, *E. coli* 19, Proteus 11, *P. aeruginosa* 7, Enterococcus 4, Unclassified coliforms 17
	Ojide (2013) [234]	2011-2012	Prospective	University of Uyo Teaching Hospital, Nigeria	Admissions to urban tertiary referral hospital	357 neonatal sepsis admissions (0 -28 days)	Blood	*S. aureus* 39, *E. coli* 23, *K. pneumoniae* 15, Enterobacter spp 5, CoNS 4, *P. mirabilis* 3, Enterococcus faecalis 2

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Ojukwu (2005) [235]	2002-2003	Prospective	Ebonyi State University Teaching Hospital, Abakiliki, Nigeria	Admissions to SCBU of a urban tertiary referral hospital	138 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 15, *E. coli* 5, Streptococcus spp 3, Klebsiella spp 3, *S. paratyphi* A 2, Proteus spp 1, Enterobacter spp 1, *H. influenzae* 1, Pseudomonas spp 1.
	Okolo (1985) [236]	1978-1983	Retrospective	University of Benin Teaching Hospital, Benin City, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	177 inborn and outborn neonatal sepsis admissions (0 -28 days)	Blood	*S. aureus* 50, Klebsiella 38, Pseudomonas 34, *Staphylococcus albus* 23, *E. coli* 21, *Alcalogenes faecalis* 10, Proteus 8, Salmonella spp 4, Acinetobacter 3, Providence A, 3, Diptheroids, 1, Citrobacter 1, *S. viridans* 1, *S. faecalis* 1, Haemolytic streptococcus 1, Coliforms 24,
	Okon (2014) [237]	2008-2009	Retrospective	University of Maiduguri Teaching Hospital, Maiduguri, Nigeria	Laboratory data from admissions to urban tertiary referral hospital special care baby unit	2134 children (< 12 years)	Blood	*S. aureus* 39, Klebsiella spp 15, Salmonella spp 1, *E. coli* 6, Pseudomonas spp 2, Proteus spp 1, Coliforms 5, CoNS 1,
	Omoregie (2013) [238]	2010-2011	Prospective	University of Benin Teaching Hospital, Benin City, Nigeria	Admissions to neonatal unit of an urban tertiary referral hospital	534 inborn and outborn neonatal sepsis admissions (1-28 days)	Blood	Klebsiella spp 74, *S. aureus* 66, Alcaligenes spp 12, *E. coli* 10, Acinetobacter spp 9, *P. mirabilis* 8, *P. aeruginosa* 8, Providencia spp 6, Citrobacter spp 5, Proteus vulgaris 5, Enterobacter spp 4
	Onalo (2011) [239]	2004-2005	Prospective	Ahmadu Bello University Teaching Hospital, Zaria, Nigeria	Admissions to SCBU of a urban tertiary referral hospital	211 neonatal septicaemia admissions (0-28 days). Inborn (69) and outborn (142)	Blood	*S. aureus* 33, *E. coli* 15, Streptococcus spp 8, *K. pneumoniae* 6, *P. mirabilis* 5, Others 10
	Onyedibe (2016) [240]	2011	Prospective	Jos University Teaching Hospital, Jos, Nigeria	Admissions to SCBU of a urban tertiary referral hospital	165 inborn and outborn neonatal sepsis admissions	Blood & CSF	**Blood:** *K. pneumoniae* 22, *S. aureus* 20, *E. coli* 8, CoNS 5, Citrobacter spp 3, *P. aeruginosa* 1, Enterobacter spp 2, *E. faecalis* 2, *L. monocytogenes* 1, NTS 1, *P. mirabilis* 1, Salmonella typhi 1, *S. pneumoniae* 1. **CSF:** *K. pneumoniae* 1, *E. coli* 1, *S. pneumoniae* 1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Onyedibe (2015)[241]	2011	Prospective	Jos University Teaching Hospital, Jos, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	218 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*K. pneumoniae* 24, *S. aureus* 23, *E. coli* 8, CoNS 5, Citrobacter spp 3, *P. aeruginosa* 3, Enterobacter spp 2, *E. faecalis* 2, *P. mirabilis* 2, Salmonella spp 2, *S. pneumoniae* 1
	Osinupebi (2014)[242]	2008	Prospective	Olabisi Onobanjo University Teaching Hospital, Sagamu, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	356 Inborn and outborn neonatal admissions. (0-28 days)	Blood	**Community-acquired***:* *S. aureus* 8, Klebsiella spp 8, Pseudomonas spp 3, *E. coli* 2, Proteus spp 2, Atypical coliforms 3. **Nosocomial:** Klebsiella spp 10, Proteus spp 5, *S. aureus* 4, *E. coli* 2, Pseudomonas spp 1, Coliforms 2
	Owa (1988)[128]	1986	Prospective	Wesly Guild Hospital, Ilesha, Nigeria	Admissions to SCBU of a rural referral hospital	101 inborn and outborn neonatal sepsis admissions (1-28 days) investigated by blood culture	Blood	*S. aureus* 8, *E. coli* 4, Klebsiella spp 4, *P. aeruginosa* 2, Streptococcus faecalis 2, *Clostridium welchi* 2, Citrobacter spp 1, *H. influenzae* 1, *S. epidermidis* 1, Atypical coliform 6,
	Peterside (2015)[243]	2011-2013	Retrospective	Niger Delta University Teaching Hospital, Bayelsa, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	223 neonates (0-28 days)	Blood	*S. aureus* 50, *E. coli* 16, *K. pneumoniae* 14, *P. mirabilis* 8, *P. aeruginosa* 7, *S. pyogenes* 2
	Pius (2016)[244]	2012	Prospective	University of Maiduguri Teaching Hospital, Maiduguri, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	110 inborn and outborn neonatal sepsis admissions (0-28 days)	Blood	*S. aureus* 16, *E. coli* 9, *K. pneumoniae* 7, *S. pyogenes* 5, *S. epidermidis* 1, *S. pneumoniae* 1, *H. influenzae* 1, Salmonella spp 1, Coliforms 5
	Rabasa (2007)[245]	1991	Prospective	University of Benin Teaching Hospital, Benin City, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	141 inborn and outborn neonatal sepsis admissions	Blood & CSF	*S. aureus* 13, Coliform spp 26
	Shittu (2014)[246]	2012-2014	Prospective	Outreach Childrens' Hospital, Festac Town, Lagos, Nigeria	Admissions to private referral hospital	432 inborn and outborn neonates with suspected sepsis (0-28 days)	Blood	Klebsiella spp 33, *S. aureus* 27, *E. coli* 18, Pseudomonas spp 6, Proteus spp 3, CoNS 9

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Uzodimma (2013)[247]	2011-2012	Prospective	Lagoon Hospital, Lagos, Nigeria	Admissions to urban tertiary referral hospital	36 neonates with suspected sepsis (<4 weeks)	Blood	*S. aureus* 9, Streptococcus spp 2, Klebsiella spp 3, *E. coli* 1, Enterococci 1
	West (2012) [248]	2007	Prospective	University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria	Admissions to SCBU of an urban tertiary referral hospital	406 inborn and outborn neonatal septicaemia admissions. (0-28 days)	Blood	Klebsiella 99, *S. aureus* 33, *E. coli* 13, Proteus spp 9, *P. aeruginosa 8*, *E. faecalis* 3, *S. epidermidis* 3, Streptococcus spp 1
	Camara (2003) [249]	1995-2000	Retrospective	L'hopital d'enfants Albert-Royer, CHU de Fann, Dakar, Sénégal	Admissions to urban tertiary referral hospital	1095 children with bacterial meningitis (0-15 years)	CSF	Streptococcus 13, Staphylococci 9, *E. coli* 9, *S. pneumoniae* 6, *K. pneumoniae* 4, Citrobacter 4, *P. aeruginosa* 2, Salmonellae 2, Enterobacteriaceae 2,
	Cisse (2001) [250]	1997-1998	Retrospective	CHU de Dakar, Sénégal	Admissions to urban tertiary referral hospital	2312 neonates with suspected bacterial infection	Blood	*K. pneumoniae* 134, Enterobacter spp 25, Staphylococcus 19, *E. coli* 13, GBS 12, Enterobacteria 9, Pseudomonas spp 6,
	Cisse (1992) [251]	1983-1991	Prospective	Pediatric Hospital Albert Royer, Dakar, Sénégal	Admissions to neonatal unit of an urban tertiary referral hospital	471 neonatal blood cultures (0-30 days)	Blood	Klebsiella spp 41, Enterobacteriaceae 30, *E. coli* 28, *S. aureus* 25, GBS 7, *S. pneumoniae* 2, GDS 2, GAS 1, Gram-negative bacilli 7
	Landre-Peigne (2011) [252]	2005	Retrospective & Prospective	Hôpital Principal de Dakar, Senegal	Admissions to urban tertiary referral hospital	273 neonatal admissions with suspected sepsis	Blood	ESBL *K. pneumoniae* 9, *K. pneumoniae* 1*, E. cloacae* 2, Acinetobacter spp 1, MRSA 1
	Le Doare (2016) [253]	2014-2015	Prospective	MRC Hospital, Fajara, The Gambia	Admissions to urban secondary referral hospital	51 infants with suspected infection (0-89 days)	CSF	GBS 1
	Mulholland (1999) [254]	1990-1991, 1992	Prospective	MRC Hospital, Fajara & Royal Victoria Hospital, Banjul, The Gambia	Admissions to urban tertiary referral hospital	695 sick and 63 well young infants enrolled (<91 days); 476 infants investigated	Blood & CSF	**Blood:** *S. aureus* 9, *S. pneumoniae* 1, Salmonella spp 4, *P. mirabilis* 1, Enterobacter spp 1, GAS 1, GBS 1, GGS 1 **CSF:** *S. pneumoniae* 4, *E. cloacae* 2, *E. coli* 1, GBS 1, Salmonella spp 1.
	Palmer (1999) [255]	1991-1994	Retrospective	Royal Victoria Hospital, Banjul, The Gambia	Admissions to neonatal unit of an urban tertiary referral hospital	420 paediatric meningitis admissions (0-15 years)	CSF	*S. pneumoniae* 15, *E. coli* 7, *E. cloacae* 7, *H. influenzae* 1, GAS 1, GBS 1, Klebsiella spp 1, *P. mirabilis* 1, Salmonella spp 1, Coliform 1

Table 3.1 Summary of 136 hospital-based studies of neonatal sepsis, meningitis and pneumonia in sub-Saharan Africa, 1980 – 2016 (continued)

Region	Author (year)	Data collection period	Data collection	Location	Source of case capture	Population (or neonatal sub-population where reported)	Culture category	Organisms
West Africa (continued)	Balaka (2004) [256]	1987-1993	Retrospective	CHU Lomé, Togo	Admissions to urban tertiary referral hospital	41 neonatal bacterial meningitis admissions (0-28 days)	CSF	*E. coli* 6, Enterobacter 4, *K. pneumoniae* 2, *S. enteritidis* 2, *P. mirabilis* 2, Citrobacter 1, *H. influenzae* 2, GBS 2, *S. pneumoniae* 2, *S. aureus* 2, *N. meningitidis* 2
	Balaka (2004) [196]	1993-1995	Retrospective	CHU Lomé, Togo	Admissions to urban tertiary referral hospital	433 inborn and outborn neonates with suspected sepsis (0-28 days)	Blood	*E. coli* 15, *K. pneumoniae* 6, Enterobacter 4, *S. aureus* 14, *S. epidermidis* 8, *P. aeruginosa* 1, Salmonella spp 2

SCBU= Special Care Baby Unit; NICU=Neonatal Intensive Care Unit; spp = species; ESBL=Extended spectrum β-lactamase producing; CoNS=Coagulase negative Staphylococci; GBS=Group B Streptococcus; GAS=Group A Streptococcus; GCS=Group C Streptococcus; GGS=Group G Streptococcus; MRSA=Methicillin Resistant Staphylococcus aureus;

Characteristics of included studies and quality of reporting

The quality of the data and completeness of reporting was assessed by applying selected key items regarding methods and results, from the STROBE-NI checklist to all published studies excluding the grey literature. Each study was assessed independently by both reviewers, and the reporting of each item was classified as either "'not reported/unclear" or "some information mentioned but insufficient" or "clear and detailed information provided". The heat map showing grading of the completeness of reporting of selected STROBE-NI across studies is presented in Table 3.2.

Table 3.2 Heat map showing grading of the completeness of reporting of selected STROBE-Neonatal Infection items in 118 studies on neonatal infections in sub-Saharan Africa

STROBE-NI checklist item		Assessment		
		Not reported/ Unclear	**Some information provided but insufficient**	**Clear and detailed report of STROBE-NI item**
Methods (Study design)	STROBE-NI 4.1 (Case ascertainment)			
	STROBE-NI 4.3 (Distinction between CAI and HAI)			
	STROBE-NI 4.5 (Sampling strategy)			
	STROBE-NI 4.6 (Microbiological methods)			
Methods (Setting)	STROBE-NI 5.2 (Newborn population included in the study)			
	STROBE-NI 5.5 (Facility characteristics and level of neonatal care)			
Methods (Variables)	STROBE-NI 7.1 (Clinical significance of pathogens)			
Results (Descriptive data)	STROBE-NI 14.2a (Key neonatal characteristics - Gestational age at birth)			
	STROBE-NI 14.2b (Key neonatal characteristics birth-weight)			
Results (Outcome data)	STROBE-NI 15.1 (Microbiological results in context of participants)			
	STROBE-NI 15.3 (Timing of infection)			

CAI = Community-acquired infection; HAI = Hospital-acquired infection

Case ascertainment (STROBE-NI Item 4.1)

There was significant variation in case ascertainment across studies. Twenty-three studies identified cases from retrospective review of culture data from laboratory databases, 38 studies from retrospective reviews of clinical and laboratory records, and the remainder were prospective studies of newborns admitted with clinically suspected invasive bacterial disease, sepsis, septicaemia, and/or meningitis.

Distinction between community and hospital-acquired infection (STROBE-NI Item 4.3)

Fewer than 10 studies indicated if the study focused on community-acquired infection (CAI), hospital-acquired infection (HAI) or both, and defined HAI using an international standard.

Sampling strategy (STROBE-NI item 4.5)

Less than half of the studies provided some information on the microbiological sampling – strategy (clinical indication vs routine); number of participants from whom samples were taken and sample type. Thirty-seven studies reported the blood sample volume which ranged from 0.4 mL – 3.5 mL, but only 12 of these reported on the timing of sampling in relation to antimicrobial administration. Forty-five studies used manual culture methods to isolate and identify bacteria and 21 used automated methods namely the BACTEC, BacT/Alert 3D, and Oxoid Signal systems. After excluding contaminants (where this information was available (including CoNS where mentioned), the proportion of positive cultures ranged from 4.2% - 44% among studies using automated culture methods and from 1.0% - 69% among studies using manual methods. Egbule and colleagues[218] reported 69% blood culture positivity rate among 98 neonates investigated for sepsis in a Teaching Hospital in Nigeria. For each neonate, 2 ml of venous blood was collected and aseptically introduced into two culture bottles.

Organisms were considered pathogens if the same organism was obtained in the two broth culture bottles, and contaminants if either the growth was obtained in only one culture bottle or a mixed growth obtained. Interpretation of detection rates and comparison to determine whether automated methods are superior to manual methods, especially for organisms such as GBS, is complicated by differences in the years of data collection, the use of different inclusion criteria, sampling volume, microbiological capability, and overall data quality.

Microbiological methods (STROBE-NI item 4.6)

Two studies reported that they used advanced pathogen identification methods using the automated VITEK II system,[161, 177] including Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF), Mass spectrometry and 16S rDNA polymerase chain reaction (PCR) sequencing.[161] In sSA, molecular methods have not been widely used as adjunct tests in the diagnosis of neonatal sepsis unlike in the diagnosis of pneumonia,[257] meningitis,[258] and diarrhoea.[57]

Newborn population included in the study (STROBE-NI item 5.2)

Sick newborns present to health-care services through several pathways, and therefore acquire infection from several settings. Consequently, stratification into “inborn" or “outborn” does not provide adequate information regarding place of birth (i.e. home vs the admitting facility or a referral facility) or take account of timing of admission (i.e. admission from birth vs return to the facility following discharge). Seventeen studies categorised their study population as either “inborn” or “outborn”.[124, 127, 131, 156, 157, 172, 177, 191, 208, 214, 219, 221, 226, 235, 239, 242, 244] Only eight studies provided specific place of birth categories of their study population such as “born at this facility”, “born at another

facility", or "born at home".[121, 130, 152, 164, 167, 169, 199, 216] The remaining reports did not include data on place of birth.

Facility characteristics and level of neonatal care (STROBE-NI Item 5.5)

All 137 studies were facility-based, and all but 19 were carried out in tertiary referral hospitals;[120-126, 129, 133, 165, 167-169, 179, 180, 193, 194, 246, 253] however, 10 studies reported data from predominantly rural populations - five from Kenya,[120-124] two from Mozambique,[125, 126] two from Nigeria,[127, 128] and one from Cameroon.[129] Only two studies: one from Malawi[167] and another from South Africa,[177] provided detailed information on the level of neonatal care available including invasive respiratory support and indwelling devices. Figure 3.3 shows regional variation in reported level of neonatal care. Few studies included information on the number of healthcare staff (nurse-to-patient ratio) on the neonatal unit.

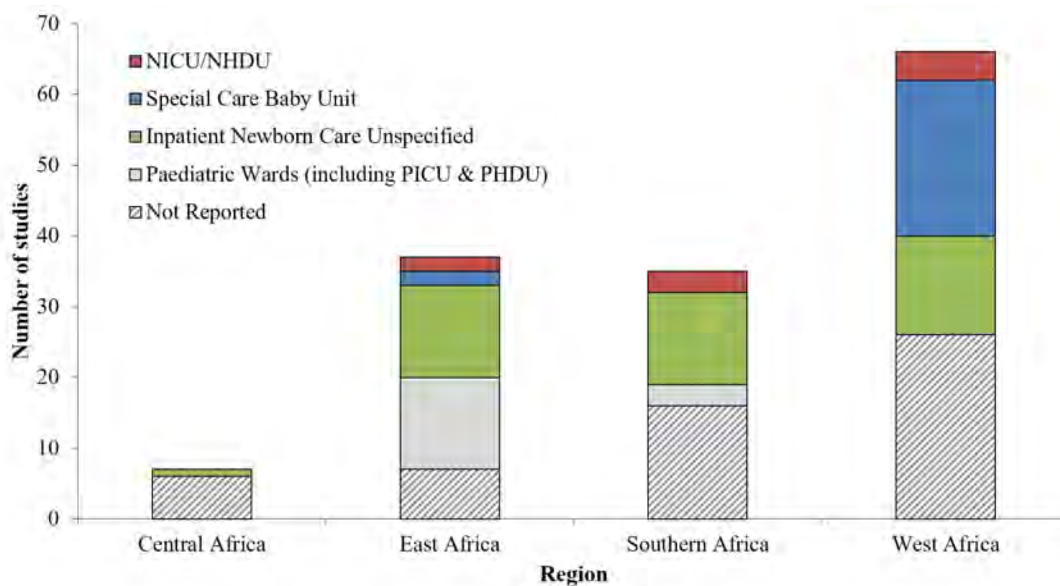


Figure 3.3 Regional variation in levels of neonatal care reported in studies of serious neonatal infection in sub-Saharan Africa.

Abbreviations. NICU=Neonatal Intensive Care Unit; NHDU=Neonatal High Dependency Unit; PICU=Paediatric Intensive Care Unit; PHDU=Paediatric High Dependency Unit

Clinical significance of pathogens (STROBE-NI item 7.1)

The majority of studies listed all detected organisms, but no study categorised organisms (as suggested in the STROBE-NI checklist) as being clinically significant, probably significant, and clinically non-significant (the preferred term to "contaminant"). Some studies listed organisms considered to be "contaminants" but did not mention the criteria[259, 260] used to define these organisms as contaminants.

Key neonatal characteristics – gestational age at birth and birth weight (STROBE-NI item 14.2)

More than half of the studies reported on key neonatal characteristics – gestational age at birth and birthweight – presenting the data in discrete categories with appropriate summary statistics (medians and ranges) for each numeric variable.

Microbiological results in the context of samples taken (STROBE-NI item 15.1)

Seventy-three studies reported blood culture positivity rates. These are presented by country, in Table 3.3.

Table 3.3 Reported blood culture positivity rates among neonates investigated for suspected sepsis from 73 studies in sub-Saharan Africa, by country

Country	Number of studies	Number of blood samples cultured	Number of positive cultures [a]	% of positive cultures Median (Range)[a]
Benin	1	95	14	14.7
Botswana	1	1119	91	8.1
Cameroon	2	343	38	10.8 (7.8 – 13.8)
Congo	1	130	29	22.3
Equatorial Guinea	1	293	28	9.6
Ethiopia	5	1206	402	32.0 (31.3 – 35.3)
Ghana	1	63	28	44.4
Kenya	5	2077	495	26.8 (22.3 – 60.5)
Madagascar	1	105	55	52.4
Mali	1	367	70	19.1
Mozambique	1	952	154	16.2
Nigeria	32	9742	3407	35.8 (27.0 – 44.0)
Senegal	3	3056	373	9.4 (5.1 – 29.9)
South Africa	7	9658	949	8.1 (1.3 – 15.3)
Tanzania	6	1634	391	20.8 (12.9 – 38.1)
Togo	1	433	50	11.5
Uganda	3	547	174	32.5 (21.8 – 37.5)
Zambia	1	313	102	32.6

IQR= Interquartile range
[a] Excluding contaminants where mentioned
[b] Median (IQR) only presented for countries with >1 study reporting data on number of blood samples cultured and number of culture positive cases

CSF positivity

Fifty-eight studies reported data on CSF cultures among neonates with suspected meningitis, 45% (26/58) of which reported on the number of neonates investigated by lumbar puncture and the CSF culture positivity rate (Table 3.4).

Table 3.4 Reported CSF culture positivity rates among neonates investigated for suspected meningitis from 26 studies in sub-Saharan Africa

Author (year)	Country	Region	Number of LP performed	Number of positive CSF cultures (%)
Chiabi (2011)	Cameroon	Central Africa	180	2 (1%)
Mulu (2014)	Ethiopia	East Africa	154	9 (6%)
Zewdie (2014)	Ethiopia	East Africa	112	6 (5%)
Gebremariam (1998)	Ethiopia	East Africa	55	30 (55%)
Mulu (2005)	Ethiopia	East Africa	35	2 (6%)
Mwaniki (2011)	Kenya	East Africa	845	20 (2%)
Laving (2003)	Kenya	East Africa	84	4 (5%)
Sigauque (2008)	Mozambique	Southern Africa	48	4 (8%)
Haffejee (1991)	South Africa	Southern Africa	277	9 (3%)
Wolzak (2012)	South Africa	Southern Africa	62	36 (58%)
Adhikari (1995)	South Africa	Southern Africa	60	55 (92%)
Fubisha (2012)	Zambia	Southern Africa	447	22 (5%)
Agossou (2016)	Benin	West Africa	85	0 (0%)
Balaka (2004)	Burkina-Faso	West Africa	37	28 (76%)
Do Rego (1988)	Cote d'Ivoire	West Africa	87	6 (7%)
Choketu (2005)	Mali	West Africa	367	35 (10%)
Ajayi (1997)	Nigeria	West Africa	747	32 (4%)
Onyedibe (2015)	Nigeria	West Africa	165	3 (2%)
Iregbu (2006)	Nigeria	West Africa	127	7 (6%)
Airede (2008)	Nigeria	West Africa	69	50 (72%)
Longe (1984)	Nigeria	West Africa	53	45 (85%)
Airede (1993)	Nigeria	West Africa	36	26 (72%)
Ambe (2007)	Nigeria	West Africa	14	6 (43%)
Camara (2003)	Sénégal	West Africa	71	52 (73%)
Palmer (1999)	The Gambia	West Africa	54	30 (56%)
Le Doare (2016)	The Gambia	West Africa	1	1 (100%)

[a] Excluding contaminants where mentioned

Aetiology of neonatal infection

Table 3.5 summarises the top five bacterial isolates (blood and CSF) reported across all 136 studies by country, and Figure 3.4 shows the top five bacterial isolates by sub-Saharan African region.

Table 3.5 Summary of studies reporting microbiologically-confirmed neonatal infection aetiology in sub-Saharan Africa (1980 – 2016), with the top 5 bacterial pathogens isolated by country

Region/Country (number of studies)	NMR	Top five bacterial pathogens by country (number of isolates)
Central Africa (n=7)		
Cameroon (n=4)	24	*E. coli* (29), GBS (18), *S. aureus* (10), Klebsiella sp. (10), S. pneumoniae (9), Acinetobacter sp. (7)
CAR (n=1)	42	*S. pneumoniae* (3), GBS (2), H. *influenzae* (2), Salmonella (2)
Congo (n=1)	21	Klebsiella sp. (12), Streptococcus sp. (4), *S. aureus* (3), *E. coli* (2), Enterobacter (2)
Equatorial Guinea (n=1)	32	Klebsiella sp. (16), Acinetobacter sp. (4), *E. coli* (4), CoNS (4)
East Africa (n=32)		
Kenya (n=11)	23	Klebsiella sp. (170), *S. aureus* (120), *E. coli* (104), Enterobacter (84), GBS (76)
Ethiopia (n=10)	28	Klebsiella sp. (124), *S. aureus* (61), *E. coli* (44), CoNS (29), *S. pneumoniae* (19)
Tanzania (n=6)	22	Klebsiella sp. (119), *S. aureus* (83), *E. coli* (63), CoNS (28), Enterococcus (23)
Uganda (n=3)	21	*S. aureus* (97), *E. coli* (26), *N. meningitidis* (11), GBS (8), Klebsiella sp. (6)
Madagascar (n=2)	19	CoNS (51), Klebsiella sp. (25), Enterobacter sp. (17), Enterococcus (9), *E. coli* (6)
Southern Africa (n=33)		
South Africa (n=19)	12	Klebsiella sp. (681), GBS (593), CoNS (365), *S. aureus* (278), *E. coli* (229)
Malawi (n=5)	23	GBS (305), *S. aureus* (210), NTS (189) *E. coli* (156), *S. pneumoniae* (130)
Zimbabwe (n=3)	23	GBS (114), *S. aureus* (71), *S. epidermidis* (55), Klebsiella sp. (42), *E. coli* (19)
Botswana (n=1)	26	Klebsiella sp. (43), GBS (15), *E. coli* (11), *S. aureus* (8)
Mozambique (n=2)	27	*S. aureus* (61), GBS (32), Enterococcus (10), NTS (9), *E. Coli* (9), *S. pneumoniae* (9), Enterobacter sp (6)
Zambia (n=2)	23	Klebsiella sp. (84), CoNS (7) *S. aureus* (6), *E. coli* (5), *S. pneumoniae* (5), Acinetobacter sp (1)
Namibia (n=1)	18	Klebsiella sp. (4), *S. pneumoniae* (2), *N. meningitidis* (2), *E. coli* (1)
West Africa (n=64)		
Nigeria (n=43)	34	*S. aureus* (1484), Klebsiella sp. (738), *E. coli* (492), Pseudomonas sp. (170), CoNS (83)
Ghana (n=4)	27	Enterobacter sp. (191), CoNS (90), Klebsiella sp (75), *S. aureus* (75), Enterococcus (71), Acinetobacter (60)
The Gambia (n=3)	28	*S. pneumoniae* (20), Enterobacter sp. (10), *S. aureus* (9), *E. coli* (8), Salmonella sp. (6)
Burkina Faso (n=2)	26	*E. coli* (8), *S. pneumoniae* (6), Enterobacter sp. (5), GBS/Klebsiella sp. (4), *S. aureus* (2), Salmonella (2)
Cote d'Ivoire (n=3)	37	GBS (15), Klebsiella sp. (5), *S. pneumoniae* (4), *H. influenza* (3), Pseudomonas (2)
Senegal (n=4)	21	Klebsiella sp. (189), *E. coli* (50), Enterobacter sp. (27), *S. aureus* (26), GBS (19)
Togo (n=2)	26	*E. coli* (21), *S. aureus* (16), Klebsiella sp. (8), Enterobacter sp. (8), *S. epidermidis* (8), Salmonella (4), GBS (2), *S. pneumoniae* (2), *H. influenzae* (2), *N. meningitidis* (2)
Benin (n=1)	31	*E. coli* (5), *S. aureus* (3), Klebsiella sp. (2), GBS (1), Pseudomonas (1), *S. epidermidis* (1)
Mali (n=1)	36	*S. aureus* (42), *S. pneumoniae* (21), *E. coli* (14), *H. influenzae* (6), Salmonella (5)
Niger (n=1)	26	*S. pneumoniae* (34), *N. meningitidis* (12), *H. influenza* type b (10)

CAR = Central African Republic; sp. = species

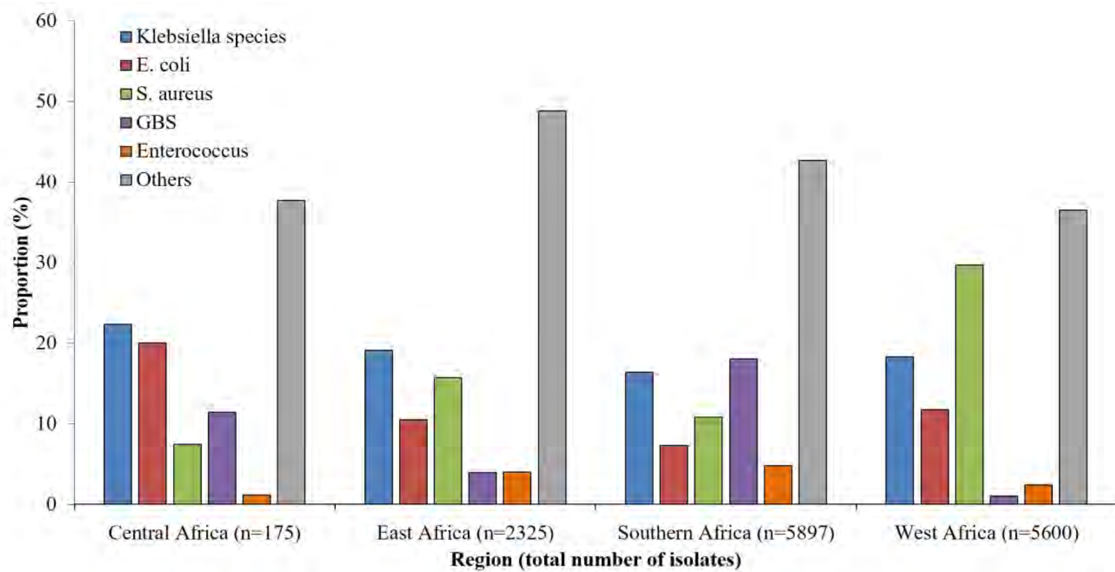


Figure 3.4 Top 5 reported neonatal infection aetiologies in sub-Saharan Africa by region (1980 – 2016).

Antimicrobial resistance

AMR was reported in most studies although some studies did not disaggregate resistance data by pathogen. Table 3.6 shows the reported AMR for *Klebsiella*, *E. coli*, *S. aureus* and GBS pooled from 25 studies where this disaggregated data was available. AMR data for Enterococcus was not reported by any study.

WHO-recommended first-line antibiotics for the treatment of neonatal infections are ampicillin and gentamicin. *Klebsiella* are intrinsically resistant to ampicillin, and the average reported gentamicin-resistance rate was 59%. Resistance to the third-generation cephalosporins, which are second-line treatment, was as high as 87% for ceftazidime but nearly 50% lower for more commonly used ceftriaxone. Just over 60% of reported *S. aureus* isolates were reportedly resistant to methicillin. Reported GBS resistance to gentamicin was 93%; however, resistance to ampicillin was low.

Table 3.6 Reported antimicrobial resistance in organisms causing neonatal infections in sub-Saharan Africa

	Klebsiella species		*Escherichia coli*		*Staphylococcus aureus*		*Group B Streptococcus*	
	No. Tested	**No. Resistant**	**No. Tested**	**No. Resistant**	**No. Tested**	**No. Resistant**	**No. Tested**	**No. Resistant**
Penicillins								
Ampicillin	582	449 (77%)	262	221 (84%)	342	266 (78%)	31	8 (26%)
Amoxicillin [a]	524	366 (70%)	191	119 (62%)	429	185 (43%)	20	7 (35%)
Cloxacillin	67	59 (88%)	34	30 (88%)	410	259 (63%)	-	-
Methicillin	-	-	-	-	45	28 (62%)	-	-
Penicillin	73	72 (97%)	30	28 (93%)	264	226 (86%)	153	3 (22%)
Piperacillin-tazobactam	119	46 (39%)	22	3 (15%)	1	0 (0%)	-	-
Cephalosporins								
Cefotaxime	326	248 (87%)	129	40 (31%)	60	11 (18%)	14	5 (36%)
Ceftazidime	342	203 (59%)	160	74 (46%)	510	277 (54%)	9	5 (56%)
Ceftriaxone	337	142 (42%)	180	58 (32%)	638	272 (43%)	10	5 (50%)
Aminoglycosides								
Gentamicin	747	439 (59%)	309	125 (40%)	773	203 (26%)	158	147 (93%)
Amikacin	339	49 (14%)	62	5 (8%)	46	0 (0%)	14	0 (0%)
Fluoroquinolone								
Ciprofloxacin	368	113 (31%)	127	32 (25%)	247	42 (17%)	1	0 (0%)
Carbapenem								
Imipenem/ Meropenem	256	13 (5%)	71	7 (10%)	9	1 (11%)	1	0 (0%)
Others								
Vancomycin	13	12 (92%)	14	14 (100%)	156	26 (17%)	17	1 (6%)

[a] Includes Amoxicillin/Clavulanate

3.4 Discussion

This systematic review has shown that aetiology-specific data for neonatal infections are challenging to combine and interpret due to the considerable variation in case ascertainment, data recording, and reporting. However, the most commonly reported bacterial causes of serious neonatal infection from facility-based studies in sSA are, in ranked order; *S. aureus*, *Klebsiella* species, and *E. coli*.

Facility-based neonatal infection studies have the advantage of optimising the potential for case finding if the majority of births in the study population occur in the hospital, increasing the probability of rapid identification of early onset infection; if the majority of infants return to the same hospital in a timely manner if they become ill after postnatal discharge; and if the hospital is a specialist referral centre to which infants born elsewhere are referred if presenting with pSBI.[261] In many sub-Saharan African countries however, the majority of women still give birth at home without a skilled attendant despite progress in scaling up skilled care at delivery.[262] Many of these newborns die in the community within the first week primarily due to delays in recognising problems, deciding to seek care, and in transportation to reach appropriate care.

Few studies clearly distinguished between community-acquired and nosocomial infections, which is important as associated pathogens and antimicrobial resistance patterns differ according to the source of infection. Developing country hospital environments are usually reservoirs of infection with highly drug resistant pathogens; hospital-born babies will therefore acquire different pathogens than those born in the community.[21] *Klebsiella* species, *E. coli* and *S. aureus* were reported as major pathogens responsible for culture-confirmed neonatal bloodstream infection and meningitis across

all regions, with some inter-regional variation. *Klebsiella* predominated in all regions except West Africa where *S. aureus* was most predominant. Although *Klebsiella* can be part of the normal maternal gastrointestinal and vaginal flora, the extended spectrum β-lactamase (ESBL)-producing *K. pneumoniae* infections reported in several studies[136, 154, 170, 172, 177, 183, 184] are usually acquired from heavily contaminated hospital environments. *S. aureus* was most commonly reported across West Africa however, 90% of reported cases were from Nigerian studies and it is unclear what proportion of these were nosocomial outbreaks. *S. aureus* is both a human commensal and a major cause of community and HAIs and usually spread through the hands of health-care providers.[82] Given the context of studies in this review (facility-based studies of predominantly facility-born infants), these reported high rates of neonatal *Klebsiella* and *S. aureus* infections in sSA further support the view that the vast majority of infections among facility-born neonates in developing countries may be hospital-acquired. [21]

Notable were the inter- and intra-regional differences in the reported occurrence of infection due to GBS. The Southern African region recorded the most number of GBS isolates (1059), although almost 60% of isolates were reported by 15 studies from South Africa[130, 171, 172, 174, 175, 177, 179-182, 184, 185, 187-189] where the incidence of invasive GBS disease has been shown to vary markedly by province.[261] Within the region, GBS was also the top reported pathogen in studies from Malawi[165-169] and Zimbabwe;[192-194] but no single isolate was reported in studies from Namibia[170] and Zambia.[190, 191] GBS was also not reported in studies from Congo,[135] Equatorial Guinea,[136] Ethiopia,[137-146] Tanzania,[155-157, 159-161] Madagascar,[153, 154] and Niger.[205] This might be explained by the small number of eligible studies from these countries (two or less, with the exception of Tanzania where there were six studies). The lowest number of isolates overall were

reported from West Africa (58). Surprisingly, although Nigeria had the most number of published neonatal infection studies overall, only two studies reported isolation of GBS.[230, 235] These findings may reflect true geographic differences in disease epidemiology, empiric antibiotic treatment before referral or operational factors such as sampling techniques, blood culturing practices, variability in laboratory capacity, and the quality of microbiological investigations.[99, 112, 261] In South Africa,[263] Kenya[264] and Malawi,[265] GBS serotypes III and Ia cause over 75% of both early and late-onset disease. A recent meta-analysis of GBS serotype distribution in sub-Saharan Africa showed that serotypes III and Ia contribute over 70% and 90% to the overall serotype distribution for early-onset and late-onset GBS disease respectively with serotype III being predominant.[266] The authors did not report regional differences in serotype distribution for invasive disease, possibly due to the paucity of serotype data.

Few studies have reported the contribution of viruses to neonatal infection morbidity in sSA. In a prospective study of viral pneumonia aetiology among infants and children in Kenya, Berkley *et al.* reported respiratory syncytial virus (RSV) as the most common cause of neonatal pneumonia with an incidence rate of 2.46 per 1,000 live births.[267] Other reported viruses were; human coronavirus 229E (0.51 per 1,000 live births); influenza type A (0.31 per 1,000 live births); parainfluenza virus 3 (0.10 per 1,000 live births); adenovirus (0.10 per 1,000 live births); and human metapneumovirus (0.10 per 1,000 live births). The fact that viruses other than RSV were as common among well infants and children and those with mild URTI as among those with severe disease led the authors to infer that these make only a minor contribution to the burden of severe clinical pneumonia in rural Kenya. In Nigeria, Okuonghae and colleagues[268] reported RSV infection among 14 out of 56 newborns admitted to a neonatal nursery, with 11 of

the 14 cases being nosocomially acquired. Two other studies have reported nosocomial outbreaks of viral infection in neonatal units involving RSV[269] and adenovirus.[270]

Antimicrobial resistance is a global threat and has reached alarming levels in neonatal units in developing countries. The reported high rates of resistance of the leading pathogens to WHO-recommended first-line antibiotics, with increasing resistance of Klebsiella to second-line antibiotics is worrisome for developing countries with limited treatment options. Even more worrying is the fact that in places where newer alternative antibiotics are available, such as meropenem and piperacillin-tazobactam, moderate rates of resistance are already being reported.

This is the first systematic review of neonatal infection aetiology in sSA, employing a comprehensive search strategy with the inclusion of African databases, grey literature, and no language restrictions. Another strength of this review is in the assessment of the quality of reporting among published studies using the STROBE-NI guidelines. Except for differences in the reported occurrence of GBS, these data suggest that the major pathogens associated with neonatal infection in sSA are similar within and between regions but differ from that observed in neonates in developed regions where GBS, *E. coli* and coagulase-negative staphylococci (CoNS) are the predominant pathogens.[73, 79, 271, 272] Further research would provide a more current picture of aetiological distribution and allow for regular guideline updates.

Aetiology-specific data for neonatal infections are however challenging to combine or interpret due to inconsistencies in data recording and reporting.[117] This review however has several limitations. Excluding studies before 1980 to narrow the literature review may have resulted in missing some relevant studies. Noteworthy is the fact that

a third (43/136) of studies were based in Nigeria, and there were little or no data for other countries with comparable neonatal mortality levels.

The data showed a wide range of positive blood cultures; several studies had very low culture positivity rates, resulting in small numbers of organisms being reported. This might be related to different study criteria for doing blood cultures, with selective culture of the sickest newborns, or only those with risk factors versus all admissions. Nearly all studies were carried out in routine clinical settings where the costs of investigations are borne by the parents. In some studies, the inability of several parents to pay for the blood cultures (even when the cost had been reduced by half) resulted in blood culture not being performed for all the newborns in the study.[195] Some studies presented considerably larger numbers of isolated organisms than others, thereby giving greater weight to their reported aetiological data. The potential to generalise results is limited by small sample sizes.

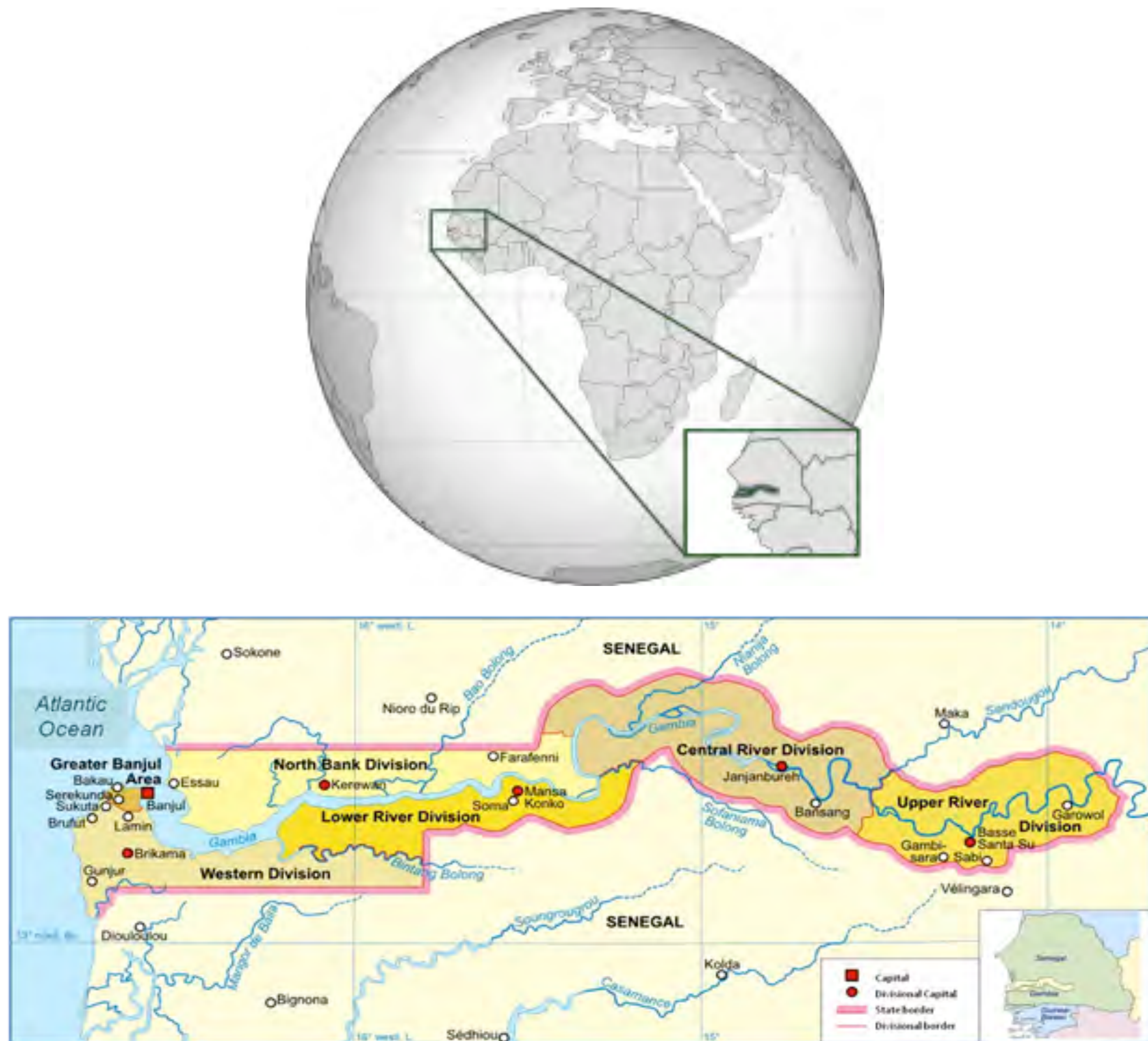
Case ascertainment was ill-defined across studies and included mixed neonatal populations (preterm vs term) with incomplete reporting of pathogens and clinical significance of isolates in relation to gestational age and birthweight. The risk of infection increases greatly with decreasing birthweight but variables such as gestational age, birthweight and level of care provided were not defined in most of the studies. The risk of hospital-acquired infections is higher among preterm infants admitted to intensive care units due to the use of invasive devices. Another limitation in the data is the population representability due to the lack of community-based studies. Infection-related early neonatal deaths among home-births not presenting to hospital are not reflected in facility-based research, especially GBS-related deaths. Research shows that the aetiology of neonatal sepsis is continually evolving, and therefore continuing

updating of aetiological data are necessary to inform appropriately targeted management and interventions.

The largest numbers of studies found by this review (n=90) were conducted between 2011 and 2010 and only 27 studies were relevant to the most recent period of 2011-2015, although several studies spanned different periods, highlighting the need for new research in this area and implying potential issues with the representativeness of data presented here particularly with regard to a possible epidemiological evolution in aetiology of neonatal infection. This further made it difficult to examine whether the introduction of childhood vaccines against *Haemophilus influenzae* type b and *Streptococcus pneumoniae* may have had effect on aetiology especially as these vaccines were deployed at different times across countries in the region. Although these vaccines do not have a direct effect on the newborn as they are not given in the neonatal period or antenatally to the mother like the tetanus vaccine, the resultant herd immunity may potentially have an indirect effect on the newborn.

Despite decades of literature on neonatal infection aetiology in sSA, burden and risk factors, appropriate treatment algorithms and effective interventions are still not well understood. The findings from this review further emphasize the need to improve the data on neonatal infection aetiology (bacterial, viral and fungal), antimicrobial sensitivity and outcomes.[117] The recently published Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) statement will improve scientific reporting of observational neonatal infection studies, to increase comparability and to strengthen research in this area.[117] Future research should focus on areas of high disease burden with relative paucity of data.

CHAPTER 4. THE GAMBIAN CONTEXT FOR SERIOUS NEONATAL INFECTIONS



Map of The Gambia

Source: https://www.worldofmaps.net/en/africa/map-the-gambia/map-regions-gambia.htm

Overview

The Gambia is the smallest country in mainland Africa extending 400km inland from the West African coast along the Gambia River. It is surrounded on three sides by Senegal and its entire western extremity is marked by coastline on the Atlantic Ocean.

Agriculture and tourism are economic foundations for The Gambia, which has a gross national income per capita of $US610, half the average for sub-Saharan Africa, with half the population under the national poverty line. The population is predominantly Muslim, and ethnically there are over a dozen tribes the largest of which are the Mandinka and Wollof.

4.1 Health service delivery

In the public sector, health service delivery is organised into a three-tier system: Primary (Village Health Services; VHS), Secondary (minor and major health centres) and Tertiary (Hospitals).[273] The VHS consist of community health workers traditional birth attendants (TBA) and village health workers (VHW) who are often the first point of contact between individuals, families and communities within the health system. These community health workers are supervised by trained community health nurses (CHN). TBAs provide care for pregnant women, conduct normal deliveries, identify and refer obstetrics emergencies. The VHWs on the other hand are involved in health promotion and prevention measures, the treatment of minor ailments, and refer cases beyond their scope of management. The VHS are complemented by the Reproductive and Child Health (RCH) trekking visits from the health centres. The RCH package includes: antenatal care, child immunization, growth monitoring, registration of births and deaths, and limited treatment for sick children.

The minor health centre is the unit for the delivery of basic health services including basic emergency obstetric care. The national standard for a minor health centre is 20-40 beds per 15,000 population. The minor health centre is to provide up to 70 percent of the Basic Health Care Package need of the population. The major health centre serves as the referral point for minor health centres for comprehensive emergency obstetric care (surgical, blood transfusion services and further medical care). Additionally, they also offer services such as infant welfare and antenatal services, surveillance and dental services. The standard bed capacity for major health centres ranges from 110 -150 beds per 150,000 - 200,000 population.

The general and regional hospitals serve as referral points for the Major health centres as they provide specialised services. The Edward Francis Small Teaching Hospital (EFSTH) also serves as the referral hospital for the general hospitals. There are currently 38 minor and 6 major health centres, 5 general hospitals, 1 regional eye hospital and a university teaching hospital spread across 8 local government areas and 43 districts.[273] Access to health facilities is relatively good, and over 85% of the population live within three kilometres of a primary health-care or outreach health post and 97% of the population within five kilometres.[274]

4.2 Progress during the era of the Millennium Development Goals

During the era of the Millennium Development Goals (MDGs), considerable progress was made in reducing under-five mortality but much less in reducing neonatal mortality (Figure 4.2). Data from the Farafenni Demographic Surveillance System located in a rural area on the North Bank region of The Gambia showed a 56% drop in under-five mortality between 1992 and 2008 but the corresponding decline in neonatal mortality was only 38%.[275] Nevertheless, neonatal deaths now account for 45% of under-five

mortality compared to 31% in 1990.[276] The neonatal mortality rate (NMR) of 30 deaths per 1000 live births ranks 8th in descending order among the 18 countries of West Africa, and is two and a half times the Sustainable Development Goals (SDGs) target of 12 neonatal deaths per 1000 live births in 2030. [277]

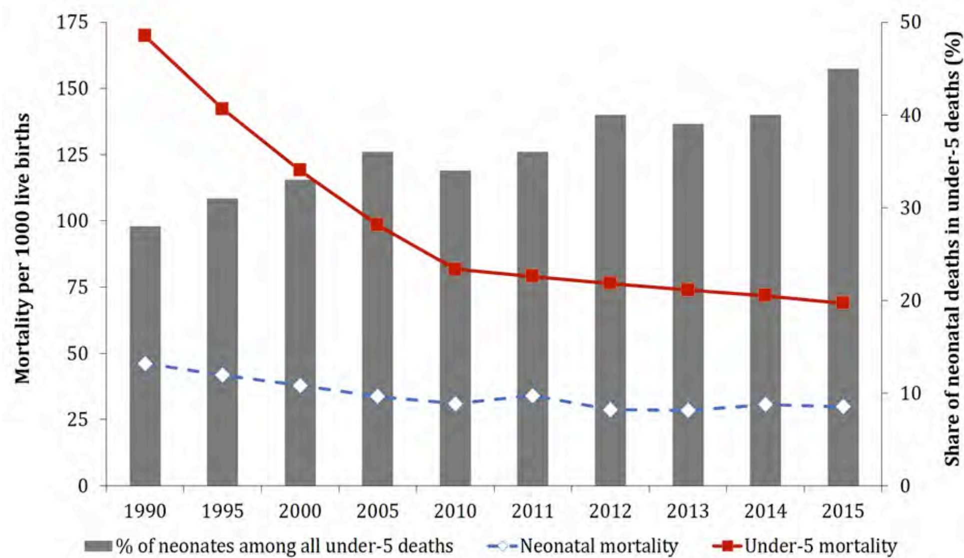


Figure 4.1 Trends in Under-5 and neonatal mortality in The Gambia, 1990-2015.

Data sources: Child Mortality Estimates. http://www.childmortality.org/; A Promise Renewed Progress Reports 2012 – 2015. http://www.apromiserenewed.org/publications/

Over the same period, there has been a marked improvement in maternal and child health despite high levels of poverty, limited resources and a shortage of adequately and appropriately trained staff.[278-284] (Table 4.1).

Table 4.1 Maternal, Newborn & Child Health in The Gambia

Total population (2015)	1 991 000
Mothers, newborns and children	
Annual births (2015)	83 000
Maternal mortality ratio per 100,000 live births (2015)	706
Annual number of maternal deaths (2015)	590
Still birth rate per 1000 total births (2013)	27
Annual number of stillbirths (2013)	1734
Neonatal mortality rate per 1000 live births (2015)	30
Annual number of newborn deaths (2015)	2168
Neonatal deaths as a proportion of all under-5 deaths (2015)	45%
Infant mortality rate per 1000 live births (2015)	48
Annual number of infant deaths (2015)	4000
Under-5 mortality rate per 1000 live births (2015)	69
Annual number of under-5 deaths (2015)	6000
Human resources for health[285]	
Physicians density (general and specialist) per 10,000 population (2008)	1.07
Nursing & Midwifery personnel density per 10,000 population (2008)	8.65
Coverage indicators	
Institutional delivery (women 15 – 49 years who gave births in a health facility; 2015)	63%
Skilled attendant at birth (doctor, nurse or midwife; 2015)	57%
Antenatal care coverage – at least 1 visit (2015)	86%
Antenatal care coverage – at least 4 visits (2015)	78%
Births by caesarean section (2015)	2%
Context	
Increasing urban migration with higher urbanized population	
Low literacy rate and high poverty	

[1]Adapted and updated from Okomo *et al.*[99]

There is also limited neonatal infection aetiology data for the same period. Although pathogens associated with infection in Gambian newborns have been described within the context of studies investigating infections in infants and children (Table 4.2), with the earliest study in 1999 being part of the first WHO multicentre study of aetiology and clinical signs of serious infections in young infants in developing countries,[254] no formal study on the aetiology of neonatal infections in The Gambia has been conducted to date.

Table 4.2 Summary of studies reporting neonatal infection aetiology in The Gambia (1990 – 2015)

First author (year)	Study period	Study site(s)	Study design and population (neonatal sub-population where reported)	Neonatal isolates (n)
Mulholland (1999)[254]	1990 – 1991, 1992	MRC Unit, Fajara & The Royal Victoria Hospital, Banjul[a]	Prospective study of serious infections in very young infants (0 – 90 days) 476 infants investigated for possible sepsis (unspecified number of neonates < 1 month)	**9 CSF culture positive**: *S. pneumoniae* (4), *E. cloacae* (2), *E. coli* (1), *Salmonella* spp (1), GBS (1) **19 Blood culture positive:** *S. pneumoniae* (1), *P. mirabilis* (2), *Enterobacter* spp (1), GBS (1), GAS (1) *Salmonella* spp (4), S. aureus (9)
Palmer (1999)[255]	1991 - 1994	Royal Victoria Hospital, Banjul[a]	Retrospective case review of paediatric meningitis admissions 420 children with suspected meningitis (54 neonates < 1 month)	**31 CSF culture positive:** *S. pneumoniae* (15), E. *coli* (7), GAS (1), Hib (1), *P. mirabilis* (1), *Klebsiella* spp (1), Coliform (1) *Salmonella* spp (1), GBS (1), *E. cloacae* (1), *Bacillus* spp (1),
Goetghebuer (2000)[286]	1990 - 1995		Retrospective study of prevalence & severity of late sequelae after Hib and Pneumococcal meningitis. 257 children with culture-positive meningitis. (33 neonates ≤ 1 month)	**33 CSF culture positive:** *S. pneumoniae* (32), Hib (1)
Le Doare (2016)[253]	2014 - 2015		Prospective longitudinal cohort study of GBS carriage in mother/infant pairs. 750 mother/infant pairs (41 neonates with signs of systemic infection)	**1 CSF culture positive:** GBS (1)
Darboe et al (Unpublished)	2005 - 2015	MRC Unit, Fajara	Retrospective laboratory surveillance	**11 CSF culture positive :** *S. pneumoniae* (6), *E. coli* (3), *S. aureus* (2) **67 Blood culture positive [b] :** *S. pneumoniae* (4), S. aureus (33) *Proteus* spp (1), GBS (3), E. coli (3), *Pseudomonas* spp (4), *Shigella* (1), *Enterobacter* spp (2), Coliforms (3), *Acinetobacter* (1), *Klebsiella* spp (11), Raoultella *ornithinolytica* (1)

[a] Now called Edward Francis Small Teaching Hospital

[b] Excluding 9 blood culture isolates from my PhD study pilot study patients reported later in this thesis

In these studies, the most important causes of serious neonatal infections were *S. pneumoniae, S. aureus*, and *E. coli*. With improved maternal and perinatal care over the last two decades as well as the introduction of several vaccines (*Haemophilus influenza* type b and pneumococcal conjugate vaccines), it is possible that the demographics, pathogens, and outcome associated with serious neonatal infections in The Gambia have changed. The use of novel molecular techniques for diagnosing serious neonatal

infections has also not been studied in West Africa, and may provide interesting insight into potentially missed bacterial and viral aetiologies.

Determining the aetiology of serious neonatal infections and identifying acquisition pathways, particularly the role of maternal colonisation is an important step towards understanding the epidemiology of neonatal infections. This is necessary to inform and prioritize appropriate intervention strategies in this setting, such as the decision to either target treatment of maternal colonisation, and or prophylactic treatment of newborns of high-risk mothers as a means of preventing a significant proportion of early-onset serious newborn infections.[88] It will also inform the development of standardised protocols to improve aseptic practices in the labour rooms, maternity and neonatal units, as well as protocols for the management of neonatal infections.

CHAPTER 5. AUDIT OF NEONATAL ADMISSIONS, QUALITY OF CARE AND OUTCOME AT THE GAMBIA'S TEACHING HOSPITAL

The Edward Francis Small Teaching Hospital, Banjul, The Gambia with staff and patients at the Neonatal Unit

Overview

Patterns in newborn survival are a sensitive indicator of the functionality of a health system and its response to its most vulnerable population.[287] In order to be credible, referral facilities must be capable of providing appropriate, affordable and quality care.[288] National facility-based neonatal mortality audits provide a formal assessment of inpatient care and are an important source of data to identify gaps in the quality of care and therefore, areas for improvement in relation to service delivery and patient outcomes.

5.1 Audit methods

5.1.1 Setting

The Edward Francis Small Teaching Hospital (EFSTH), is the national teaching hospital and the only tertiary government referral hospital for health facilities in The Gambia.[279] Built in 1853 and located in the capital city of Banjul, it was originally called the Royal Victoria Hospital but was renamed the Royal Victoria Teaching Hospital between 1999 and 2013, when it became part of the newly founded Faculty of Medicine of the University of the Gambia and began providing clinical education for medical students. It is the largest hospital in The Gambia, and the maternity unit delivers an average of 6000 newborns each year. Until 2016, it was the only hospital in The Gambia with neonatal care facilities and is staffed by a neonatologist, two medical officers and several house officers. In-house departmental training is occasionally conducted for medical and house officers as well as midwives, sometimes in conjunction with visiting foreign medical personnel. Trained nurses assisted by nurse attendants provide nursing care. There are usually between two and four trained nurses with an equal number of nurse attendants during the day (morning and afternoon shifts), and only one trained

nurse and one nurse attendant during the night. This distribution is usually maintained during weekdays and weekends.

Figure 5.1 Staff at work in the EFSTH neonatal ward

The ward is separated into high- and low-risk areas with the aim of limiting the spread of infection, and comprises 31 cots and four functional incubators. Cot occupancy is often more than 200% with neonates sharing cots and incubators during peak admission periods. Mothers are allowed unrestricted access to the ward for the purpose of regular breastfeeding.

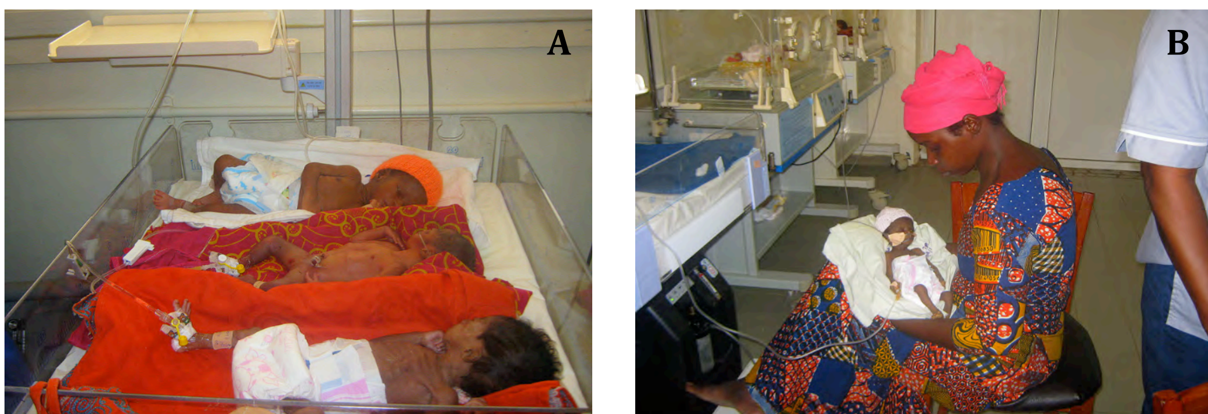


Figure 5.2 Neonatal admissions at the EFSTH neonatal ward

A) Preterm neonates sharing the bed under a radiant warmer due to shortage of incubators; B) A mother bonding with her preterm infant

Care is basic and mostly supportive, consisting of parenteral fluids and medications (antibiotics, phenobarbitone, and aminophylline), nasogastric tube feeding, and phototherapy. Fluids and medications are given via peripheral intravenous catheter, as central venous catheters are not used. Limited respiratory support in the form of oxygen administered from electricity-operated oxygen concentrators is available. During the period under review, there were only two oxygen concentrators available in the ward; using a splitter, up to ten infants can receive oxygen at one time from each concentrator. Kangaroo mother care (KMC) is not yet practiced although several staff have received the relevant training.

Microbiology is performed in the main hospital laboratory, but haematology and chemical pathology services are provided by the paediatric department's laboratory which is supported by the Medical Research Council (MRC) Unit in The Gambia. Full blood count and blood film for malaria parasites are the only laboratory investigations available during weekends. Blood glucose is measured on the ward using a glucometer; however, the glucometer strips are frequently out of stock. Laboratory sticks for protein measurement are not available. Only limited radiological services are available during weekends in the hospital.

5.1.2 Study design

This was a retrospective analysis of routine neonatal inpatient data for the period 1 January 2009 to 31 December 2013.

5.1.3 Data collection

The medical records of neonates admitted during this period were retrieved from the Records Department. To determine the completeness of record retrieval, the ward admission books were reviewed to establish the total number of admissions for the

period, and an exhaustive search was made for all missing records. Data were extracted from available records detailing dates of birth, admission and outcome; gender; birth and/or admission weight; estimated gestation; mode and place of delivery; antenatal care, obstetric complications; maternal HIV status; anti-retroviral administration for prevention of mother-to-child transmission (PMTCT) of HIV; tetanus toxoid immunization and intermittent preventive treatment for malaria during pregnancy (IPTp); investigations and treatment, as well as admission and final diagnoses. Where available, gestational age was copied from the antenatal card (this is usually calculated from the fundal height, and infrequently from an ultrasound scan), and a few neonates were assessed for clinical maturity using the New Ballard Score.

5.1.4 Definitions and outcome

Diagnoses were mostly clinical. A diagnosis of pSBI or neonatal infectious syndrome (sepsis, meningitis or pneumonia) at admission was based respectively, on the presence of one or more of the WHO YICSSG diagnostic algorithm criteria[8] or physician diagnosis. Prematurity was defined as a gestational age of less than 37 completed weeks or a birthweight of <1.5 kg when the gestational age was not available. 1.5kg (very low birthweight, VLBW) was used as the cut-off as the most likely cause of VLBW is preterm birth. Late preterm was defined as a gestational age of 34–36 completed weeks. For neonates admitted on the day of birth for which no birth-weight had been documented, the admission weight was used as the_birth-weight. Where no final diagnoses were provided, the admission diagnoses were used.

The primary outcome was death in the hospital. Age at admission and at death and length of hospital stay were calculated from the raw data. Times of admission and of death were examined using two different exposure categories. The first was based on

whether admission occurred at the weekend (commencing on Friday at 2.00 p.m., when weekday work ends, until Monday at 7.59 a.m.) or during the week (Monday 8.00 a.m. until Friday 1.59 p.m.). The second exposure category was based on an aggregation of the day of the week (weekday vs weekend) and time of the day, defined as on-call duty hours (4.00 p.m. to 7.59 a.m. Monday to Thursday or at the weekend) and regular working hours (8.00 a.m. to 4.00 p.m. weekdays except Friday when it is 8.00 a.m. to 1.59 p.m.).

5.1.5 Statistical analysis

All statistical analyses were performed with STATA version 13 (Stata Corp., College Station, TX, USA) and statistical significance was defined as alpha 0.05 (two-sided). Categorical and continuous variables were summarised, respectively, as percentages and median (inter-quartile range). Cross-tabulations with outcome were performed using the χ^2 statistic for categorical variables. Neonates whose outcome could not be ascertained from their hospital records and those who were removed against medical advice (or absconded from care) were excluded from further analysis.

Neonatal characteristics of interest were assessed as risk factors for death using logistic regression, adjusting for age, sex and admission weight as potential confounders. Odds ratios with accompanying 95% confidence intervals and Wald test P-values (two-tailed) for the univariate and multivariable analyses are reported.

5.2 Results

Between 1 January 2009 and 31 December 2013 there were 7161 admissions to the neonatal ward; medical records for 5285 neonates were recovered from the Records Department representing 74% of all admissions for the period under review. The proportions of records retrieved by year are shown in Table 5.1. Owing to low records

capture, data from 2009 were subsequently excluded from analysis, and the results for 4944 admissions during 2010–2013 are presented.

Table 5.1 Annual neonatal ward admissions and numbers of records retrieved

Year	Number of admissions (ward register)	Number of records retrieved	Records retrieved as a proportion of admissions
2009	1,463	341	23%
2010	1,381	1,117	81%
2011	1,245	1,048	84%
2012	1,448	1,429	99%
2013	1,484	1,350	91%
Total	**7,021**	**5,285**	**73%**

Characteristics of neonates and mothers

The characteristics of admitted neonates are shown below in Table 5.2. The majority (64%, 3142/4944) of neonates were facility-born. Birthweight was only recorded for 67% (3336/4944) of newborns, 50% (1672/3336) of which were <2500g. Of the 1289 neonates born at <37 completed weeks of gestation, 30% (387/1289) were born between 34 and 36 weeks of gestation.

Respiratory rate was the most commonly recorded vital sign, found in 4525 (92%) of case notes; 38% (1729/4525) of newborns had a respiratory rate >60 breaths/minute. 48% (2282/4413) of newborns whose axillary temperature was documented at admission had hypothermia (temperature <36.5°C) and 28% (1310/4413) had fever (temperature ≥37.5°C). Hypothermia was more prevalent on admission in neonates born at EFSTH than in those born outside (69% *vs* 46%, *P*<0.001), and 80% of neonates admitted on the day of birth were hypothermic. Nearly two-thirds (62%) of those admitted with hypothermia weighed <2500g, and half of these were very small (<1500g). The prevalence of hypothermia decreased significantly with increasing admission weight: 83% <1500 g, 51% 1500–2499 g and 36% ≥2500 g (*P*<0.001). Of the

3455 neonates with an admission blood glucose measurement, hypoglycaemia (<2.6 mmol/L) was detected in 666 (19%) and hyperglycaemia (>6.9 mmol/L) in 608 (18%).

Table 5.2 Characteristics of 4944 neonatal inpatients at EFSTH, Banjul 2010–2013

Categories	*n*	%
Sex		
Male	2782	56.3
Female	2020	40.8
Unknown/missing	142	2.9
Age on admission (days)		
Day of birth	2242	45.4
2–7	1550	31.3
≥8	907	18.3
Unknown/missing	245	5.0
Gestational age (weeks)		
Preterm, <37	1289	26.1
Term, 37–42	500	10.1
Post term, >42	17	0.3
Unknown	3138	63.5
Weight on admission (grams)		
<1500	942	19.1
1500–2499	1411	28.5
2500–3999	2347	47.5
≥4000	148	3.0
Unknown/missing	96	1.9
Place of delivery		
Home/Traditional birth attendant	437	8.8
EFSTH	1590	32.2
Other health facility	1552	31.4
Born before arrival	14	0.3
Unknown/missing	1351	27.3
Mode of delivery		
Vaginal	3452	69.8
Caesarean	724	14.7
Unknown/missing	768	15.5
Maternal age (years)		
<18	219	4.4
18–35	2924	59.2
>35	328	6.6
Unknown/missing	1473	29.8

Information on maternal antenatal care was documented for 83% (4106) of newborns; the majority (62%, 2565/4106) of mothers received antenatal care, of whom 51% (1316/2565) attended at least four times. Evidence of maternal HIV screening was recorded for 132 (3%) newborns. Of the 132 mothers who were documented to have received HIV counselling and testing with or without accessing PMTCT services; 30

(23%) were HIV-positive (mostly HIV-1, with only one case of HIV-1 and 2 dual infection). Only 11 (37%) of the 30 HIV-exposed newborns received a PMTCT drug (nevirapine). The presence or absence of obstetric complications was indicated in 59% (2937/4944) of records, most (70%, 2047/2973) of whom had at least one recorded complication.

Clinical diagnoses

pSBI accounted for 44% (2166/4944) of admissions, 27% (1340/4944) of prematurity/low birthweight (LBW), and 20% of intrapartum-related conditions. Characteristics of newborns with pSBI are described further in Table 5. 3.

Table 5.3 Characteristics and clinical assessment of neonates with possible severe bacterial infection (pSBI)

Characteristics	Total no.	pSBI *n* (%)	Non-pSBI *n* (%)	*P*
Place of birth, *n*=3593				
Inborn (EFSTH)	1590	379 (28)	1212 (54)	
Other hospital facility	1556	715 (53)	851 (38)	
Home/TBA	437	249 (19)	188 (8)	<0.001
Mode of delivery, *n*=4167				
Vaginal	3452	1522 (89)	1930 (78)	
Caesarean section	724	193 (11)	531 (22)	<0.001
Age at admission, days, *n*=4699				
1–3	2242	984 (49)	2313 (87)	
4–7	1550	413 (20)	83 (3)	
≥8	907	629 (31)	278 (10)	<0.001
Admission weight, g, *n*=4848				
<1500	942	187 (9)	756 (28)	
1500–2499	1411	643 (31)	768 (28)	
≥2500	2495	1280 (60)	1215 (44)	<0.001
Symptoms/signs*				
History of convulsions	393	264 (67)	129 (33)	<0.001
History of difficulty feeding	2192	1079 (49)	1113 (51)	<0.001
Fever, temp. ≥37.5°C	1259	1052 (81)	243 (19)	<0.001
Hypothermia, temp. <36.5°C	2165	455 (21)	1711 (79)	<0.001
Restlessness/irritability	474	359 (76)	115 (24)	<0.001
Difficulty breathing	2351	917 (39)	1434 (61)	<0.001
Lethargy/reduced movement	238	134 (56)	104 (44)	<0.001

* Row percentages

Jaundice was documented in 5% (243/4944) of newborns, two of whom had kernicterus; however, all were treated as cases of pSBI. Of the 2026 pSBI cases with known age on admission, nearly half (984, 49%) were early-onset infections (defined as infections within the first 72 hours of life).

Outcome

Overall, more than one-third (35%, 1734/4944) of neonates died during admission. There was evidence of a trend of increasing case-fatality from 33% in 2010 to 39% in 2013 (*P*=0.03). Outcome could not be ascertained for 17 neonates, and 38 neonates were taken home against medical advice. These newborns represented 2% of the dataset and did not differ significantly from newborns alive and well at discharge with regard to the variables of interest, and were excluded from analysis of risk factors for mortality. The main causes of death in the unit were complications of pre-term birth, serious infections and intra-partum related events (Fig. 5.3A).

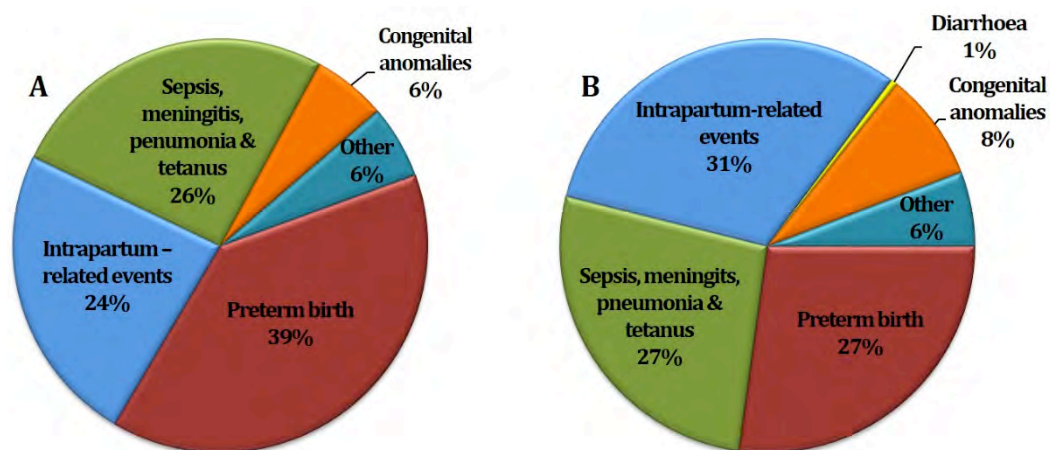


Figure 5.3 Distribution of causes of neonatal death in The Gambia

A) Data from Edward Francis Small Teaching Hospital, 2010–2013.

B) WHO/CHERG estimates for causes of neonatal death distribution in The Gambia, 2010–2013

Newborns admitted at weekends were more likely to die than those admitted during the remainder of the week (38% *vs* 35%, *P*=0.03); similarly, the risk of death was

greater for those admitted during on-call duty than for those admitted during regular working hours (38% *vs* 33%, $P=0.004$).

More than half of all deaths (57%, 989/1734) occurred during the first 48 hours of admission. Fifty-four per cent (482/897) of preterm neonates died compared with 38% (186/494) of term and 41% (7/17) of post-term neonates ($P<0.001$). Case fatality was associated with lower weight on admission; 58% (539/932) of neonates weighing <1500g died compared with 34% (469/1399) of those weighing 1500–2499g, 29% (674/2320) of those weighing 2500–3999 g, and 15% (22/144) of those weighing ≥4000 g (test for trend $P<0.001$). Of the 1673 deaths with known age at death, 1267 (76%) were early neonatal deaths (defined as deaths occurring during the first 7 days of life), 67% (853/1267) of which occurred during the first 48 hours of life. Risk factors for death (excluding newborns who absconded or were taken home against medical advice) are presented below in Table 5.4.

In the multivariable analysis, independent factors for neonatal death were weight <1500g on admission (OR 1.61, 95% CI 1.15–2.26), delivery outside EFSTH [at home or by a traditional birth attendant (OR 2.17, 95% CI 1.40–3.38), delivery at another health facility (OR 1.79, 95% CI 1.34–2.39)], lack of antenatal care (OR 1.68, 95% CI 1.17–2.41), hypothermia (OR 2.48, 95% CI 1.76–3.49), hypoglycaemia (OR 1.60, 95% CI 1.19–2.15) and hyperglycaemia (OR 1.62, 95% CI 1.19–2.19).

Table 5.4 Risk of death among neonatal inpatients 2010 – 2013

Categories	Dead, *n*	Discharged, *n*	Unadjusted OR	*P*	Adjusted OR [a]	*P*
Sex						
Male	695	1304	1.04 (0.92–1.17)	0.57	1.06 (0.85–1.34)	0.60
Female	978	1772	1		1	
Age on admission (days)						
Day of birth	996	1225	2.87 (2.40–3.43)	<0.001	1.51 (0.93–2.45)	0.09
2–7	480	1052	1.61 (1.33–1.95)	<0.001	1.27 (0.81–2.00)	0.30
≥8	198	699	1		1	
Admission weight (grams)						
<1500	539	393	3.35 (2.86–3.92)	<0.001	1.61 (1.15–2.26)	0.01
1500–2499	469	930	1.23 (1.07–1.42)	0.004	0.86 (0.64–1.14)	0.30
2500–3999	674	1646	1		1	
≥4000	22	122	0.41 (0.37–0.48)	0.001	0.75 (0.38–1.47)	0.40
Place of birth						
Inborn (EFSTH)	529	1046	1		1	
Other health facility	640	899	1.41 (1.22–1.63)	<0.001	1.79 (1.34–2.39)	<0.001
Home/TBA	180	254	1.40 (1.13–1.74)	0.002	2.17 (1.40–3.38)	0.001
Mode of birth						
Vaginal	1325	2081	1		1	
Caesarean	196	525	0.60 (0.49–0.70)	<0.001	0.66 (0.48–0.91)	0.01
Resuscitation at birth						
Resuscitated	535	717	1.52 (1.33–1.73)	<0.001	1.33 (1.00–1.76)	0.05
Not resuscitated	1199	2438	1		1	
Maternal age (years)						
<18	99	119	1.45 (1.10–1.91)	0.01	1.35 (0.85–2.15)	0.21
18–35	1059	1840	1		1	
>35	110	212	0.90 (0.70–1.15)	0.40	1.08 (0.73–1.58)	0.71
Maternal ANC						
None	535	978	1.30 (1.07–1.56)	0.01	1.68 (1.17–2.41)	0.01
1–4 visits	691	1084	1.51 (1.26–1.81)	<0.001	1.15 (0.87–1.51)	0.34
>4	228	540	1		1	
Temperature (°C)						
<36.5	966	1178	2.99 (2.50–3.56)	<0.001	2.48 (1.76–3.49)	<0.001
36.5–37.4	203	739	1		1	
≥37.5	341	942	1.32 (1.08–1.61)	0.01	1.17 (0.79–1.73)	0.42
Blood glucose (mmol/L)						
<2.6	325	336	2.14 (1.79–2.56)	<0.001	1.60 (1.19–2.15)	0.002
2.6–6.9	673	1490	1		1	
>6.9	281	320	1.94 (1.62–2.34)	<0.001	1.62 (1.19–2.19)	0.002
Time of the day						
Regular work hours	508	1013	1		1	
On-call hours	1095	1805	1.21 (1.06–1.38)	0.004	0.97 (0.76–1.25)	0.84

[a] adjusted for all variables in the univariate analysis

Investigations

A full blood count result was documented in 841 (17%) of the case notes, and haemoglobin concentration was documented in 1020 (21%). Serum bilirubin had been estimated in 36 (15%) of the 243 neonates with documented jaundice. Only 5% (104/2166) of pSBI cases had had at least one microbiological or radiological

investigation: 41 had a chest radiograph, 26 had a blood culture and 43 had a lumbar puncture. Two blood cultures were positive for *S. aureus*, one for *E. coli* and three for coagulase-negative staphylococci. All neonates with positive blood cultures recovered and were discharged. Only one infant, a 2-day-old home/TBA-born male neonate, had a positive cerebrospinal fluid (CSF) culture (*Klebsiella* species); he received antibiotics for 25 days but finally died.

Prescription of oxygen, intravenous (IV) fluids, and antibiotics

There were 2559 neonates who, according to their history and examination findings (respiratory distress, cyanosis), met the indications for oxygen therapy; however, only 77% (1965/2559) of those in who needed oxygen were documented to have received it. Seventy per cent (3455/4944) of neonates had documentation of receiving IV fluids. Antibiotics were the most frequently administered medication, and 94% (4635/4944) of neonates received parenteral antibiotics during admission. The majority (65%, 2995/4635) of these neonates received two antibiotics during admission, 27% (1235/4635) received three drugs, 5% (161/4635) received just one, and <1% (14/4635) received five or more. Antibiotics are usually provided free of charge by the hospital; however, when they are out of stock, parents have to provide them. Ampicillin and gentamicin were the most frequently used medications. Among neonates who received a single antibiotic, gentamicin was the most commonly administered (122/161), followed by ceftriaxone (28/161). Fifty-five per cent (2533/4635) of neonates who received antibiotics, lacked clinical evidence of pSBI and did not have any diagnostic work-up. The median duration of treatment was 4 days (IQR 2–7, range 0–30 days) for both pSBI and non-pSBI neonates. Twenty-eight newborns received antibiotics for more than 21 days, of whom 50% (14/28) had a diagnosis of pSBI, and 25% (7/28) had congenital malformations. Ten (36%) of these newborns were also

preterm and of low birthweight. Owing to a high case load and limited cot availability, most neonates were discharged once clinical improvement was obvious with oral antibiotics and advice on danger signs. I did not assess whether choice of fluid, fluid volumes, antibiotics, and antibiotic dosage complied with WHO guidelines.

5.3 Discussion

This study provides the first comprehensive description of morbidity and mortality and quality of care of sick newborns in a tertiary centre in The Gambia. As far as we know, this is the largest neonatal inpatient audit published from West Africa, and it provides a baseline from which to improve to clinical care and data collection.

Case fatality in the neonatal unit is higher than reported from similar tertiary referral hospitals in West Africa: 7% in Senegal,[289] 13–20% in Nigeria[290-293] and 13–15% in Burkina Faso.[294, 295] The three most common causes of neonatal deaths in the unit are preventable, and are similar to the global and national WHO/Child Health Epidemiology Reference Group (CHERG) estimates for The Gambia for the same period[296] (Figure 3.3B) but rank differently. Whereas complications of preterm birth were the major cause of death in our unit, the WHO/CHERG estimates show intrapartum-related events to be the major cause of death in the country. This difference might be explained by the fact that most of the neonates admitted were facility-born and therefore more likely to reflect a selected population with obstetric complications, especially preterm birth. The risk of death in the unit was greatest on the day of birth and among very small infants. Small size at birth – owing to preterm birth or small-for-gestational-age (SGA), or both – is the greatest risk factor for most neonatal deaths.[287] Globally, antenatal corticosteroids have been of great benefit in preventing complications of preterm birth;[297] however, their use in preterm newborns admitted in the unit was not assessed. The majority of

deaths occurred within 48 hours of admission. The inability of health-care facilities to provide timely, good-quality care on arrival at hospital is reported to be a leading[298] or secondary[299] cause of in-hospital newborn deaths. Although an increased odd of deaths of newborns admitted at the weekend (as well as among those admitted during on-call hours, data not shown) was noted, there was a lack of a weekend effect on death in the adjusted analysis. Consequently, improved survival of newborns requires fast interventions at any time of day or night as the time to death can range from a few minutes for the inadequately resuscitated neonate who does not breathe at birth, to an hour for the infant suffering a severe hypoxic event, and a few hours for severe early-onset sepsis.[287] All facilities which provide maternity services should at least have a functioning mechanical suctioning device and a self-inflating bag and mask. Helping Babies Breathe (HBB) is an evidence-based educational programme to teach neonatal resuscitation techniques in resource-limited areas, with the goal of having at least one person who is skilled in neonatal resuscitation at every birth. Pre-service and in-service training curricula should be revised to support implementation of the HBB curriculum. As a tertiary referral hospital, EFSTH should in addition ensure that the appropriate equipment and expertise for endotracheal intubation are available for severely depressed infants.

The seasonal variation in admissions in our unit mirrors seasonal variations in births in EFSTH and nationwide (data not shown). Although seasonal variation is a cardinal feature of paediatric diseases in West Africa,[300] and accentuates the vulnerability of children in poor families, the reasons for it are not known. No seasonal variation was observed when stratified by admission weight; however, divergent patterns of seasonality have previously been reported in The Gambia for preterm birth and SGA.[301]

In this study, the peaks in prematurity closely paralleled increases in agricultural labour (July) and malaria infections (October). The incidence of SGA was highest at the end of the annual hungry season, from August to December (peaking in November), and has been attributed to seasonal deterioration of nutritional status owing to food shortages, and to an increase in agricultural labour which often coincides with seasonal epidemics of infectious and parasitic diseases.[302]

Almost half of the neonates were admitted with clinical signs compatible with pSBI, and half of these presented as "early-onset sepsis" (within the first 3 days of birth). Early-onset sepsis is usually associated with vertically acquired infection from the birth canal, while late-onset sepsis is associated with acquisition from the home or hospital environment, particularly through the umbilical cord.[82] In developing countries, unclean delivery practices and initial care of the infant in hospital contribute to very early-onset infections.[21] The very high cot occupancy in the unit makes it difficult to isolate infected patients. Being the only neonatal referral centre, there is a strict policy not to turn patients away, leading to sharing of cots and incubators during peak admission periods. Consequently, infection control practices would have been sub-optimal, making it difficult to ascribe 'maternally acquired' or 'hospital-acquired' status to those presenting with early-onset neonatal infections.

There was a striking mismatch of high antibiotic usage and low laboratory investigations. Sick neonates do not routinely undergo microbiological investigations, mainly owing to an absence of ward protocols, unreliable diagnostic laboratory facilities with delayed results, and the general expectation of a 'negative' result by attending clinicians. The majority of newborns received only 'first-line' empirical treatment with the WHO-recommended ampicillin and gentamicin; however, many received additional

antibiotics or other regimens including third-generation cephalosporins, and some were on treatment for as long as 30 days. In the absence of clinical guidelines, one major reason for the widespread use of ampicillin and gentamicin is the fact that the pharmacy does not dispense cephalosporins if a consultant has not signed the prescription. Unlike culture-proven sepsis, which is treated with a full course of antibiotics on the basis of antimicrobial sensitivity, the appropriate duration of treatment for suspected neonatal sepsis when cultures are negative or not available is a challenge.[303] Prolonged initial empirical antibiotic treatment (defined as ≥5 days of initial empirical antibiotic with sterile culture results) is associated with an increased risk of an adverse outcome, including invasive candidiasis and death, particularly in premature and extremely small newborns.[304, 305] Moreover, antibiotic costs are not cheap and represent a significant proportion of the hospital drug budget.

Point-of-admission hypothermia was present in 48% of neonates. The prevalence of hypothermia on admission among newborns in sSA ranges from 22% to 85%.[306-308] Newborns must be kept warm at birth (at home or in hospital) and, especially, if ill or being transferred from home or between hospitals or within a hospital to a neonatal care unit. Preterm and small newborns are especially vulnerable, and can rapidly become hypothermic, increasing the risk of respiratory distress, hypoglycaemia, infections and death. About one-third of the newborns with hypothermia in the unit weighed <1500 g, and most were intra-hospital transfers from the maternity unit. Despite taking place within the hospital environment, over relatively short distances with low apparent risk of complications, intra-hospital transfer can constitute an additional risk, especially to pre-term infants.[309] Even in high-income settings, hospitals without a neonatal transport team and incubator may have significantly more deaths or

adverse events in low-birthweight infants with respiratory disease than do comparable hospitals with neonatal transport facilities.[310] Multi-disciplinary transport teams are, however, a luxury in low-income settings. Prevention of hypothermia should commence before delivery by ensuring that the place of delivery is warm, and by maintaining a 'warm chain' of procedures at birth and during the hours and days that follow.[311] These simple, cost-effective procedures include skin-to-skin contact with the mother (KMC) during transfer, wearing of hats, and the use of plastic bags/wraps particularly for very small and low-birthweight (LBW) newborns, and should be encouraged. KMC is particularly suitable when there is a lack of sophisticated equipment such as radiant heaters and incubators, and has been shown to substantially reduce mortality among clinically stable preterm/LBW infants in hospital.[312] It has also been shown to reduce the workload on the ward, thereby allowing nursing staff to focus attention on more unstable infants.[313] Minimal instruction is required and it can also be used in the community or at home. The current Gambian health policy framework does not include implementation of KMC;[273] there is therefore an urgent need to address barriers to scaling-up facility-based initiation of KMC in line with the post-2015 newborn health research priorities.[314]

Hypoglycaemia is an important contributor to hypothermia, particularly among those at risk (pre-term or term small-for-gestational-age (SGA) or infant of a diabetic)[315] and vice-versa: it maintains a vicious circle, which leads to weak sucking, weight loss and finally increased mortality. Nearly one-fifth of newborns in the unit were hypoglycaemic on admission, compared with one-third in a similar facility in Nigeria.[316] Breastfeeding prevents hypothermia by warming through the mother, especially by skin-to-skin contact, and also by replenishing a newborn's glucose levels. The Gambian national

health policy subscribes to the principles of the Baby-friendly Hospital Initiative to implement practices which protect, promote and support breastfeeding.[317] Initiation of early breast-feeding is, however, difficult in newborns requiring admission, particularly if a mother is recovering from anaesthesia following caesarean delivery or is too ill with other post-partum complications. Delays in referral and transfer to the neonatal unit and establishing intravenous access further contribute to hypoglycaemia in those at risk.

There are several possible reasons for the sub-optimal neonatal care in The Gambia. One is the lack of appropriate equipment and trained staff. The level of laboratory support in the unit falls short of essential newborn care standards for a district hospital, let alone a referral-level hospital.[318] Although this deficiency highlights health system challenges in the provision of appropriate equipment (e.g. lack of blood culture bottles, laboratory reagents and equipment) and appropriately trained personnel (e.g. microbiologists and laboratory technicians), it also draws attention to the capabilities of the clinicians managing sick newborns as investigations were not requested for most of the neonates in whom there was clinical suspicion of infection.

Poor knowledge and training of neonatal care-providers and a shortage of qualified staff are associated with sub-optimal care in developing countries.[298, 299] As is the case in most other countries with very high maternal and neonatal mortality, The Gambia lacks the minimum requirement of 23 doctors, midwives and nurses per 10,000 population to provide a basic package of care.[319] In 2015 there were 157 Gambian and 479 non-Gambian medical doctors registered with the Gambian Medical and Dental Council. There are also non-registered Cuban, Egyptian and Syrian doctors providing medical care as part of bilateral agreements with their respective governments. From inception

in 1999 until 2015, 111 indigenous doctors have graduated from the University of The Gambia. Upon completion of a 2-year internship programme, the medical officers are usually retained at the EFSTH; since January 2014 however, the Ministry of Health has been posting medical officers to district hospitals and health centres to serve communities. The goal is for 80% of all hospitals and major health facilities to be managed by indigenous doctors by 2016. Unfortunately, postgraduate medical training is not available in The Gambia and so locally trained doctors lack affordable avenues to acquire specialized skills and competencies for neonatal care; the provision of specialist neonatal care depends solely on expatriate doctors who also work in EFSTH. Nurses are the most accessible health-care providers in The Gambia with more nurses than doctors per 1000 people. Basic training for nurses follows either a 2-year track to the level of state-enrolled nurse (SEN) or a 3-year track to the level of state-registered nurse (SRN). SENs wishing to become SRNs have to undergo an additional 2 years of training. Most nurses undergo additional training in midwifery but there is no formal training in paediatric or neonatal nursing, and competence is acquired through experience. The mix of nursing staff is not regulated, and, in health centres, care-givers might not have the necessary competence to provide neonatal care, and may not even be nurses but attendants with no clinical training at all. Uncompetitive salaries and benefits have resulted in high attrition rates, staff shortages and low motivation which have further contributed to the poor quality of care. Urgent systematic attention is required, including non-rotation of nurses with skills in neonatal care, and where appropriate, the development of a neonatal nurse cadre, as well as rewarding those who work against the odds in hard-to-serve areas.[320]

In developed countries, detailed quality-of-care protocols and 'core nursing skills sets' for almost every aspect of newborn care have improved quality and given more responsibility for care to skilled neonatal nurses, particularly with regard to infection prevention, feeding support and use of intravenous fluids.[321] Hospitals providing neonatal care have service standards that take account of the nurse-to-patient ratio (maximum number of patients who may be assigned to a nurse during one shift) as well as nursing skills, training and development depending on the category of newborn care.[322, 323] The British Association of Perinatal Medicine's (BAPM) recommendations for neonate-to-nurse ratios for intensive care (IC), high dependency care (HDC) and special care (SC) in England are currently 1:1, 2:1 and 4:1, respectively.[322] Similar recommendations and service standards for essential neonatal care are lacking in The Gambia and West Africa, despite studies in Ghana and Nigeria, which have shown that levels of nursing staff in a neonatal care unit affect patient outcomes (mortality and adverse events), patient experience, and quality as well as efficiency of care delivery.[324, 325] Appropriate nurse-to-patient ratios cannot be generalized because of factors such as patient load and characteristics, availability of nurses and work environment; the ratio that is sufficient for one unit might be insufficient for another. A nurse-to-patient ratio of 1:12.5–1:25 has been reported in the neonatal intensive care unit of a Ghanaian teaching hospital.[324] WHO has developed a tool for calculating optimal health worker levels, known as the Workload Indicators of Staffing Need (WISN). The software calculates the number of health workers per cadre, based on health facility workload, and provides two indicators to assess staffing: the gap/excess between the current and required number of staff, and the WISN ratio, a measure of workload pressure. WISN has been used extensively in East and Southern Africa but there are no case studies of

its use in West Africa.[315-317, 326] Owing to a lack of essential information, the staffing requirements for the EFSTH neonatal unit could not be calculated.

Given the strong emphasis on improving referral pathways and promoting institutional delivery for high-risk births as a means of improving neonatal survival, more needs to be done to ensure that the EFSTH neonatal unit is suitably equipped and staffed. With just over 70,000 births annually, nearly 60% of which take place in health facilities, the 30-cot EFSTH neonatal unit is grossly inadequate to meet the national need for referral-level hospital care of sick newborns. As the proportion of neonates delivered in health facilities increases, it is necessary to accelerate coverage of essential newborn care. Urgent attention needs to be focused on scaling up the provision of appropriate packages of newborn care at district hospitals, and possibly major health centres, as recommended by the WHO.[318] The packages of care with the greatest impact on preventing neonatal deaths and stillbirths include care during labour, childbirth and the first week of life, and care for the small and sick newborn.[327] These packages, the focus of the WHO's Every Newborn Action Plan to end preventable deaths, include management of preterm births (including the use of antenatal corticosteroids), essential newborn care (hygienic care, thermal control, support for breastfeeding and newborn resuscitation), interventions to deal with complications arising from preterm birth and/or SGA, and neonatal infections (sepsis, meningitis, pneumonia and diarrhoea).[327] Appropriate management of small sick newborns includes extra thermal care and support for feeding small or pre-term newborns, including KMC, antibiotic treatment for infections and full supportive facility care. Since endorsement of the newborn health action plan in 2014, only two West African countries, Ghana and Nigeria, have hosted

national newborn events and are in the process of developing their own national newborn action plans.[328]

Insufficient government funding of the health service, lack of an integrated maternal and newborn health policy, and greater investment on addressing maternal rather than fetal and newborn outcome are further reasons for sub-optimal neonatal care in The Gambia. In 2001, The Gambia and other African Union governments pledged to commit at least 15% of their annual budgets to improve the health sector.[329] At the end of 2013, only two West African countries, Togo and Liberia, had achieved this target.[330] In The Gambia, priority areas for child health have been scaling-up immunization coverage and prevention of deaths from malaria, pneumonia and diarrhoea. It is only in the last decade that attention has turned to the contribution of neonatal deaths to child mortality.[331, 332] The Gambian national health policy for 2012–2020 is focused on 'acceleration of quality health services and universal coverage';[273] however, newborn survival and health are not specifically addressed. There is therefore an urgent need to strengthen newborn health components in existing health sector plans and strategies, especially those which relate to reproductive, maternal and child health, as outlined in the Every Newborn action plan.

The study has some limitations. Although data presented are from the main national tertiary referral hospital, it cannot be assumed that the findings represent neonatal morbidity and mortality in The Gambia as a whole. Furthermore, the results should be interpreted in the light of the following. Firstly, this is a retrospective study with data abstracted from routine medical records. About 26% of records for the period under review could not be located and data were missing for nearly all variables. Secondly, poor documentation of medical histories and examination findings, lack of systematic

assessment of gestation and possible ascertainment bias at the point of data abstraction from written records might have led to misclassification of diagnoses. Furthermore, diagnoses were based almost entirely on clinical assessment without laboratory support. A report on microbiological aetiology could not be presented as only six newborns had positive blood cultures and only one had a positive CSF culture. Lastly, poor documentation of care and the implicit assumption that written records reflect actual practice might have resulted in bias regarding quality of care.

This study has provided a comprehensive overview of inpatient care for newborns in The Gambia, showing that over one-third of them die, even in the country's teaching hospital. Further operational and research data are required on infections, notably regarding aetiologies and antimicrobial resistance. Education and training of health-care workers, development of guidelines and standards of care, and regular audit are necessary for the provision of high-quality neonatal services. Creation of national and regional perinatal/neonatal databases for stillbirths, pre-term and very low-birthweight infants, as well as those meeting other eligibility requirements, will provide data for improving outcomes and increasing the quality, safety and value of newborn care through quality improvement collaborations with other countries in the sub-region.

5.4 Changes to the neonatal ward after the audit and during the period of the PhD (2014 – 2017)

The number of incubators increased from 4 to 11, with the addition of 3 radiant warmers. The unit also acquired a Bubble-CPAP machine that is, however, used sporadically. A KMC unit was opened in July 2017, and several staff from the ward underwent refresher training on KMC in preparation for a randomised controlled trial of early KMC for mild-to-moderately unstable hospitalised newborns with birth-weight

<2000g which commenced in August 2017 (personal communication with the Principal Investigator, Dr Helen Brotherton).

CHAPTER 6. CASE CONTROL STUDY OF NEONATAL INFECTION AETIOLOGY

Study nurse preparing a Case for phlebotomy

Study nurses reviewing participants’ clinical record forms

Bactec 9050 Instrument containing innoculated blood culture bottles

QuantStudio™ & Flex Real-time PCR Instrument for running TaqMan Array Cards

Overview

The case-control study was carried out in two phases: a pilot case-control study involving 100 newborn-mother pairs, and a larger 'main' case-control study involving 306 newborn-mother pairs. The aim of the pilot study was to test the feasibility and practicality of participant recruitment and other study-specific procedures in order to inform an appropriate design and sample size estimation for the main study. Other than slight differences in the inclusion criteria, participating health facilities, and samples collected (Table 6.1), the same methods were used for both pilot and main study. This chapter integrates the description of the methods employed in both studies. The study design and timelines are summarised in Figures 6.1 and 6.2.

Table 6.1 Differences between the Pilot and Main Studies

	Pilot Study	Reason for choice	Main Study	Reasons for modification
Inclusion criteria (Cases): admission weight	≥1500g	-Concerns regarding minimum blood sampling volume in smaller babies	≥1000g	-Many potentially eligible neonates were being excluded
Site of Case recruitment	EFSTH Neonatal Ward	-The only neonatal unit in The Gambia	-EFSTH Neonatal Ward -Kanifing General Hospital -Brikama Health Centre	- To meet recruitment targets. - Referrals to EFSTH began to drop when these two facilities increased their capacity for neonatal inpatient care.
Site of Control recruitment	Polyclinic, Banjul	-Nearest EPI Clinic to the EFSTH and therefore source of healthy controls	Postnatal wards and/EPI clinics of the following facilities: -EFSTH -Polyclinic, Banjul -Brikama Health Centre -Kanifing General Hospital -Serekunda Health Centre -Fajikunda Health Centre -Bundung Maternal and Child Health Hospital	-To make controls more comparable with cases in respect to age (In the Pilot study, controls were older (median 14 days IQR: 9 – 17 days) than the cases (median 3 days IQR: 1 – 10 days). -These additional facilities are the main ones serving the study area
Samples: Nasopharyngeal swabs (NPS)	NPS collected from neonates only	To assess correlation between newborn nasal and maternal genital colonisation	NPS collected from neonates and their mothers	Maternal NPS included in order to assess correlation between newborn and maternal nasal colonisation
Samples: Newborn rectal swabs	Not collected		Collected	To assess correlation between gut colonisation and invasive isolates

EPI = Expanded Programme on Immunisation; EFSTH = Edward Francis Small Teaching Hospital

Case pair
(Sick newborns 0 – 27 days of age admitted with possible serious bacterial infection at any participating government health facility)

Infant-Mother Control pair
(Healthy newborn 0 – 27 days of age recruited from one of 6 participating health facilities matched to a case)

Newborn
1. Physical examination
2. Infection work-up:
-Culture (Blood, CFS, NPS, RS
-Bacterial/Viral PCR (Blood, NPS)
-CXR (where clinically indicated)

Mother
1. Culture (RVS & NPS)
2. Risk factor interview

Mother
1. Culture (RVS & NPS)
2. Risk factor interview

Newborn
1. Culture (NPS & RS)
2. Blood (PCR)

Compare proportion with and without rectovaginal colonisation

No organism detected
Culture neg/ PCR neg

Organism detected
Culture pos/PCR pos
Culture pos/PCR neg
Culture neg/PCR pos

Colonised with potential pathogen

No pathogen

Clinically non-significant pathogen

Clinically significant pathogen

Bacterial isolates

1. Blood culture vs Mother RVS
2. Baby NPS vs Mother RVS
3. Baby NPS vs Mother NPS
4. Blood culture vs Baby RS
[Strain confirmation, Serotyping, ± WGS]

Figure 6.1 Study design for Pilot and Main Studies

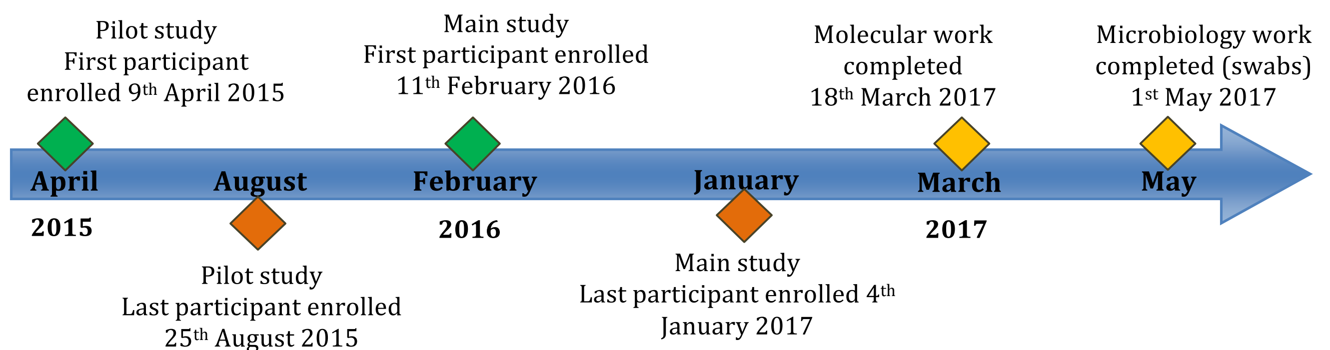


Figure 6.2 Timelines for Pilot and Main studies

6.1 Epidemiological methods

6.1.1 Choice of study design

The ideal study of neonatal infection aetiology would capture all cases of neonatal infections in a pre-defined study base (the reference population). The incidence of neonatal infection is quite low, even in high burden sub-Saharan African countries where it is reported to range from 5.46 – 21 cases per 1000 live births.[112] A cohort study design therefore would be long and expensive to carry out. The case-control design is particularly efficient for outcomes that occur in a small fraction of exposed and unexposed individuals because participants are selected on the basis of their disease status, thereby permitting identification of adequate numbers of 'diseased' individuals, as well as not having to recruit/collect data on everyone without the outcome of interest.[333] The role of controls is to provide valid information on the distribution of exposures within the population at risk of becoming a case.[334] Identification of cases may occur in the community or the hospital; however, the site of identification and diagnostic challenges contribute to the likelihood of selection bias. Selection bias for cases is present whenever the cases identified are not representative of cases occurring in the reference population. Correct control selection is crucial to the internal validity of case-control studies,[334-337] and case-control comparisons are likely biased when controls are selected from an ill-defined study base and consequently do not represent the exposure experience of the true source population. For validity to be high, cases and controls must be recruited from the same source populations. This may, but does not have to, be the whole population and restrictions can be applied to case recruitment to ensure that they come from the same population as the controls, and vice versa.

For a community- or population-based study of neonatal infection aetiology, the primary study base from which cases and controls should be drawn would be all babies

aged 0 – 27 days in a defined area. Ideally, the "cases" in the population would be all newborns within the base who develop symptoms and signs suggestive of pSBI, and "controls" would be all healthy newborns in the same primary study base. Defining the population and the complete ascertainment of all cases who meet the eligibility criteria arising from the study base would be challenging in the absence of a demographic health surveillance system or registry of all births in the area.[338] Population-based identification of acute conditions such as neonatal infections can only be achieved with intense community surveillance, and most population-based case-control studies will not recruit all cases in the population, even if they try to. A second challenge would be the set-up of an appropriate strategy for sampling and evaluation of controls from the study base. Usually for studies of this kind, a large proportion of cases and a small proportion of available controls are evaluated. Selection bias is avoided when equal sampling fractions are achieved for exposed and non-exposed cases as well as for exposed and non-exposed controls, so that both cases and controls represent the exposure experience of their source populations, within strata that will be used for stratification in the analysis.[335] Therefore, while the population-based approach is preferred because it is less prone to bias from factors that may have influenced the access to care such as the choice of a particular health facility or physician, the logistical and cost considerations make it impractical in most instances.[333]

Hospital-based studies differ from population-based studies in that the study base is defined secondarily to the identification of cases. Cases are usually selected regardless of the population from which they arise (e.g. all cases from a given hospital that receive patients from different settings), although it is possible to place restrictions on who is recruited for example restricting recruitment to patients who reside in a defined

geographical area. An effort is then made to identify the study base corresponding to the selected cases in order to define the population from which the controls are to be selected.[338] Several sources of bias may be present with hospital-based studies. Presentation at a health facility is influenced by health-seeking behaviour, access to care and resources with the potential for under-representation of cases that are distant from the hospital and over-representation of those with the resources to get there. Access to hospital care is also influenced by survival. Bias towards selection of survivors is inherent in hospital-based neonatal infection aetiology studies in developing countries where most neonatal births and deaths occur at home.[331] However, compared with population-based studies, hospital-based studies are more practical and relatively inexpensive as procedures for both case and control selection and evaluation tend to be logistically less demanding.[333]

With the exception of MRC-led malaria and vaccine surveillance (*Haemophilus influenzae* type b[339] and *Streptococcus pneumoniae*[340] vaccines) no intensive community-level disease surveillance exists in The Gambia. Consequently, health facilities are the most appropriate places to identify cases of severe paediatric illness. In The Gambia, 63% of births take place in a health facility and 37% occur at home.[279] In addition, births in urban areas are substantially more likely to be delivered at a health facility than those in rural areas (83% versus 44%). Given these considerations a hospital-based case-control study design with measures to minimise biases, was judged most appropriate to answer the questions posed within the bounds of the time and resources available in the context of this doctoral work. While such a study cannot definitively establish all pathogens associated with neonatal infection in the study area,

it can potentially generate the much-needed evidence required to justify undertaking intervention trials including maternal vaccination.

6.1.2 Study setting and choice of facilities

The study was conducted in the Western Health Region of The Gambia where 58% of the population reside[279] (Figure 6.3).

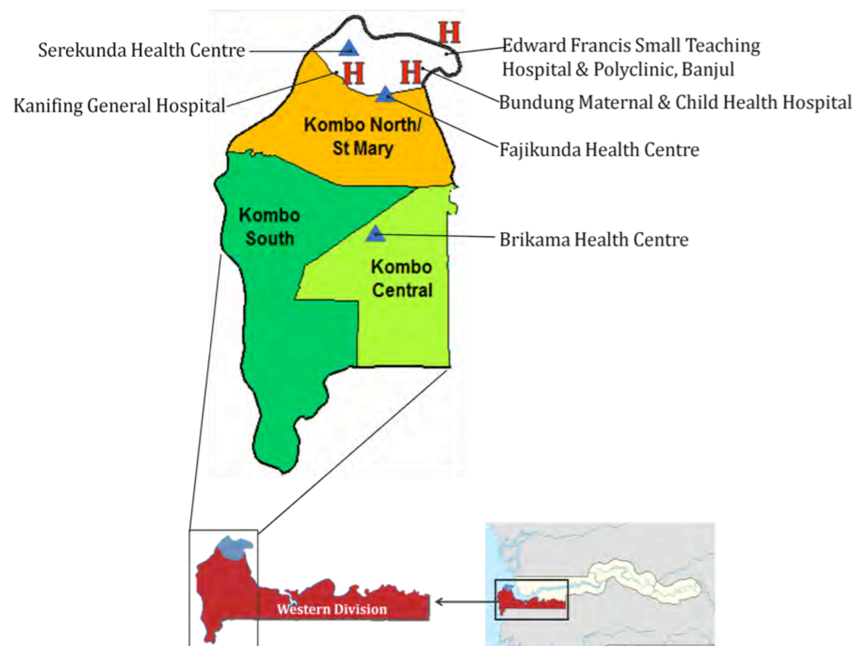


Figure 6.3 Map of study area showing study sites (Hospitals and Health Centres)

Image sources : Public Domain, https://commons.wikimedia.org/w/index.php?curid=15379602

The region comprises three local government areas served by 13 government health facilities. An estimated 75% - 93% of women in the region deliver in a health facility, and the regional neonatal mortality rate is estimated at 24 – 27 deaths per 1000 live births.[279] The EFSTH is the main referral hospital for the region and close to 70% of outborn neonates referred to the neonatal unit were delivered at the following five government health facilities: Kanifing General Hospital, and Bundung Maternal and Child Health Hospital, Brikama, Fajikunda, and Serekunda Health Centres.[99] With the

exception of Kanifing General Hospital and the EFSTH, these other facilities also run Expanded Programme on Immunization (EPI) clinics (the EFSTH provides EPI services through its Polyclinic, while Kanifing General Hospital provides EPI services through Serekunda Health Centre). These were the considerations for selection of these facilities as study sites

6.1.3 Entry criteria and definitions

Selection and enrolment of cases

Cases were recruited from EFSTH, Kanifing General Hospital and Brikama Major Health Centre. Up until 2015 when the Pilot study was conducted, the EFSTH was the only facility providing neonatal inpatient care. By the end of that year Kanifing General Hospital and Brikama Health Centre had increased their capacity for limited inpatient care of neonates with suspected infection. This resulted in reduced neonatal sepsis referrals to the EFSTH and necessitated the inclusion of these additional facilities as sites for the recruitment of cases during the main study. Potentially eligible newborns and their caregivers were screened at the point of admission by a study nurse. A newborn was enrolled as a case of suspected infection if all of the eligibility criteria and none of the exclusion criteria in Table 6.2 were met.

Table 6.2 Case enrolment criteria

Eligibility criteria	Exclusion criteria
1. Sick neonate presenting for admission with one or more clinical signs of possible serious bacterial infection (pSBI) 2. Postnatal age 0 - 27 days 3. Admission weight ≥1000g (≥1500g in pilot study) 4. Mother is normally resident in the Western Health Region 5. Mother attended antenatal care at any health facility (irrespective of location) 6. Parental/Caregiver written informed consent given	1. Already enrolled in another research study

The criteria for definition of pSBI used in this study (Table 6.3) were modified from the YICSSG[8] and in keeping with definitions used in other neonatal infection studies: clinical trials of home-based simplified antibiotic regimens (African Neonatal Sepsis Trial (AFRINEST)[341] and The Simplified Antibiotic Therapy Trials (SATT) in Bangladesh[342] and Pakistan[343]), and the Aetiology of Neonatal Infections in South Asia (ANISA) study.[344] Definitions of clinical signs are provided in Appendix 4

Table 6.3 Comparison of definitions of possible serious bacterial infection used in this PhD study and other studies of neonatal infection treatment and aetiology

WHO YICSSG Study	My PhD Study [a]	AFRINEST/SATT Clinical Trials [a]	ANISA Study [a]
History of convulsion	History of **or observed convulsion**	Excluded because home-based treatment is felt to be potentially unsafe for this group	History of **or observed convulsion**
Fast breathing (respiratory rate ≥60/min)	Fast breathing (respiratory rate ≥60/min **on repeat count**) **only if > 3 days postnatal age**	Excluded to increase specificity of diagnosis (data from previous studies in Bangladesh suggests that this sign alone is not predictive of severe illness)	Fast breathing (respiratory rate ≥60/min **on repeat count**)
Severe chest in-drawing	Severe chest in-drawing	Severe chest in-drawing	Severe chest in-drawing
Axillary temperature >37.5°C	Axillary temperature **≥38°C** (**confirmed by a second reading**)	Axillary temperature **≥38°C; confirmed by a second reading** (Cut-off increased to increase specificity of diagnosis)	Axillary temperature **≥38°C** (**confirmed by a second reading**)
Axillary temperature <35.5°C	Axillary temperature **≤35.5°C** (**confirmed by a second reading**)	Axillary temperature **≤35.5°C** (**confirmed by a second reading**)	Axillary temperature **≤35.5°C** (**confirmed by a second reading**)
Lethargic or less than normal movement	Movement only when stimulated (lethargy) or no movement at all (unconscious)	Lethargic (movement only with stimulation)	Movement only when stimulated or no movement at all
History of feeding problems	History of not feeding well, **confirmed by feeding assessment**	**Not feeding well on observation (confirmed by poor suck on examination in SATT Bangladesh)**	History of not feeding well, **confirmed by feeding assessment**

[a] Text in bold red indicate differences between individual study criteria and the original WHO criteria

Selection and enrolment of controls

When cases are ascertained from hospitals serving a defined geographic area, and where a list of the population exists, a probability sample of unaffected individuals from

the population of that geographic region can be selected as controls to enhance the likelihood that cases and controls come from the same source population. This is more challenging when such a list does not exist, and a concern with this approach is that population controls sampled from a convenient population register may not be representative of the true population at risk of being a member of a hospital case series; population controls may also lack comparability in recruitment fraction and recall of information.[334-337, 345] But there are also concerns that hospital controls, especially those with other diseases, may fail to provide an unbiased sample of the population at risk with respect to exposure status. The options for selection of a control group along with the strengths and weaknesses of each option when subjected to the classic tests, are detailed in Table 6.4

Taking into consideration the strengths, weaknesses, and practicalities of the different control groups, the decision was made to match controls to cases on neighbourhood using the EPI clinic location/coverage as a proxy for neighbourhood. For each enrolled case, the mother was asked to indicate which of the six participating EPI clinics she would normally take her baby to for their first infant vaccination. The Gambian EPI schedule requires that all newborns receive BCG (Bacille Calmette-Guerin), HepB (Hepatitis B) and OPV (oral polio) vaccines at birth or shortly thereafter.[346] Recent data shown that in the urban sections of the study area, the median age at BCG vaccination is just less than 2 weeks.[346] A control was then recruited from among newborns brought to the aforementioned EPI clinic as follows: study nurses would screen successive mothers waiting at the EPI clinic until an eligible mother-newborn pair was identified and recruited. To ensure that cases and controls were similar in age, where a case was enrolled within the first week of life, the matched control would be recruited from the

postnatal ward of the health facility where the EPI clinic is located. For this study recruiting one control for each case was operationally simpler and less expensive than recruiting multiple controls of any one type. As it was anticipated that obtaining the required number of cases to meet the sample size requirements of the study would be feasible multiple controls were not needed.[347]

Table 6.4 Options for selection of control groups for the hospital-based neonatal infection case-control study

Site & description	Control test [b]	Case test [c]	Comments
Neonatal ward: Non-infection/sepsis admissions	Yes, likely to be detected	Yes, likely to be detected	***Strengths:*** - logistically convenient ***Weaknesses:*** - The baby is by definition ill. Diseased (ill) controls may not have the same exposure distribution as the wider population of non-cases
Post-natal ward: Healthy newborns awaiting maternal discharge	Yes, likely to be detected	Yes, likely to be detected	***Strengths:*** - logistically convenient ***Weaknesses:*** - This control group is biased in favour of facility- born babies; - The median postnatal age of this control group would be 0 days (lower than that of cases presenting after the day of birth)
EPI clinics: Healthy newborns [a]	Yes, likely to be detected	Yes, likely to be detected	***Strengths*:** - Clear case versus non-case boundaries; better recall of maternal and some neonatal risk factors. ***Weaknesses:*** - The median postnatal age of this control group would be older that cases
Neighbourhood of the case: Random sample of all healthy newborns in the neighbourhood	Uncertain: Might not get to the health centre or hospital clinic and may die at home	Yes, same chance of being detected as other non-cases	***Strengths*:** - Representative of all non-sepsis neonates from the same populations as the case reference population. - potentially controls for difficult to measure confounders, e.g. environmental factors ***Weaknesses:*** - Theoretically and logistically difficult in the absence of a well-established demographic surveillance system that records all births.
Neighbourhood of the case: Healthy newborns in the neighbourhood matched to cases on age/facility of birth	Problem: Might not get to the health centre or hospital clinic and may die at home	Probably; dependent on health-seeking behaviour of mother.	***Strengths*:** - May identify more home-born babies - potentially controls for difficult to measure confounders, e.g. environmental factors ***Weaknesses:*** - Logistically inconvenient; - Will prevent examination of neighbourhood as a potential risk factor

[a] For some babies, BCG, HepB and OPV0 (meant to be taken at or shortly after birth) are taken any time after the age of 7 days (when the naming ceremony occurs)

[b] Control Test: would a control, having developed the disease under study, be identified and recruited as a case?

[c] Case Test: if someone identified in the study as a case were in fact to not have the disease under study would they have the same chance as other non-cases of being recruited as a control?

Potentially eligible newborns and their caregivers were screened at the EPI clinic (prior to vaccination) or the postnatal ward by a study nurse. A newborn was enrolled as a control if all of the eligibility criteria and none of the exclusion criteria in Table 4.5 were met.

Table 6.5 Control enrolment criteria

Eligibility criteria	Exclusion criteria
1. Healthy neonate with no clinical signs of infection on the postnatal ward OR presenting for vaccination at a participating EPI clinic 2. Postnatal age 0 - 27 days 3. Mother is normally resident in the Western Health Region 4. Mother attended antenatal care at any health facility (irrespective of location) 5. Parental/Caregiver written informed consent given	1. Already enrolled in another research study

6.1.4 Follow-up and change in status during the study

All enrolled newborns were followed up on day 27 to determine final outcome. All mothers were encouraged to report to the study nurses any concerns or clinical signs of illness in the newborn as it was anticipated that some of the controls might develop serious infection within the period of follow-up.

6.1.5 Sample size and analysis plan

The basis for the sample size calculation for this study was to detect a difference in maternal recto-vaginal colonisation with the main organisms associated with serious neonatal infections (*Klebsiella* species, *E. coli*, *S. aureus* and GBS) between cases and controls. *Klebsiella* species, and *S. aureus* were identified as causes of neonatal infection in the pilot study while *E. coli* and GBS, although not identified in the pilot, are known causes of neonatal infections in the study area.[348] Data obtained from recent studies of maternal carriage in The Gambia show that at least 20% of women in the population are colonised with either GBS[253] or *S.aureus.* (A. Roca, personal communication) A total

sample size of 300 mothers (150 mothers of sick newborns (Cases) and mothers of 150 mothers of healthy newborns (Controls) would have 80% power to detect a 30% absolute difference in maternal colonisation between the two groups at 5% significance level.

Statistical analyses were carried out using STATA version 13 (Stata Corp, Texas). Descriptive statistics reported include the prevalence and aetiology of culture-confirmed bacterial infection as well as the prevalence and aetiology of nasopharyngeal and rectal colonisation in Cases. The frequency of detection of concordant newborn blood and maternal rectovaginal isolates were calculated. In a secondary analyses, concordance rates were examined in early and late-onset infection. The prevalence of maternal rectovaginal colonisation with any of GBS, *E. coli, S. aureus* or *Klebsiella* species between matched Case-Control pairs was compared by the McNemar test for paired data, reporting odds ratios and 95% confidence intervals.

6.1.6 Study procedures

Cases

At recruitment, each Case had a clinical assessment followed by laboratory work-up for infection comprising cultures of blood, cerebrospinal fluid (CSF), nasopharyngeal and rectal swabs. C-reactive protein (CRP) is not measured by the MRCG or EFSTH laboratories and was therefore not part of the infection work-up. Newborn and maternal samples were collected following detailed study-specific standard operating procedures (Appendix 5).

- ***Blood: -*** Blood samples were collected, whenever possible, before the commencement of antibiotics and in the following sequence: Up to 3ml of venous

blood was collected and divided into 2 aliquots for culture and molecular diagnostic assay as shown in Table 6.6.

Table 6.6 Blood sample volumes

Blood volume obtained	Blood culture (BACTEC Peds Plus™/F culture bottle)	Molecular diagnostics (K_2EDTA microtainer)
0.5 ml– 1.0 ml	Full volume (venpuncture repeated if <1.0ml)	Nil (venepuncture repeated if possible)
>1.0 ml– 2.0 ml	1.0ml – 1.5 ml	0.5 ml (venepuncture repeated if sample insufficient)
>2.0 ml	>1.0ml	0.5 ml

Culture samples were collected using a strict no-touch aseptic technique as follows: once a suitable peripheral vein was chosen, the overlying skin was cleaned with 70% alcohol and allowed to dry for 30 seconds after which the same area was cleaned with 10% povidone iodine and allowed to dry for 60 seconds. Once the skin was completely dry, blood was drawn from the vein taking absolute care not to touch the cleaned site. Only one blood culture bottle was routinely drawn for each Case. Blood culture bottles were not weighed before or after addition of the blood sample. Inoculated *BACTEC Peds* Plus™/F culture vials were placed in a designated incubator in the neonatal ward set at 37°C and transported to the MRCG Clinical Microbiology Laboratory before the close of work each day (within 6 hours of collection). Incubator temperature was monitored twice daily by study nurses and culture bottles were checked for validity. Blood cultures collected after hours were also placed in the same incubator, and transported to the MRCG Clinical Microbiology Laboratory as early as possible the following morning. Blood samples for molecular testing were put in a cold box before transfer to the Biobank at the MRCG within 6 hours of collection for storage at -70°C until tested. Samples collected after hours were

stored temporarily at -20°C in a designated sample freezer in the research study office, then transported to the MRCG Biobank as early as possible the following morning for storage at -70°C until tested.

- ***Cerebrospinal fluid (CSF):*** CSF samples were only collected from neonates with suspected meningitis where there were no contra-indications to do so, and only during working hours. Up to 2ml of CSF were collected and aliquoted for culture and biochemistry. Aliquots for microbiology and biochemistry were transported within 1 hour of collection to the respective clinical laboratories at the MRCG.
- ***Nasopharyngeal (NPS), rectovaginal (RVS) and rectal swabs (RS):*** Two NPS and RS samples were collected from each newborn, while two NPS and two RVS samples were collected from each mother all using Copan flocked swabs™. Upon collection, swabs were immediately placed into vials containing Skim-milk Tryptone-Glucose-Glycerol (STGG) transport medium and put in a cold box before transfer to the MRCG. One of each swab was used for microbiological culture (Clinical Microbiology Laboratory); the second newborn NPS was used for molecular detection of pathogens while other swabs were biobanked for future molecular work. Swabs for molecular testing were for stored at -70°C until tested. Samples collected after hours were stored temporarily at -20°C in a designated freezer in the research study office, then transported as early as possible the following morning to the Clinical Microbiology Laboratory (culture samples) or Biobank (molecular detection samples) for storage at -70°C until tested.

Clinical care: Each case received the routine antibiotics currently used to treat serious neonatal infections at the respective facility of recruitment according to the discretion

of the attending physician; usually ampicillin (and cloxacillin where there is suspected/confirmed Staphylococcal infection), and gentamicin or ceftriaxone, according to the WHO pocket book of hospital care for children dosing guidelines (Table 6.7) which are used in health facilities in The Gambia.

Table 6.7 Antibiotic dosing for serious neonatal infections in Gambian health facilities

Antibiotic	Dose	Frequency		Route	Duration
		< 7 days of age	≥ 7 days of age		
I. Sepsis or pneumonia					
Ampicillin	50mg/kg/dose	12 hourly	8 hourly	IV or IM	7 – 10 days
Cloxacillin	50mg/kg/dose	12 hourly	8 hourly	IV	7 – 10 days
Gentamicin	2.5mg/kg/dose	12 hourly	8 hourly	IV or IM	7 – 10 days
Ceftriaxone	100mg/kg/dose	daily	daily	IV	7 – 10 days
II. Meningitis					
Ampicillin	100mg/kg/dose	12 hourly	8 hourly	IV	21 days
Gentamicin	2.5mg/kg/dose	12 hourly	8 hourly	IV	21 days
Ceftriaxone	100mg/kg/dose	daily	daily	IV	21 days

IV= intravenous; IM=intramuscular

The results of microbiological culture were made available in real time to the staff at each recruiting facility in order to inform patient care. For each patient the final diagnosis (sepsis, meningitis) was determined by the gold standard (cultures of blood and CSF). Pneumonia was diagnosed clinically and whenever possible, radiologically (chest X-ray).

Controls

To guide the interpretation of molecular aetiology results and understand the clinical significance of specific pathogens, similar samples were also collected from healthy newborn controls (nasopharyngeal and rectal swabs) and their mothers (nasopharyngeal and rectovaginal swabs). There was however, no clinical justification to collect blood culture samples from healthy babies and only 0.5 mls of venous blood was collected for molecular diagnostic tests. Samples were transported, processed and stored using the same procedures as that of cases.

6.1.7 Ethical approval

This study was considered and approved by the MRC Scientific Coordinating Committee and the Gambian Government/MRC Joint Ethics Committee (SCC 1384 – see Appendix 6). It was also approved by the LSHTM Observational/ Interventions Research Ethics Committee (LSHTM Ethics Reference: 8622 – see Appendix 7).

6.2 Laboratory methods

The laboratory analysis of samples collected during this study was carried out by others. I visited the laboratory and field sites of the ANISA study in Bangladesh before the start of the Pilot study, to familiarise myself with the flow of samples for the molecular diagnostic assays from collection to processing. The customized TACs (20 Respiratory and 20 Blood cards) used for the pilot study molecular assays were provided courtesy of Professor Samir Saha, Principal Investigator of the ANISA Study, Child Health Research Foundation, Dhaka, Bangladesh. I spent further time at the MRC Clinical Microbiology and Molecular diagnostics laboratories to familiarise myself with sample processing and learn about quality control.

The TAC experiments were done by Mrs. Awa L. Mendy and supervised by Dr Muna Affara, Mr. Sheikh Jarju and Dr Kirsty Le Doare (Imperial College, London). I checked all data for integrity and carried out the analyses of the output from the TAC experiments with the support of Dr Kirsty Le Doare (Imperial College, London), and Drs Maureen Diaz and Jonas Winchell (Centers for Disease Control and Prevention, Atlanta, Georgia, USA).

Microbiology samples were processed by Mr. Buntung Ceesay, Mr. Ngange Kebbeh, Mr. Frank Thornton-Wood, Miss Shuling Appleby, Miss. Awa L. Mendy, and supervised by Mrs. Saffiatou Darboe. Some of the consumables used for the microbiology work were

provided courtesy of Dr Kirsty Le Doare and funded from her Thrasher Award (BK1350).

6.2.1 Sample processing

All clinical samples were processed at the MRCG Fajara Field Station laboratories. The MRCG Laboratories consist of the 'Clinical laboratories' (microbiology, haematology and biochemistry/immunochemistry), and the 'Research laboratories' (TB diagnostics, serology and molecular diagnostics). The Clinical laboratories are located in the Clinical Services Department, and process samples from outpatients, inpatients, patients from peripheral health care facilities as well as samples from participants in MRCG research studies.

The Clinical laboratories are accredited to the international quality standard for the quality and competence of medical laboratories (ISO15189) with regular quality control monitoring by assessment by The Kenya Accreditation Service in accordance with international quality systems for laboratories. The laboratories are also certified compliant with the Good Clinical Laboratory Practice (GCLP) standard,[349] as published by the British Association of Research Quality Assurance (BARQA), 2003.[350] The GCLP accreditation scheme involves the assessment of a clinical laboratory which undertakes the analysis of samples from clinical trials. Certification of compliance demonstrates to sponsors of clinical trials worldwide that the clinical laboratories operate to a standard that assures the reliability, quality and integrity of the work and results generated. The MRCG Research Laboratories, are also one of the WHO Regional Reference Laboratories for Invasive Bacterial Vaccine Preventable Diseases.

The flow of samples in both the pilot and main studies, and their processing is summarised below in Figure 6.4. The methods for specimen processing and testing were same for cases and controls.

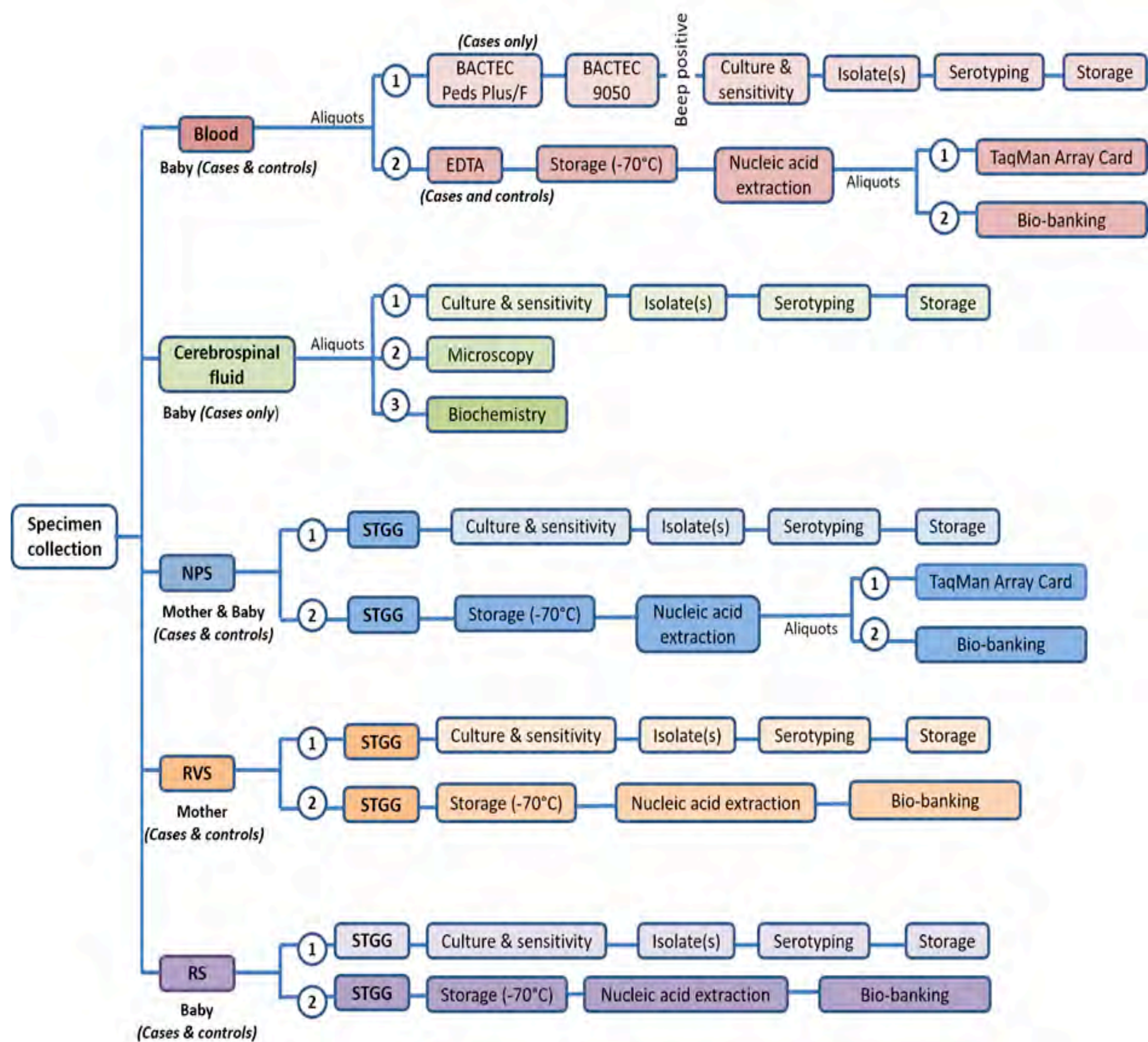


Figure 6.4 Flow chart of sample collection and processing

6.2.2 Microbiological methods

Blood cultures

All blood cultures were processed using BACTEC Peds Plus /F bottles and BACTEC 9050 fluorescent series instrument (Becton Dickinson, Temse, Belgium). The BD BACTEC

Peds Plus /F medium is an enriched soybean-casein digest resin-containing broth for the qualitative culture and recovery of aerobic microorganisms (mainly bacteria and yeast) from blood samples which are generally less than 5 ml in volume. Each vial contains 40 ml of broth. The medium also contains an anticoagulant, Sodium polyanetholesulfonate (SPS) that inhibits bacteriocidal activities in the blood. The resins serve to neutralize a wide variety of antibiotics, allowing growth of microorganisms that may not occur with conventional media and thereby enhancing the recovery of organisms among patients under antibiotic treatment.

Inoculated vials were inserted into the BACTEC 9050 fluorescent series instrument (Becton Dickinson, Temse, Belgium) for incubation, agitation, and periodic measurement. Each vial contains a chemical sensor that can detect increases in CO_2 produced by the growth of microorganisms. When microorganisms are present in the blood sample, they metabolize nutrients in the culture medium, releasing CO_2 into the medium. A dye in the sensor reacts with the CO_2, modulating the amount of light that is absorbed by the fluorescent material in the sensor. The instrument's photo detectors monitor the sensor every ten minutes for an increase in its fluorescence and measure the level of fluorescence, which is proportional to the amount of CO_2 present. Algorithms resident in the instrument rack's microprocessor determine the positivity of a vial. The algorithms use the rate of CO_2 production as well as the absolute increase in CO_2 to interpret the data. A positive reading indicates the presumptive presence of viable microorganisms in the vial. Detection is limited to microorganisms that will grow in a particular type of culture medium. Culture vials flagged as presumptively positive were removed from the instrument for sub-culture, Gram-stain, and identification. For each positive vial, sub-cultures were carried out on solid media: blood agar (aerobic and

anaerobic), chocolate agar and MacConkey agar. Identification was by means of cultural morphology, biochemical test kits (BioMerieux Analytical Profile Index (API), API 20 E™ API 20 NE, API NH) and serological agglutination as appropriate for the pathogen. Culture vials that did not flag as positive remained in the instrument for five days (according to the manufacturer's test protocol) after which negative vials were discarded.

Clinical significance of blood culture isolates

The list of pathogens associated with infection in the newborn is diverse and varies geographically.[6, 21, 22, 80] Many of the bacterial pathogens are also skin flora or colonisers of mucosal surfaces, and may 'contaminate' blood culture bottles during phlebotomy, inoculation of blood culture bottles and processing of samples and culture plates thereby leading to false-positive results.[259] The clinical significance of organisms detected by blood culture is listed in Table 6.8.

Table 6.8 Clinical significance of bacteria detected by blood culture

Clinically significant organisms		Clinically non-significant organisms
Acinetobacter species	Klebsiella pneumoniae	Bacillus species
Citrobacter species	Proteus mirabilis	Coagulase negative staphylococci
Enterobacter species	Pseudomonas aeruginosa	Diphtheroids
Enterococcus species	Salmonella species	Micrococcus species
Escherichia coli	Serratia marcescens	Propionibacterium species
Group A Streptococcus	Staphylococcus aureus	Viridans streptococcus
Group B Streptococcus	Streptococcus pneumoniae	
Group D Streptococcus		

In this study, Coagulase negative *Staphylococcus* (CoNS), usually associated with indwelling devices, was also considered clinically non-significant given that these devices are not used in The Gambia. This is in line with the MRCG clinical microbiology protocol, previous aetiology studies in The Gambia,[348] and the classification used by the ANISA study group.[260] In the ANISA study, a third category of pathogens was described

a being ‘Probably significant’. Organisms in this group were identified from regional data on environmental organisms that frequently colonise the skin of South Asian newborns and which may also cause bloodstream infections in newborns.[351, 352] Viridans Streptococci and *S. aureus* were among the ‘Probably significant’ pathogens in the ANISA study. Data on surface colonisation among Gambian newborns is limited.[253, 353] Consequently, Viridans Streptococci was considered clinically non-significant, and *S. aureus* considered clinically significant based on the MRCG clinical microbiology laboratory protocol.

Study personnel reviewed documentation for each case with a clinically non-significant blood culture to identify possible reasons for contamination as an ongoing quality check. However, laboratory personnel identified and recorded all organisms grown on blood culture, even if they were in the list of clinically non-significant organisms or there was growth of multiple organisms. Beyond the pre-determined contaminant list, some unexpected organisms of questionable clinical significance were isolated. The clinical and laboratory characteristics of these cases were reviewed at the end of the study by an independent panel of clinicians and microbiologists at the MRC review to determine their clinical significance. Any isolate classified as a clinically non-significant was not stored; all other organisms were identified then stored at –80°C for future work.

Antibiotic susceptibility

Antibiotic susceptibility patterns of significant bacterial isolates were determined using approved disk-diffusion methods described in the Clinical Laboratory Standard Institute (CLSI) guidelines (2014). For each isolate, the antibiotic zone of inhibition diameter was recorded to enable translation of results with any possible future changes in CLSI

guidelines. Appropriate American Type Culture Collection (ATCC) controls were used consistently.

Swabs

All swabs were stored at -70°C and processed in batches. Stored samples were thawed at room temperature and then vortexed. 50µl of each vortexed specimen was plated on Chromagar media, which was the primary agar used in this study. From the primary plate, presumptive bacterial colonies were streaked onto appropriate agar plates and re-incubated overnight at 37°C for confirmatory testing. Priority was given to identification of bacteria only.

All concordant bacterial isolates from mother-newborn dyads have been stored for future investigation including strain confirmation and Whole Genome Sequencing in order to determine whether the maternal recto-vaginal isolate is the cause of neonatal disease.

CSF

Part of each CSF specimen aliquoted for culture were immediately plated on chocolate, blood and MacConkey agar media. The second aliquot was sent to the biochemistry laboratory for glucose and protein estimation.

6.2.3 Molecular diagnostic assays

The multipathogen molecular detection platform used in this study was the TAC. Each TAC contains eight sample loading ports each with a microfluidic channel leading to 48 different wells (Figure 6.5). The wells contain specific dried-down primers and hydrolysis probes for the detection of defined pathogen-specific or control targets. Once specimen nucleic acids are added to the wells, the system is closed (i.e. the reactions are completely contained within each well). This is of particular benefit in resource-poor

settings where risk of contamination with PCR amplicons is high due to the lack of dedicated rooms for different PCR steps. Other advantages of this methodology include the ability to customise a panel of pathogen targets, and ease of use, including minimal "hands-on" setup.[53]

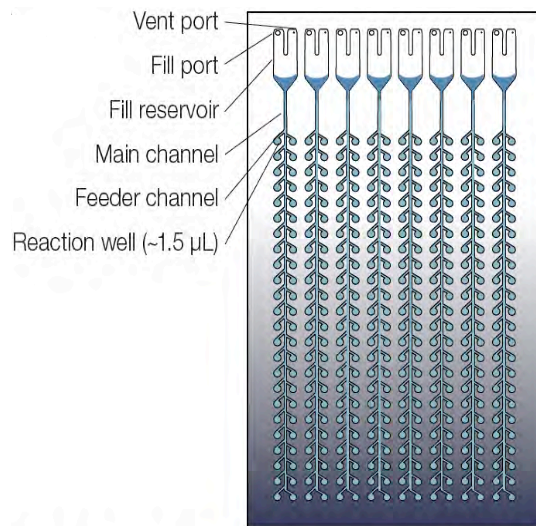


Figure 6.5 Layout of a Taqman Array Card

The TAC has only ever been used in the diagnosis of neonatal infection in South Asia during the ANISA study,[48] and not in sSA. One of the aims of this PhD was to explore the applicability of this technology for pathogen detection among sick Gambian newborns. Given the limited number of cards available for this study and the very high costs of customised manufacture and shipping, it was not possible to apply them to all study samples. Priority was therefore given to the neonatal blood and respiratory swab samples from the Pilot Study. The optimised ANISA-specific TAC configurations for the respiratory and blood cards in shown in Figures 6.6 and 6.7. Each customised TAC comprised fill ports for six specimens, a no template control (NTC), and a positive control (PC).[53] The respiratory TAC had two replicates of each assay while the blood TAC had four replicates of each assay. The higher number of assay replicates on the

blood TAC is to increase the proportion of the sample which is evaluated, and therefore improve detection.[53] The ANISA study protocols for carrying out the TAC experiments were adhered to as much as possible. The only differences were in the equipment used for the nucleic acid extraction and running of the TACs; however, this was discussed with the ANISA/CDC collaborators who confirmed that this would not affect the validity and comparability of the results.

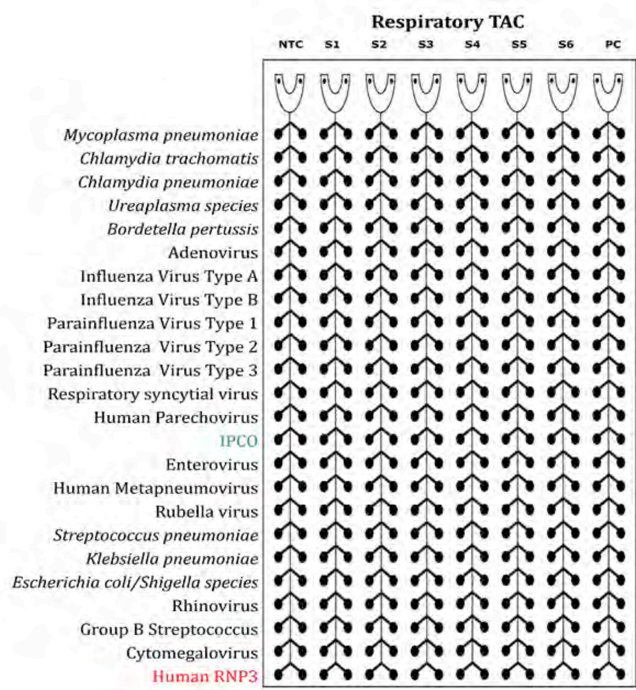


Figure 6.6 ANISA-specific TaqMan Array Card configurations for the Respiratory cards

NTC= Non-Template Control; S1 – S6= Specimens 1 – 6; PC=Positive control; IPCO=Internal Positive Control
*Duplex assay consisting of Human RNP3 assay with FAM-labeled probe and IPCO assay with VIC-labeled probe.

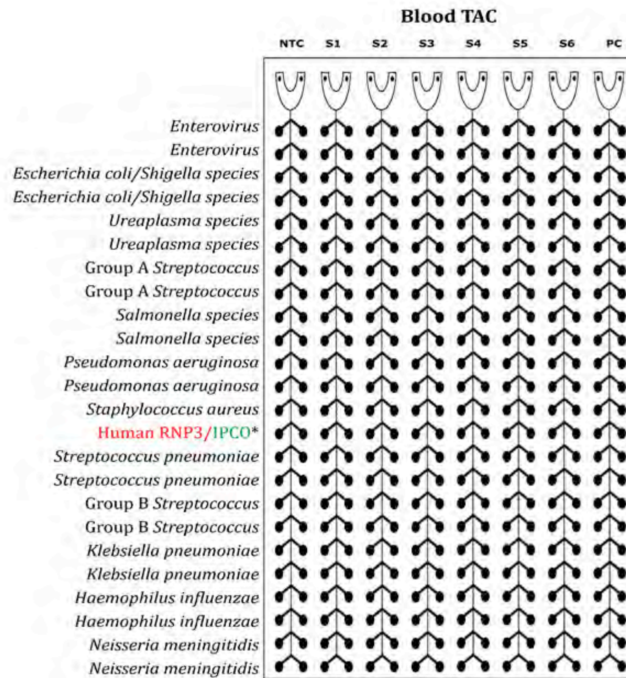


Figure 6.7 ANISA-specific TaqMan Array Card configurations for the Blood cards

NTC= Non-Template Control; S1 – S6= Specimens 1 – 6; PC=Positive control; IPCO=Internal Positive Control
*Duplex assay consisting of Human RNP3 assay with FAM-labeled probe and IPCO assay with VIC-labeled probe.

Specimen processing and nucleic acid extraction

Total nucleic acid (TNA) was extracted from thawed blood and NPS specimens using the QIAxtractor® Robot instrument (QIAGEN, GmbH) with the *cador* Pathogen 96 QIAcube HT Kit and Pre-treatment protocols for difficult-to-lyse bacteria in whole blood and liquid extracts from swabs. Pre-treatment was followed by nucleic acid purification using the nucleic acid purification. For NPS specimens, 400µl of STGG was extracted and eluted in 100µl. For extraction of whole blood, 300µl of blood in EDTA was mixed with 100µl of freshly prepared solution of lytic enzymes consisting of consisting of 1.5 mg/ml lysostaphin, 2500 U/ml mutanolysin, and 200 mg/ml lysozyme (Sigma-Aldrich, St. Louis, MO, USA) in Tris-EDTA (TE) buffer and incubated at 37°C for 60 minutes prior to extraction on the QIAxtractor® Robot, with elution in 100µl.

TAC assay performance

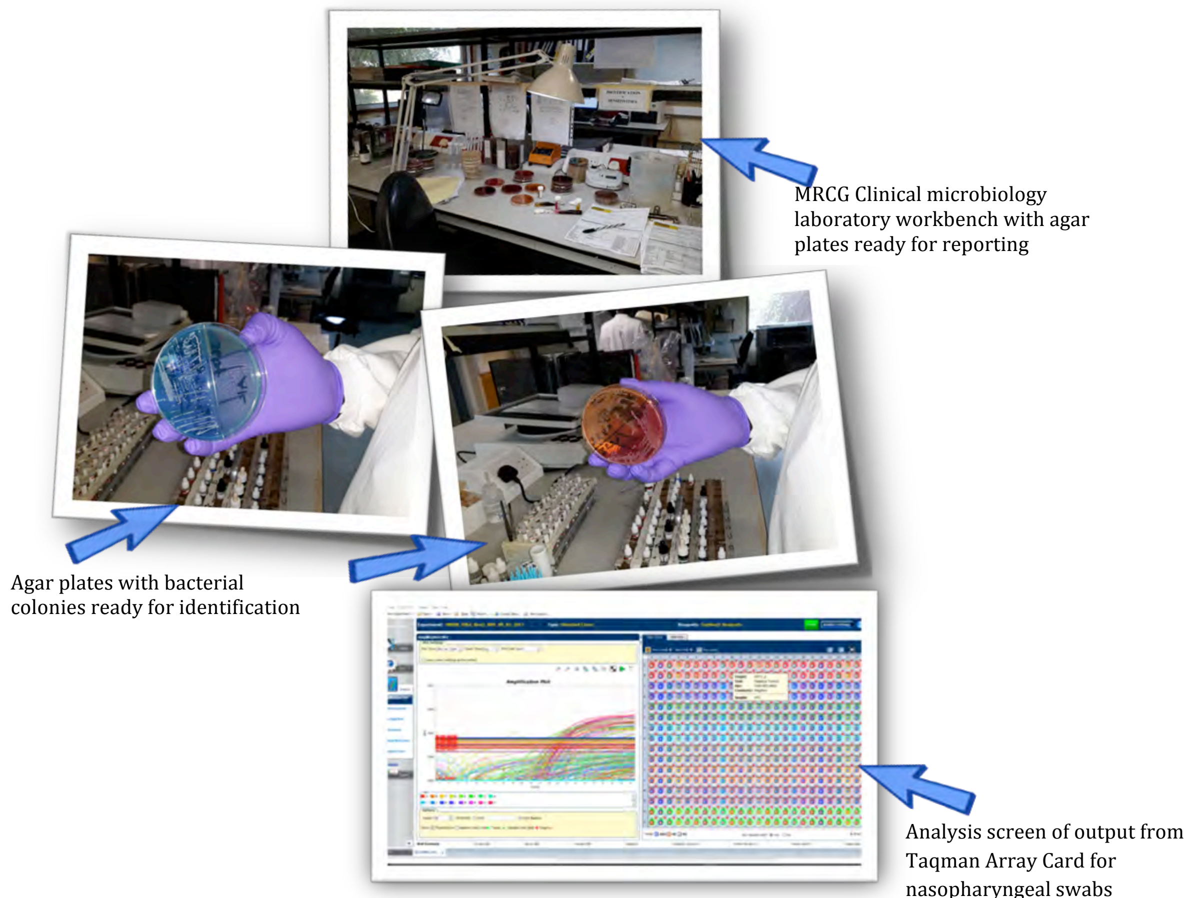
The PCR mastermix for each TAC consisted of 1 x qScript XLT 1-Step RT-qPCR ToughMix, low ROX (Quanta Biosciences, Gaithersburg, MD, USA). The 2nd – 7th fill ports on each TAC were loaded with 50µL qScript XLT 1-Step RT-qPCR ToughMix and 50µL of TNA extract. In the first fill port, the TNA was replaced with nuclease-free water as a template (no-template control; NTC). The role of the NTC is to ensure no fluorescence amplification signal is observed in the absence of nucleic acid. In the eighth fill port, the TNA extract was replaced with a positive control consisting of combined RNA transcripts. Each card was centrifuged at 1200rpm for 1 minute twice, to distribute the fluid in the reaction wells, and then sealed to sequester individual reactions. All TACs were run on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument System (Life Technologies, Foster City, CA, USA). The purpose of the TAC is not to quantify the infectious agent and the assays are qualitative in nature.

Analysis and interpretation of TAC assay output

The data generated from each TAC experiment were exported to a designated folder on my desktop and analysed using QuantStudio™ Real-Time PCR Software v1.2 CDC (Thermo Fisher Scientific Inc). I carried out all analyses and interpretation of results according to Standard Operating Procedures used by the ANISA study (Appendix 8), with support from Dr. Kirsty Le Doare and collaborators at Centers for Disease Control and Prevention, Atlanta, USA, who reviewed and confirmed the final results. Relative levels of amplification directly correlate to the fluorescence data generated and measured during the reaction(s) using the QuantStudio™ 7 Flex Real-Time PCR Instrument System. Fluorescence amplification indicates the presence of the specific nucleic acid target. A specimen is considered positive if any number of the pathogen-specific replicates (1 of 2 replicates in NPS or <4 of 4 in blood specimens) is positive.

6.3 Results

This section integrates the epidemiological and laboratory results for both Pilot and Main studies.



Eligibility, recruitment, sampling and diagnoses of Infant Case-Mother pairs and Infant Control-Mother pairs are summarised in Figures 6.8 and 6.9 respectively. The first results section presents the descriptive and comparative analyses of the epidemiological characteristics of the study participants. The second section presents descriptive and comparative analyses of the laboratory results, with a detailed analysis of conventional microbiology results. The third section presents the results from an investigation into two outbreaks that occurred in the neonatal unit during the course of the main study.

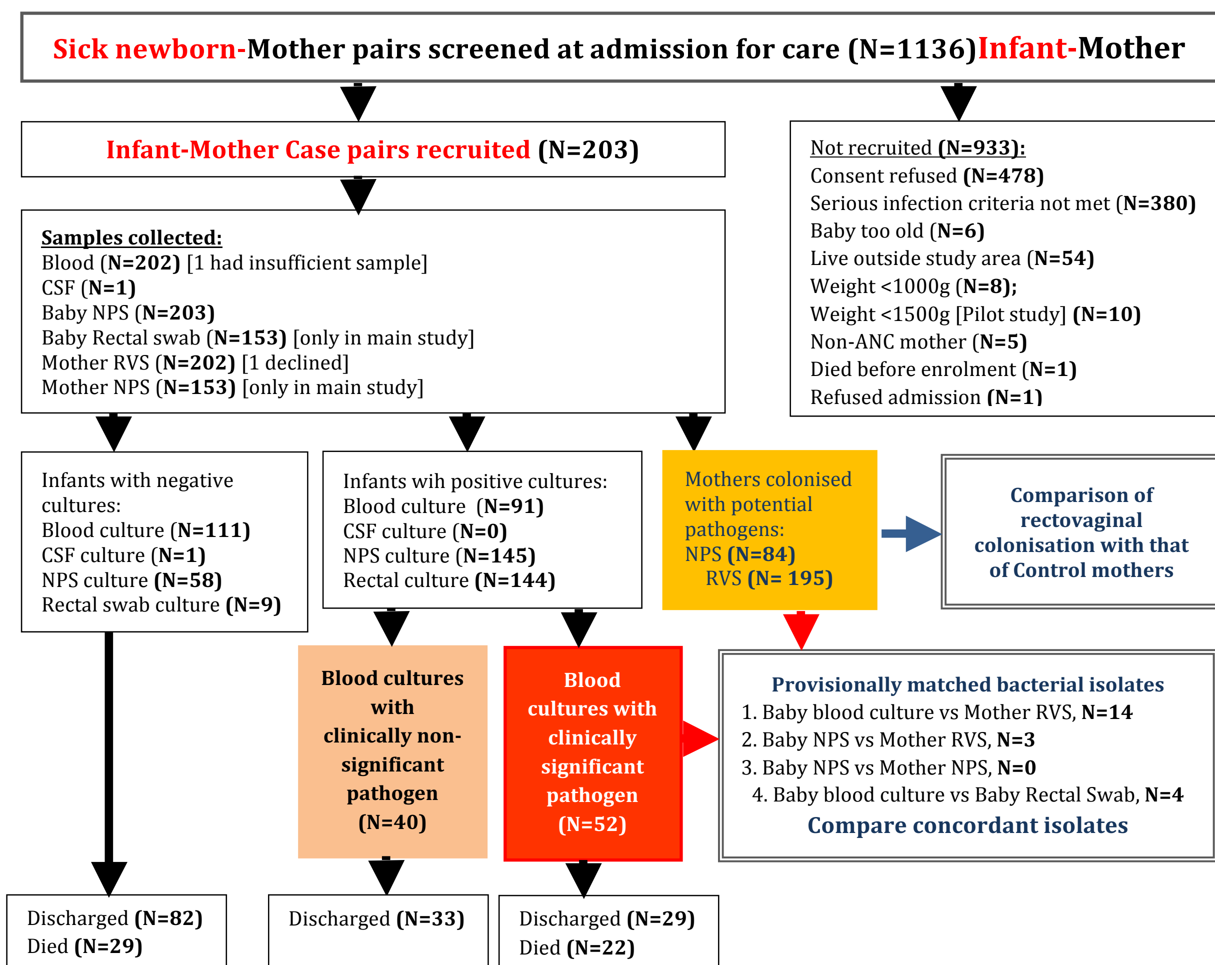


Figure 6.8 Flow chart showing overview of recruitment, participation and results of Infant Case-Mother pairs for pilot and main studies combined

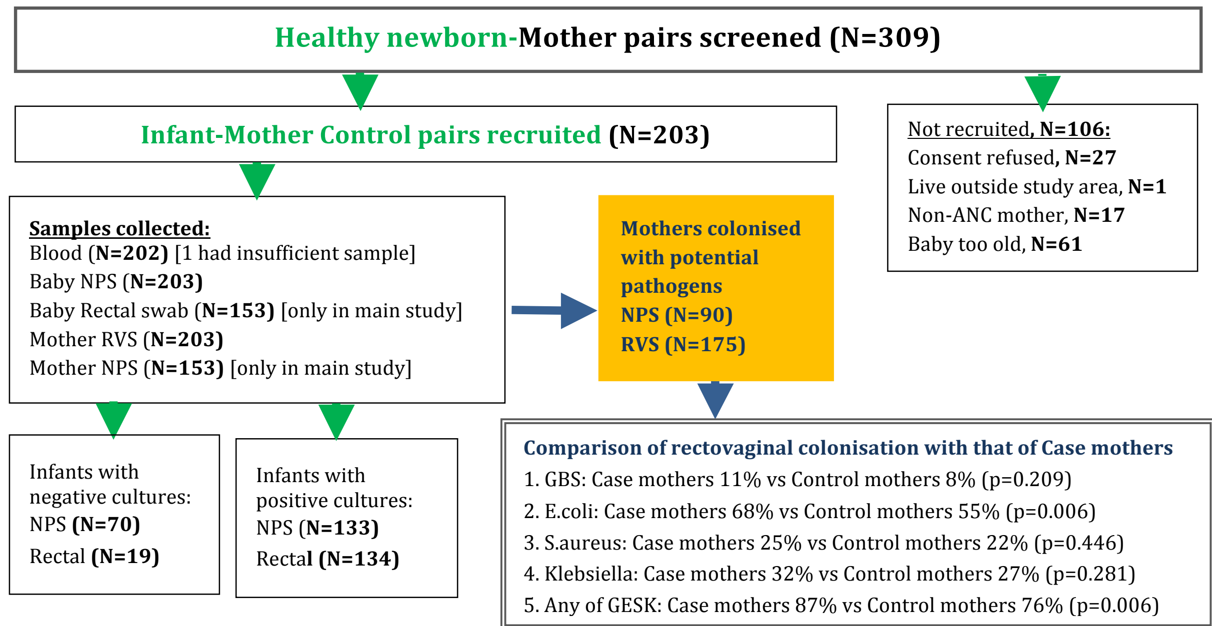


Figure 6.9 Flow chart showing overview of recruitment, participation and results of Control-Mother pairs

6.3.1 Epidemiological Results

Descriptive analyses

406 infant-mother case-control pairs were enrolled; 100 in the Pilot study (April – August 2015) and 306 in the Main study (February 2016 – January 2017). The clinical and maternal characteristics of the newborn cases (hereafter simply referred to as "Cases") are detailed in Table 6.9, by phase of recruitment. Overall, the participants recruited during the both phases of the study were similar in these characteristics. More than half of the Cases [57% (144/203)] were aged ≤3 days at recruitment; and 7% (15/203) were recruited on the day of birth. Among the 184 facility-born Cases, 57% (105/184) were born at one of the three recruiting facilities; EFSTH 16% (29/184), Kanifing General Hospital 17% (32/184), and Brikama Health Centre 24% (44/184). Half of the Cases (51%, 103/203) were referred from Government health facilities, 31% (62/203) self-referred, 11% (23/203) were referred from private clinics, and 7% (15/203) were referred directly from the EFSTH labour ward.

Table 6.9 Demographic and clinical characteristics of 203 infant-mother case pairs stratified by phase of recruitment

Characteristic	Pilot Study (N=50) [a]	Main Study (N=153) [a]	Total (N=203) [a]
Neonatal			
Sex			
Male	62% (31)	60% (92)	61% (123)
Female	38% (19)	40% (61)	39% (80)
Median postnatal age in days(IQR)	3 (1 – 10)	3 (1 – 8)	3 (1 – 9)
Aged 0 – 3 days	56% (28)	57% (87)	57% (115)
Aged 4 – 6 days	14% (7)	14% (22)	14% (29)
Aged 7 – 27 days	30% (27)	29% (44)	29% (59)
Median gestational age in weeks (IQR)	38 (36 – 39)	38 (37 – 40)	38 (36 – 39)
28 - <32 weeks	4% (2/48)	0 0	1% (2/193)
32 - <37 weeks	31% (15/48)	23% (33/145)	25% (48/193)
≥37 weeks	65% (31/48)	77% (112/145)	74% (143/193)
Median birth-weight in Grams (IQR)	3000 (2700 – 3400)	3000 (2600 – 3400)	3000 (2600 – 3400)
≤1500g	2% (1/47)	2% (3/141)	2% (4/188)
1501 – 2500g	19% (9/47)	21% (30/141)	21% (39/188)
>2500g	79% (37/47)	77% (108/141)	77% (145/188)
Recruiting site			
EFSTH	100% (50)	70% (107)	77% (157)
Kanifing General Hospital	0 (0)	3% (4)	2% (4)
Brikama Heatth Centre	0 (0)	27% (42)	21% (42)
Place of birth			
Facility-born	90% (45)	91% (139)	91% (184)
Home/TBA	10% (5)	9% (14)	9% (19)
Mode of delivery			
Spontaneous vaginal delivery	82% (41)	86% (132)	85% (173)
Caesarean section/Assisted vaginal delivery [b]	18% (9)	14% (21)	15% (30)
Clinical presentation			
Fast breathing (RR ≥60/min)	60% (33)	46% (70)	51% (103)
Severe chest wall in-drawing	34% (17)	16% (25)	21% (42)
Fever (≥38.0°C)	42% (21)	56% (85)	52% (106)
Hypothermia (≤35.5°C)	10% (5)	16% (25)	15% (30)
Abnormal movement	16% (8)	18% (28)	18% (36)
Convulsions	30% (15)	24% (37)	26% (52)
Feeding difficulty	38% (19)	39% (60)	39% (79)
Pre-admission antibiotic exposure	10% (5)	19% (29)	17% (34)
Final diagnosis			
Culture-positive bacteraemia/sepsis [C]	26% (13)	25% (38)	25% (51)
Clinical Meningitis	0% (0)	7% (11)	5% (11)
Pneumonia	6% (3)	2% (3)	3% (6)
Maternal			
Median age in years (IQR)	27 (23 – 29)	26.5 (22 – 32)	27 (22 – 32)
Aged < 18 years	4.5% (2/44)	6% (9/152)	6% (11/196)
Aged 18 – 34 years	91% (40/44)	78% (119/152)	81% (159/196)
Aged ≥35 years	4.5% (2/44)	16% (24/152)	13% (26)
Nationality			
Gambian	84% (42)	91% (139)	89% (181)
Mother's education			
None	30% (15)	28% (43)	29% (58)
Primary	26% (13)	18% (28)	20% (41)
Secondary	40% (20)	43% (66)	42% (86)
Tertiary	4% (2)	11% (16)	29% (18)
Married	100% (50)	93% (142)	95% (192)
Median parity (IQR)	3 (2 - 5)	2 (1 – 4)	2 (1 – 5)
Primiparous	24% (12)	33% (50)	31% (62)
Multiparous	76% (38)	67% (103)	69% (141)

[a] Denominators (X/Y) are presented for variables with missing data
[b] Assisted vaginal delivery: Vacuum extraction (4) and assisted breech delivery (2)

In Table 6.10, the characteristics of the newborn controls (hereafter simply referred to a "Controls") and their mothers are presented by phase of recruitment.

Table 6.10 Demographic and clinical characteristics of 203 infant-mother control pairs stratified by phase of recruitment

Characteristic	Pilot Study (N=50) [a]	Main Study (N=153) [a]	Total (N=203) [a]
Neonatal			
Sex			
Male	50% (25)	52% (79)	51% (104)
Female	50% (25)	50% (25)	49% (99)
Median postnatal age in days(IQR)	14 (9 – 18)	2 (0 – 10)	4 (1 – 4)
Aged 0 – 3 days	6% (3)	64% (98)	50% (101)
Aged 4 – 6 days	2% (1)	5% (7)	4% (8)
Aged 7 – 27 days	92% (46)	31% (48)	46% (94)
Median gestational age in weeks (IQR)	38 (36 – 39)	38 (37 – 38)	38 (37 – 39)
28 - <32 weeks	2% (1/47)	0% (0)	1% (1)
32 - <37 weeks	28% (13/47)	18% (26/146)	20% (39/193)
≥37 weeks	70% (33/47)	82% (120/146)	79% (153/193)
Median birth-weight in Grams (IQR)	3150 (2950 – 3400)	3000 (2700 – 3300)	3000 (2800 – 3300)
≤1500g	0% (0)	0% (0)	
1501 – 2500g	4% (2/48)	18% (28/152)	15% (30/200)
>2500g	96% (46/48)	82% (124/152)	85% (170/200)
Recruiting site			
EFSTH Postnatal ward	0% (0)	1% (1)	<1% (1)
Polyclinic banjul	10% (5)	4% (6)	5% (11)
Serekunda Health Centre	34% (17)	21% (33)	25% (50)
Fajikunda Health Centre	12% (6)	8% (12)	9% (18)
Bundung Maternal & Child Health Hospital	22% (11)	25% (39)	25% (50)
Brikama Health Centre	22% (11)	41% (62)	36% (73)
Place of birth			
Facility-born	92% (46)	96% (147)	95% (193)
Home/TBA	8% (4)	4% (6)	5% (10)
Mode of delivery			
Vaginal delivery [b]	100% (50)	89% (136)	92% (186)
Caesarean section	0% (0)	11% (17)	8% (17)
Pre-admission antibiotic exposure			
Maternal			
Median age in years (IQR)	27 (21 - 30)	25 (21 – 30)	25 (21 – 30)
Aged < 18 years	6% (3)	4% (6)	4% (9)
Aged 18 – 34 years	80% (40)	84% (127)	83% (167)
Aged ≥35 years	14% (7)	12% (19)	13% (26)
Nationality			
Gambian	96% (48)	95% (146)	96% (194)
Mother's education			
None	18% (9)	44% (67)	37% (76)
Primary	28% (14)	9% (14)	14% (28)
Secondary	50% (25)	41% (62)	43% (87)
Tertiary	4% (2)	6% (10)	6% (12)
Married	92% (46)	94% (144)	94% (190)
Median parity (IQR)	2 (1 – 4)	3 (1 – 4)	3 (1 – 4)
Primiparous	28% (14)	27% (41)	27% (55)
Multiparous	72% (36)	73% (112)	73% (148)

[a] Denominators (X/Y) are presented for variables with missing data
[b] Assisted vaginal delivery: Vacuum extraction (4) and assisted breech delivery (2)

6.3.2 Microbiology Results

Prevalence and aetiology of neonatal infection

Blood cultures

Bacteria were cultured from the blood of 45% (91/202) of Cases, and 25% (52/202) had a clinically significant organism. *S. aureus* was highly prevalent accounting for 25% (13/52) of all clinically significant infections (Table 6.11). Coagulase-negative staphylococcus (CoNS) was the most abundant isolate and predominant clinically non-significant organism accounting for 80% (32/40) of Cases. Others include *Bacillus* species (4), *Micrococcus* species (2), and Viridans *streptococci* (2).

Table 6.11 Clinically-significant bacteria isolated from blood cultures of cases stratified by phase of recruitment

Isolate	Pilot Study (N = 13)	Main Study (N = 39)	Total (N = 52)
Gram positive			
Staphylococcus aureus	8% (1)	31% (12)	25% (13)
Enterococcus species	0% (0)	8% (3)	5% (3)
Streptococcus species	0% (0)	5% (2)	4% (2)
Gram negative			
Klebsiella species [a]	30% (4)	13% (5)	17% (9)
Burkholderia cepacia	8% (1)	21% (8)	17% (9)
Pseudomonas species [c]	46% (6)	3% (1)	14% (7)
Acinetobacter baumanii	0% (0)	5% (2)	4% (2)
Escherichia coli	0% (0)	5% (2)	4% (2)
Achromobacter xylosoxidans	0% (0)	3% (1)	2% (1)
Citrobacter species	0% (0)	3% (1)	2% (1)
Enterobacter cloacae [b]	0% (0)	3% (1)	2% (1)
Salmonella species	0% (0)	3% (1)	2% (1)
Pantoea species	8% (1)	0% (0)	2% (1)

[a] *Klebsiella pneumoniae* (8) and *Klebsiella oxytoca* (1)
[b] This baby had mixed infection with *Escherichia coli.*
[c] *Pseudomonas luteola* (6) and *Pseudomonas* species (1)

Early-onset infection (≤ 3 days) accounted for 28% of Cases of clinically significant bloodstream infection. There was no difference in the prevalence of clinically significant bloodstream infection by age: 4- 7 days [21% (6/29), P=0.303] or ≥7 days [24% (14/59), P=0.942] compared with those aged ≤3 days [28% (32/114)], possibly due to the small sample size of clinically significant infections within the different age categories. Table 6.12 presents the distribution of clinically significant isolates by

postnatal age while Figure 6.10 shows the distribution of positive blood cultures and aetiology over the first week after birth. The predominant pathogens associated with early-onset infection were *S. aureus*, *B. cepacia,* and Gram-negative rods (*Klebsiella* and *Pseudomonas* species).

Table 6.12 Prevalence of clinically significant blood culture isolates by postnatal age

Isolate	Prevalence Within Different Age Groups		
	Aged ≤3 days (n=32)	Aged 4-6 days (n=6)	Aged ≥7 days (n=14)
Staphylococcus aureus	25% (8)	0% (0)	37% (5)
Burkholderia cepacia	22% (7)	17% (1)	7% (1)
Klebsiella species [b]	19% (6)	50% (3)	0% (0)
Pseudomonas species [c]	13% (4)	17% (1)	14% (2)
Enterococcus species	9% (3)	0% (0)	0% (0)
Streptococcus species	6% (2)	0% (0)	0% (0)
Acinetobacter baumanii	3% (1)	17% (1)	0% (0)
Pantoea species	3% (1)	0% (0)	0% (0)
Escherichia coli	0% (0)	0% (0)	14% (2)
Achromobacter xylosoxidans	0% (0)	0% (0)	7% (1)
Citrobacter species	0% (0)	0% (0)	7% (1)
Enterobacter cloacae	0% (0)	0% (0)	7% (1)
Salmonella species	0% (0)	0% (0)	7% (1)

[a] *Includes clinically non-significant pathogens*
[b] *Klebsiella pneumoniae* (8) and *Klebsiella oxytoca* (1)
[c] *Pseudomonas luteola* (6) and *Pseudomonas* species (1)

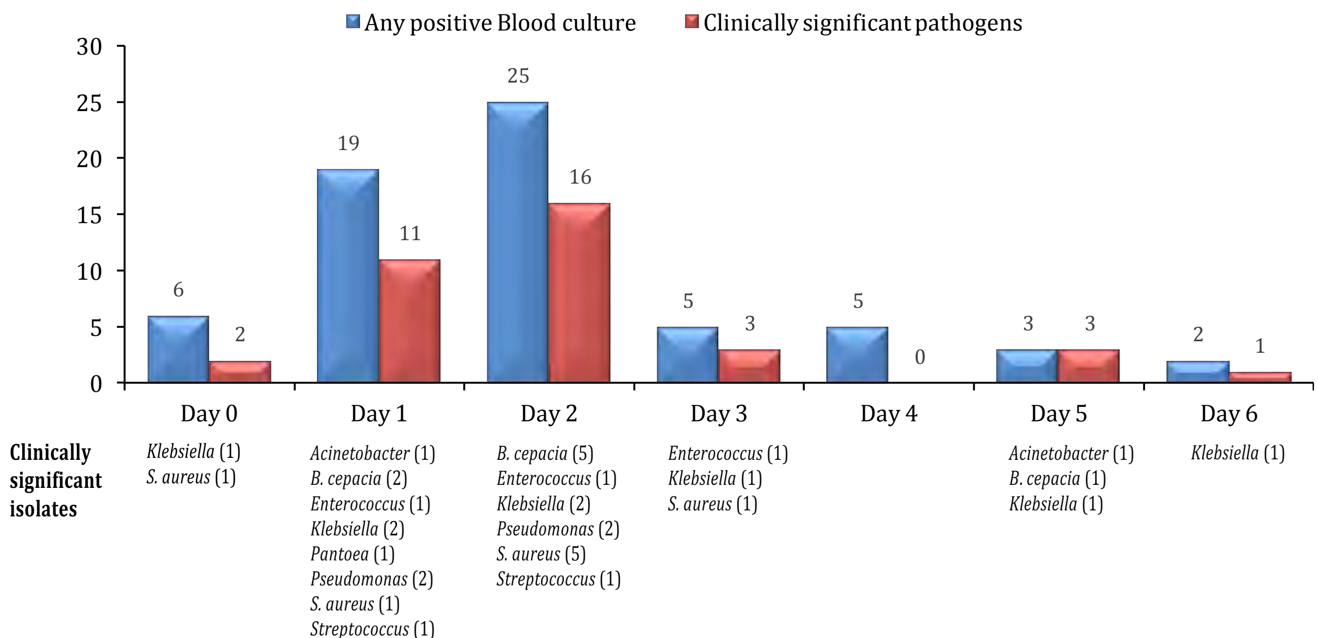


Figure 6.10 Distribution of clinically significant blood culture isolates in the first week after birth

Clinical characteristics of Cases with positive blood cultures

The demographic and clinical characteristics of Cases stratified by blood culture results are detailed in Table 6.13.

Table 6.13 Demographic and clinical characteristics of cases and their mothers stratified by blood culture result

	Clinically significant organism (N=51) [a]	Clinically non- significant organism (N=40) [a]	Negative culture (N=111) [a]	*P**
Neonatal				
Sex				
Male	65% (33/51)	60% (24/40)	59% (65/111)	*0.758*
Female	35% (18/51)	40% (16/40)	41% (46/111)	
Median postnatal age in days (IQR)	2 (1 – 8)	2 (1 - 7)	3 (1– 10)	
Aged 0 – 3 days	63% (32/51)	58% (23/40)	53% (59/111)	*0.514*
Aged 4 – 6 days	12% (6/51)	20% (8/40)	14% (15/111)	
Aged 7 – 27 days	25% (13/51)	22% (9/40)	33% (37/111)	
Median gestational age in weeks (IQR)	38 (36 – 40)	38 (36 – 39)	38 (36 – 39)	
28 - <32 weeks	0% (0/47)	0% (0/39)	2% (2/106)	*0.984*
32 - <37 weeks	26% (12/47)	26% (10/39)	24% (25/106)	
≥37 weeks	74% (35/47)	74% (29/39)	74% (79/106)	
Median birth-weight in Grams (IQR)	3000 (2500 – 3430)	3000 (2800 – 3200)	3000 (2600 – 3400)	
≤1500g	2% (1/48)	0% (0/38)	3% (3/101)	*0.582*
1501 – 2500g	25% (12/48)	13% (5/38)	21% (21/101)	
>2500g	73% (35/48)	87% (33/38)	76% (77/101)	
Place of birth				
Facility-born	94% (48/51)	93% (37/40)	88% (98/111)	*0.448*
Home/TBA	6% (3/51)	7% (3/40)	12% (13/111)	
Mode of delivery				
Spontaneous vaginal delivery	90% (46/51)	95% (38/40)	79% (88/111)	
Caesarean section/Assisted vaginal delivery	10% (5/51)	5% (2/40)	21% (23/111)	*0.033*
Clinical presentation				
Fast breathing (RR ≥60/min)	67% (34/51)	55% (22/40)	42% (47/111)	*0.014*
Severe chest wall in-drawing	27% (14/51)	15% (6/40)	20% (22/111)	*0.324*
Fever (≥38.0°C)	43% (22/51)	43% (17/40)	60% (67/111)	*0.046*
Hypothermia (≤35.5°C)	18% (9/51)	13% (5/40)	14% (15/111)	*0.731*
Abnormal movement	31% (16/51)	15% (6/40)	12% (13/111)	*0.008*
Convulsions	27% (14/51)	35% (14/40)	22% (24/111)	*0.240*
Feeding difficulty	37% (19/51)	43% (17/40)	39% (43/111)	*0.163*
Pre-admission antibiotic exposure	20% (10/51)	20% (8/40)	14% (16/111)	*0.597*
Maternal				
Median age in years (IQR)	27 (24 – 32)	27 (23 – 32)	26 (22 – 32)	
Aged < 18 years	2% (1/49)	5% (2/39)	7% (8/107)	*0.585*
Aged 18 – 34 years	80% (39/49)	85% (33/39)	80% (86/107)	
Aged ≥35 years	18% (9/49)	10% (4/39)	12% (13/107)	
Median parity (IQR)	3 (2 – 5)	2.5 (1 – 4)	2 (1 – 4)	
Previous stillbirth	10% (5/51)	10% (4/40)	7% (8/111)	*0.649*
Intrapartum fever	22% (11/51)	18% (7/40)	10% (11/111)	*0.021*
Intrapartum antibiotic exposure	2% (1/51)	3% (1/40)	2% (2/111)	*0.896*

[a] Denominators (X/Y) are presented for variables with missing data

Cases born by caesarean section or assisted vaginal delivery were significantly more likely to have a negative blood culture compared with those born by spontaneous vaginal delivery (*P*=0.033). With respect to clinical presentation, Cases with clinically significant bacteraemia were significantly more likely to present with fast breathing, abnormal movement and to have a maternal history of intrapartum fever. Interestingly, fever was a more common presentation among cases with a negative blood culture (*P*=0.046), although pre-admission antibiotic exposure did not significantly differ by culture result.

Because CoNS was the most abundant pathogen isolated from blood cultures, Cases with CoNS infection were compared with Cases that had cultures positive for clinically significant bacteria. No major differences in relation to known risk factors (gestational age and birth-weight) were observed between the two groups. For gestational age at birth, 28% (9/32) of CoNS cases were born premature compared with 26% (12/47) of those with clinically significant bacteria (*P* = 0.798). Of the four very low-birth-weight (VLBW) Cases enrolled in this study, only one had a positive blood culture which was for a clinically significant organism. Culture positive rates did not vary between facilities.

CSF cultures

Fifty-Two Cases met the clinical criteria for a diagnostic lumbar puncture at admission but only one Case underwent the procedure within 24 hours of admission and before commencement of antibiotics. The CSF sample for this neonate was sterile on culture.

Antibiotic resistance

Phenotypic antibiotic-susceptibility testing was carried out using a panel of antibiotics that are locally used (Table 6.14). For *Klebsiella* species, antibiotic resistance was 89%

for WHO-recommended first-line therapy (gentamicin), and ranges from 67% - 89% for WHO-recommended second-line therapy (third-generation cephalosporins).

Table 6.14 Antibiotic resistance patterns in Cases with clinically significant bloodstream infection

	Staphylococcus aureus	***Klebsiella* species**	***Burkholderia cepacia***	***Pseudomonas* species**	***Escherichia coli***
Antibiotic class	**No. Resistant (%)**	**No. Resistant (%)**	**No. Resistant (%)**	**No. Resistant (%)**	**No. Resistant (%)**
Penicillins					
Ampicillin	NT	8/8 (100%)	6/6 (100%)	1/1 (100%)	2/2 (100%)
Amoxicillin/clavulanic acid	NT	4/5 (80%)	4/5 (80%)	1/1 (100%)	1/2 (50%)
Penicillin	12/13 (92%)	1/1 (100%)	1/1 (100%)	NT	NT
Flucloxacillin	0/1 (0%)	NT	NT	NT	NT
Cephalosporins					
Cefuroxime	NT	8/8 (100%)	5/5 (100%)	1/1 (100%)	1/2 (50%)
Cefoxitin	0/12 (0%)	0/5 (0%)	0/1 (0%)	N T	NT
Ceftriaxone	0/2 (0%)	2/3 (67%)	1/1 (100%)	0/2 (0%)	NT
Cefotaxime	0/1 (0%)	7/8 (88%)	3/4 (75%)	0/1 (0%)	1/2 (50%)
Ceftazidime	NT	8/9 (89%)	0/7 (0%)	0/5 (0%)	1/2 (50%)
Aminoglycoside					
Gentamicin	0/11 (0%)	8/9 (89%)	1/8 (13%)	1/6 (17%)	2/2 (100%)
Fluoroquinolone					
Ciprofloxacin	0/12 (0%)	5/9 (56%)	0/9 (0%)	0/6 (0%)	1/2 (50%)

Both *E. coli* isolates were resistant to gentamicin but only one isolate was resistant to third-generation cephalosporins and fluoroquinolones. The *S. aureus* isolates demonstrated 100% sensitivity to flucloxacillin and to first-line gentamicin.

Impact of culture diagnosis on management and mortality

The median blood culture reporting time during the study was 6 days (IQR, 5 – 7), but the median times to death or discharge were 2 (IQR, 1 – 6) and 7 (IQR, 4 – 9) respectively. Only 38% (35/91) of neonates with a positive blood culture received the result before discharge or death.

The overall case-fatality rate (CFR) was 29% (59/203). The one neonate that did not have a blood culture also died during admission. When stratified by blood culture result, the overall CFR was higher among neonates with clinically significant bacteraemia [43%

(22/51)] compared with neonates with CoNS infection [13% (4/32) *P*= 0.004] and those with negative cultures [26% (30/111) *P* = 0.041].

There were no differences in the CFR between Cases with a positive culture and those with a negative culture when stratified by age of onset. However, when further stratified by clinical significance, the CFR among neonates aged 0 – 6 days was significantly higher among those Cases with clinically significant organisms [50% (18/36), P=0.020] compared with Cases with clinically non-significant organisms [27% (4/15)]. Among those aged ≥7 days, no differences in CFR by clinical significance was observed.

Table 6.15presents case fatalities among those neonates with positive blood cultures stratified by the infecting organism. The CFR was lowest amongst neonates with S. aureus, and CoNS bacteraemia. The CFR was highest for *E. coli* and *Citrobacte*r infections

Table 6.15 Outcome among culture-positive Cases according to infecting pathogen

Organism	Discharged (n=62)	Died during admission (n=29)
Clinically significant		
Gram positive		
Staphylococcus aureus	77% (10)	23% (3)
Enterococcus species	33% (1)	67% (2)
Streptococcus species	50% (1)	50% (1)
Gram negative		
Burkholderia cepacia	44% (4)	56% (5)
Klebsiella species	67% (6)	33% (3)
Acinetobacter baumanii	50% (1)	50% (1)
Escherichia coli	0% (0)	100% (2) [a]
Achromobacter xylosoxidans	100% (1)	0% (0)
Citrobacter species	0% (0)	100% (1)
Pseudomonas species	43% (3)	57% (4)
Salmonella species	100% (1)	0% (0)
Pantoea species	100% (1)	0% (0)
Clinically non-significant		
Coagulase-negative *staphylococci*	88% (28)	12% (4)
Viridans *streptococci*	50% (1)	50% (1)
Bacillus species	100% (4)	0% (0)
Micrococcus species	0% (0)	100% (2)

[a] One baby had mixed infection with *Enterobacter*

Follow up and outcome on Day 27

Among the 144 Cases discharged from care, 15 were >27 days of age at discharge and 129 were aged <27 days. Those neonates <27 days of age were followed up by telephone on Day 27 (end of the neonatal period) to ascertain their outcome. Figure 6.11 summarises the Day 27 outcome for these Cases stratified by blood culture result.

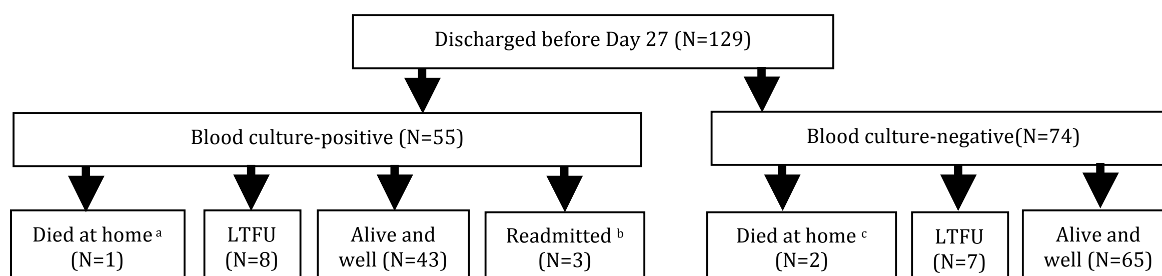


Figure 6.11 Day 27 outcome among cases discharged from care

LTFU= lost to follow-up
[a] Case of Klebsiella pneumoniae bloodstream infection with severe abdominal distension. Died at home on day of discharge
[b] Two (2) re-admitted at EFSTH due to abdominal distension/Hirschprungs disease. The place and cause for admission of the third case could not be determined. Re-admission outcomes not ascertained
[c] One (1) case is said to have had extensive skin infection. The cause of death of the second case could not be ascertained

Neonatal nasopharyngeal colonisation: prevalence and aetiologies

The distribution of nasopharyngeal colonisation among Cases stratified by age is shown in Table 6.16. Colonisation by most organisms peaked at postnatal age 4 – 6 days.

Table 6.16 Distribution of nasopharyngeal colonisation among Cases stratified by postnatal age

			Prevalence Within Different Age Groups		
	Number [a]	Overall Prevalence	Aged ≤3 days	Aged 4-6 days	Aged ≥7 days
Any positive culture	145	71% (145/203)	65% (75/115)	90% (26/29)	75% (44/59)
Staphylococcus aureus	89	44% (89/203)	34% (39/115)	69% (20/29)	51% (30/59)
Klebsiella species	30	15% (30/203)	10% (12/115)	24% (7/29)	19% (11/59)
Enterococcus species	22	11% (22/203)	12% (14/115)	10% (3/29)	8% (5/59)
Escherichia coli	18	9% (18/203)	8% (9/115)	10% (3/29)	10% (6/59)
Enterobacter species	8	4% (8/203)	4% (5/115)	7% (2/29)	2% (1/59)
Group B Streptococcus	5	2% (5/203)	3% (4/115)	0% (0/29)	2% (1/59)
Pseudomonas species	5	2% (5/203)	3% (4/115)	0% (0/29)	2% (1/59)
Streptococcus pneumoniae	4	2% (4/203)	0% (0/115)	3% (1/29)	5% (3/59)
Others [a]	31	15% (31/203)	15% (17/115)	15% (4/29)	17% (10/59)

[b] Others: *Acinetobacter species* (3), *Coagulase-negative staphylococci* (10), *Unidentified coliforms* (10), *Haemophilus species* (1), *Streptococcus species* (3), *Staphylococcus* species (4)

Neonatal rectal colonisation: prevalence and aetiologies

The distribution of rectal colonisation among Cases stratified by age is shown in Table 6.17. Colonisation by *E. coli* was most the most predominant, and increased with increasing postnatal age; over 90% of Cases admitted at 7 days of age or older were colonised.

Table 6.17 Distribution of rectal colonisation among cases stratified by postnatal age

			Prevalence Within Different Age Groups		
	Number [a]	Overall Prevalence	Aged ≤3 days	Aged 4-6 days	Aged ≥7 days
Any positive culture	153	94% (145/153)	90% (78/87)	100% (22/22)	100% (44/44)
Enterobacter species	20	13% (20/153)	9% (8/87)	14% (3/22)	20% (9/44)
Escherichia coli	113	74% (113/153)	62% (54/87)	86% (19/22)	91% (40/44)
Klebsiella species	85	56% (85/153)	47% (41/87)	68% (15/22)	66% (29/44)
Enterococcus species	76	50% (76/153)	51% (44/87)	41% (9/22)	52% (23/44)
Staphylococcus aureus	23	15% (23/153)	17% (15/87)	14% (3/22)	11% (5/44)
Group B Streptococcus	3	2% (3/153)	2% (2/87)	0% (0/22)	2% (1/44)
Unidentified coliforms	5	3% (5/153)	3% (3/87)	5% (1/22)	2% (1/44)
Others	7	5% (7/153)	5% (4/87)	9% (2/22)	2% (1/44)

[a] Rectal swabs only collected during the main study

[a] Others: *Proteus species* (3), *Coagulase-negative staphylococci* (2), *Streptococcus species* (1), *Citrobacter* species (1)

Maternal recto-vaginal colonisation and neonatal infection

Recto-vaginal swabs were collected from all Case-mothers except one who withheld consent for the procedure. Ninety-seven percent (195/202) of mothers were colonised with at least one organism, and 87% (176/202) colonised with at least one of the main organisms of interest in this study - GBS, *E. coli*, *S. aureus* or *Klebsiella* species.

Concordance between organisms recovered from blood and vaginal cultures occurred in 27% (14/51) of mother-newborn pairs suggesting possible mother-to-newborn transmission of organisms. Because confirmation of genetic relatedness of isolates can only be established by molecular methods such as whole genome sequencing, matching of concordant neonatal and maternal isolates is considered "provisional". The two Cases

with *E. coli* infection and 54% (7/13) of Cases with *S. aureus* infection were born to mothers colonised by the same pathogen. Table 6.18 presents provisional matching of clinically significant isolates from neonatal blood cultures and maternal rectovaginal swabs stratified by the age of onset of neonatal infection. A quarter of neonates (8/32) with early-onset infection, and 30% (6/20) of those with late-onset infection were born to mothers rectovaginally colonised with the same pathogen. Based on provisional microbiological matching of isolates, more than 60% of early-onset *S. aureus* bacteraemia Cases might have been of maternal origin.

Table 6.18 Provisional matching of concordant clinically significant bacterial isolates from neonatal blood culture and maternal rectovaginal swabs stratified by age of onset of neonatal infection

		Newborn with clinically significant isolate (N=51)			
		Early-onset infection [≤ 3 days] (N=32)		Late-onset infection [4 – 27 days] (N=20)	
Isolate	**(N)**	Mother colonised with same pathogen (N)	Mother not colonised with same pathogen (N)	Mother colonised with same pathogen (N)	Mother not colonised with same pathogen (N)
Staphylococcus aureus	(13)	63% (5)	37% (3)	40% (2)	60% (3)
Klebsiella species	(9)	33% (2)	67% (4)	33% (1)	67% (2)
Escherichia coli	(2)	(0)	(0)	100% (2)	(0)
Enterococcus species	(3)	33% (1)	67% (2)	(0)	(0)
Acinetobacter baumanii	(2)	(0)	100% (1)	100% (1)	(0)
Streptococcus species	(2)	(0)	100% (2)	(0)	(0)
Enterobacter species	(1)	(0)	(0)	(0)	100% (1)
Pseudomonas species	(7)	(0)	100% (4)	(0)	100% (3)
Salmonella species	(1)	(0)	(0)	(0)	100% (1)
Citrobacter species	(1)	(0)	(0)	(0)	100% (1)
Burkholderia cepacia	(9)	(0)	100% (7)	(0)	100% (2)
Achromobacter xylosoxidans	(1)	(0)	(0)	(0)	100% (1)
Pantoea species	(1)	(0)	100% (1)	(0)	(0)
Total	**(52)**	**25% (8)**	**75% (24)**	**30% (6)**	**70% (14)**

Maternal recto-vaginal colonisation and neonatal nasopharyngeal colonisation

Seventy percent of Cases were nasopharyngeally colonised, and predominantly with *S. aureus* [44% (89/203)] followed by *Klebsiella* [15% (30/203)]. Table 6.19 presents provisional matching of isolates from neonatal nasopharyngeal and maternal

rectovaginal swabs, suggesting possible mother-to-newborn transmission. The prevalence of neonatal nasopharyngeal colonisation among cases whose mothers were similarly colonised with the same organism was highest for *S. aureus* [54% (27/50)].

Table 6.19 Provisional matching of concordant bacterial isolates from neonatal nasopharyngeal and maternal rectovaginal swabs

Organism	(N)	Newborn with nasopharyngeal colonisation	
		Mother colonised with same pathogen (N)	**Mother not colonised with same pathogen (N)**
Group B Streptococcus	(5)	60% (3)	40% (2)
Staphylococcus aureus	(89)	30% (27)	70% (62)
Klebsiella species	(29)	41% (12)	59% (17)
Escherichia coli	(18)	61% (11)	39% (7)
Enterococcus species	(22)	55% (12)	45% (10)
Pseudomonas species	(5)	0% (0)	100% (5)

Maternal and neonatal nasopharyngeal colonisation

Maternal nasopharyngeal swabs were only collected during the main study. Table 6.20 presents provisional matching of isolates from maternal and neonatal nasopharyngeal swabs, suggesting possible mother-to-newborn transmission.

Table 6.20 Provisional matching of concordant bacterial isolates from neonatal and maternal nasopharyngeal swabs

Organism	(N)	Newborn with nasopharyngeal colonisation	
		Mother colonised with same pathogen (N)	**Mother not colonised with same pathogen (N)**
Streptococcus pneumoniae	(2)	0% (0)	100% (2)
Staphylococcus aureus	(73)	48% (35)	52% (38)
Klebsiella species	(26)	4% (1)	96% (25)
Escherichia coli	(15)	13% (2)	87% (13)
Enterococcus species	(19)	37% (7)	66% (12)
Pseudomonas species	(5)	0% (0)	100% (5)
Enterobacter species	(3)	0% (0)	100% (3)

Maternal colonisation was similar to that of the Cases, with *S. aureus* predominating [39% (60/153)], and less of *S. pneumoniae* [5% (7/153)]. The prevalence of neonatal

colonisation among Cases whose mothers were colonised with the same organism was highest for *S. aureus* [58% (35/60)] and for *Enterococcus* [58% (7/12)].

Neonatal infection and rectal colonisation

Table 6.21 presents provisional matching of concordant isolates from neonatal blood cultures and rectal swabs, suggesting gut translocation as a route of infection among Cases with *E. coli* and *Enterococcus* bacteraemia.

Table 6.21 Provisional matching of concordant bacterial isolates from neonatal blood culture and rectal swabs

Organism	(N)	Newborn with clinically significant isolate	
		Newborn rectally colonised with same pathogen (N)	Newborn not rectally colonised with same pathogen (N)
Staphylococcus aureus	(12)	25% (4)	75% (8)
Klebsiella species	(5)	60% (3)	40% (2)
Escherichia coli	(2)	100% (2)	0% (0)
Enterococcus spp	(3)	67% (2)	33% (1)
Enterobacter species	(1)	0% (0)	100% (1)

Comparative analyses

Table 6.22 compares the maternal and neonatal characteristics between the 203 Cases and community-matched Controls. Among Cases, significantly higher odds of a maternal history of intrapartum fever [OR 6.50, 95% CI (2.26 – 25.63), *P=0.0001*]; maternal history of multiple vaginal examinations [OR 3.40, 95% CI (1.64 – 7.72), *P=0.0003*]; LBW [OR 2.25, 95% CI (1.10 – 4.89), *P=0.016*]; and resuscitation at delivery [OR 7.57, 95% CI (3.43 – 19.74), *P<0.0001*] were observed compared with Controls. Mothers of Cases were also older and educated compared with mothers of Controls.

Table 6.22 Maternal and neonatal characteristics among 203 Cases and 203 community-matched Controls

	Case	Control	OR (95% CI)	*P*[a]
Maternal				
Maternal age (n=398)				
> 25 years	112 (57%)	95 (47%)	1.50 (0.98 – 2.32)	0.051
≤ 25 years	84 (43%)	107 (53%)		
Educational level (n=406)				
Some education	145 (71%)	127 (63%)	1.51 (0.97 – 2.39)	0.055
No education	58 (29%)	76 (37%)		
Parity (n=406)				
Multiparous	141 (69%)	148 (73%)	0.84 (0.52 – 1.33)	0.431
Primiparous	62 (31%)	55 (27%)		
Tetanus toxoid during ANC (n=400)				
Yes	160 (79%)	165 (83%)	0.76 (0.45 – 1.27)	0.272
No	42 (21%)	33 (17%)		
Intrapartum fever (n=402)				
Yes	29 (14%)	6 (4%)	6.50 (2.26 – 25.63)	0.0001
No	172 (86%)	195 (97%)		
Number of vaginal exams (n=379)				
> 3	38 (21%)	15 (8%)	3.40 (1.64 – 7.72)	0.0003
≤ 3	147 (79%)	179 (92%)		
Neonatal				
Sex (n=406)				
Female	80 (39%)	99 (49%)	0.69 (0.46 – 1.04)	0.064
Male	123 (61%)	104 (51%)		
Gestational age at birth (n=386)				
Preterm < 37 weeks	50 (26%)	40 (21%)	1.34 (0.81 – 2.25)	0.225
Full term ≥ 37 weeks	143 (74%)	153 (79%)		
Birth-weight (n=388)				
Low birth weight <2500g	33 (18%)	22 (11%)	2.25 (1.10 – 4.89)	0.016
Birth weight ≥2500g	155 (82%)	178 (89%)		
Postnatal age at recruitment (n=406)				
0 – 3 days	115 (57%)	101 (50%)	1.74 (0.96 – 3.23)	0.052
4 – 27 days	88 (43%)	102 (50%)		
Place of delivery (n=406)				
Home/TBA born	19 (9%)	10 (5%)	2.00 (0.85 – 5.05)	0.083
Facility-born	184 (91%)	193 (95%)		
Mode of delivery (n=406)				
Caesarean section	25 (12%)	17 (8%)	1.62 (0.77 – 3.51)	0.170
Vaginal delivery [b]	178 (88%)	186 (92%)		
Resuscitation at delivery				
Yes	58 (32%)	15 (8%)	7.57 (3.43 – 19.74)	<0.0001
No	124 (68%)	185 (92%)		

[a] McNemar chi-squared test for paired data

Maternal rectovaginal colonisation among Cases and Controls

The odds of being a Case were significantly higher among neonates whose mothers had a positive rectovaginal culture [OR 5.20, 95% CI (1.96 – 17.34), *P=0.0002*], and if the mother was colonised with at either GBS, *E. coli*, *S. aureus* or *Klebsiella* species [OR 2.11, 95% CI (1.19 – 3.85), *P=0.006*] (Table 6.23). However, when stratified by individual

organisms, only maternal colonisation with *E. coli* was associated with significantly increased odds of being a Case [OR 1.79, 95% CI (1.16 – 2.82), *P=0.006*].

Table 6.23 Comparison of maternal rectovaginal colonisation between Cases and matched community Controls

	Case	Control	OR (95% CI)	P[a]
Positive rectovaginal culture (n=405)				
Yes	195 (97%)	175 (86%)	5.20 (1.96 – 17.34)	0.0002
No	7 (3%)	28 (14%)		
GBS-positive rectovaginal culture (n=405)				
Yes	23 (11%)	16 (8%)	1.58 (0.73 – 3.58)	0.209
No	179 (89%)	187 (92%)		
E. coli-positive rectovaginal culture (n=405)				
Yes	138 (68%)	111 (55%)	1.79 (1.16 – 2.82)	0.006
No	64 (32%)	92 (45%)		
S. aureus -positive rectovaginal culture (n=405)				
Yes	50 (25%)	44 (22%)	1.21 (0.71 – 2.08)	0.446
No	152 (75%)	159 (78%)		
Klebsiella-positive rectovaginal culture (n=405)				
Yes	64 (32%)	54 (27%)	1.26 (0.81 – 1.99)	0.281
No	138 (68%)	149 (73%)		
GESK-positive rectovaginal culture (n=405)				
Yes	176 (87%)	155 (76%)	2.11 (1.19 – 3.85)	0.006
No	26 (13%)	48 (24%)		

[a] McNemar chi-squared test

6.3.3 Molecular assay (PCR) results using the TaqMan Array Cards

The multipathogen TaqMan Array Cards (TAC) were used to explore the applicability of this technology for pathogen detection using Pilot study samples. The blood and respiratory cards were insufficient to run all Pilot study samples (n=100) and were therefore used for the Cases (n=50) only, as the main purpose was to compare the yield from conventional microbiology cultures with this molecular tool.

Comparison of TAC PCR and blood culture results among Pilot study Cases

One neonate did not have a blood culture sample taken but had a PCR sample which was negative. PCR samples for seven Cases (six of whom had positive blood cultures and one

with a negative blood culture result) could not be tested due to insufficient TACs. The yield from blood culture and PCR for the remaining 42 Cases who had results for both investigations is compared in Table 6.24 below. PCR was positive among 17% (3/18) of Cases with a positive blood culture and 17% (4/24) of Cases with a negative blood culture.

Table 6.24 Comparison of BACTEC blood culture and TAC PCR for detection of pathogenic bacteria in blood samples from 42 Pilot study Cases

	Blood Culture [b]		**Total**
PCR [a]	**Culture positive**	**Culture negative**	
PCR positive	3	4	7
PCR negative	15	20	35
Total	**18**	**24**	**42**

The pathogens identified by each method are listed in Table 6.25 (Cases with positive results for both methods presented in bold green font, and those with only positive PCR results presented in bold red font). The most frequently identified pathogen by PCR was *S. aureus*. None of the three Cases with positive results for both methods yielded the same pathogen. PCR identified GBS in two Cases both of whom had multiple organisms. The blood culture of one of the Cases with a GBS-positive PCR result was negative while the blood culture of the other Case was positive for CoNS. PCR did not detect any viruses from the blood samples.

Table 6.25 Comparison of culture and PCR results among 22 Pilot study Cases with positive blood cultures and/or positive TAC blood PCR

Patient	Blood PCR result	Blood culture result	Comments
P0001	***E. coli***	***K. pneumoniae***	Maternal RVS culture yielded *K. pneumoniae* (GBS)
P0005	Negative	*S. aureus*	
P0007	***S. aureus***	**No growth**	
P0009	Negative	CoNS	Infant received antibiotics before admission
P0013	Negative	*Pseudomonas*	
P0035	Negative	CoNS	
P0041	Negative	*P. luteola*	
P0043	Negative	*P. luteola*	
P0049	***S. aureus***	***P. Luteola***	
P0053	Negative	*K. oxytoca*	
P0063	Negative	CoNS	
P0065	Negative	*K. pneumoniae*	
P0067	Negative	*K. pneumoniae*	
P0069	***S. aureus***	**No growth**	
P0071	Negative	CoNS	Infant received antibiotics before admission
P0079	Negative	*B. cepacia*	
P0083	Negative	CoNS	
P0085	Negative	CoNS	
P0089	***S. aureus***	**No growth**	
P0091	**GBS** ***S. aureus***	**CoNS**	Maternal RVS culture also yielded GBS. No maternal or infant antibiotic exposure. Infant discharged after 7 days on admission
P0095	Negative	CoNS	
P0099	**GBS** ***E. coli S. aureus***	**No growth**	Maternal RVS culture yielded *E. coli* and *Streptococcus* species. No maternal or infant antibiotic exposure. Infant died in hospital after 15 days on admission.

Cases with positive results for both methods presented in bold green font,

Cases with only positive PCR results presented in bold red font

Comparison of PCR and NPS culture results among Pilot Study Cases

For three Cases (all with positive NPS cultures), the corresponding respiratory PCR samples could not be tested due to insufficient TACs. The yield from PCR and NPS culture for the 47 Cases who had results for both tests is compared in Table 6.26.

Table 6.26 Comparison of conventional culture and TAC PCR for detection of pathogenic bacteria in NPS samples from 47 Pilot study Cases

	NPS Culture [b]		**Total**
PCR [a]	**Culture positive**	**Culture negative**	
PCR positive	21	3	24
PCR negative	13	10	23
Total	**34**	**13**	**47**

PCR was positive among 62% (21/34) of Cases with a positive NPS culture and 23% (3/13) of Cases with a negative NPS culture. The pathogens identified by each method are listed in Table 6.27 (Cases with positive results for both methods presented in bold green font, and those with only positive PCR results presented in bold red font). Four (19%) of the 21 Cases with positive results for both methods yielded the same pathogen; *K. pneumoniae* (P0015 and P0045), *S. pneumoniae* (P0023), and GBS (P0065 (this neonate's mother's RVS culture was also positive for GBS)).

Table 6.27 Comparison of culture and PCR results among 38 Pilot study Cases with positive NPS cultures and/or positive TAC NPS PCR

Patient	NPS PCR result	NPS culture result	Comments
P0001	Negative	*K. pneumoniae* *S. aureus*	Infant blood culture and maternal RVS both yielded *K. pneumoniae*
P0003	***S. pneumoniae*** ***Ureaplasma* spp**	**GDS**	
P0005	***S. pneumoniae*** **Rhinovirus**	***S. aureus***	Infant blood culture positive for *S. aureus*
P0007	***S. pneumoniae***	***S. aureus*** **GDS**	Maternal RVS culture positive for *S. aureus*
P0009	***S. pneumoniae*** ***K. pneumoniae***	***A. baumanii***	
P0011	**GBS** **Enterovirus** **Rhinovirus**	**CoNS**	Infant had a clinical diagnosis of pneumonia Blood culture was negative
P0013	Negative	*S. pneumoniae*	
P0015	***K. pneumoniae***	***K. pneumoniae*** **GBS**	
P0017	***S. pneumoniae*** **RSV**	***H. parainfluenzae***	
P0019	Negative	*K. pneumoniae* *E. cloacae*	
P0021	***S. pneumoniae*** ***K. pneumoniae***	***S. aureus***	
P0023	***S. pneumoniae*** **Enterovirus** **Rhinovirus**	***S. pneumoniae*** ***S. aureus***	Maternal RVS culture positive for *S. aureus*
P0025	Negative	*E. cloacae*	
P0027	Negative	CoNS	
P0035	***K. pneumoniae*** **Parainfluenza virus**	***Acinetobacter spp***	
P0037	**K. pneumoniae** **Ureaplasma spp**	**CoNS**	
P0039	Negative	*S. aureus*	
P0041	***S. pneumoniae*** ***K. pneumoniae***	***S. aureus*** ***E. coli***	Maternal RVS culture positive for *S. aureus*
P0043	***S. pneumoniae*** ***K. pneumoniae*** **Rhinovirus** **RSV**	***S. aureus***	Maternal RVS culture positive for *S. aureus*

Table 6.27 (continued) Comparison of culture and PCR results among 38 Pilot study Cases with positive NPS cultures and/or positive TAC NPS PCR

Patient	NPS PCR	NPS culture	Comments
P0045	***K. pneumoniae***	***K. pneumoniae*** ***E. cloacae***	
P0049	Negative	*S. aureus*	
P0051	***S. pneumoniae*** ***K. pneumoniae*** **Rhinovirus**	**Negative**	
P0055	**GBS** ***B. pertussis*** **Adenovirus** **Human Parechovirus** **Rhinovirus**	**Negative**	
P0057	**Chlamydia** ***S. pneumoniae*** **Ureaplasma spp**	***S. aureus*** **GCS**	
P0061	Negative	*S. aureus*	
P0063	***S. pneumoniae***	***S. aureus*** ***E. cloacae***	
P0065	**GBS**	**GBS** ***S. aureus***	Infant blood PCR positive for *S. aureus*
P0069	**GBS** ***K. pneumoniae***	**Negative**	Maternal RVS culture positive for *K. pneumoniae*
P0071	**Adenovirus**	**Negative**	
P0073	***K. pneumoniae*** **Ureaplasma spp**	**CoNS**	
P0075	Negative	*S. aureus*	
P0077	**GBS**	**CoNS**	Maternal RVS culture positive for GBS
P0079	Negative	*S. aureus*	
P0085	Negative	E. coli	Maternal RVS culture positive for *E. coli*
P0087	**GBS**	***P. aeruginosa***	Maternal RVS culture positive for GBS
P0089	Negative	CoNS	
P0091	Negative	GBS	Infant blood PCR positive for GBS Maternal RVS culture positive for GBS
P0093	***K. pneumoniae***	**CoNS**	Maternal RVS culture positive for *K. pneumoniae*

Cases with positive and concordant results for both methods presented in bold green font.
Cases with positive but discordant results for both methods presented in bold blue font.
Cases with only positive PCR results presented in bold red font.

6.3.4 Nosocomial infection Outbreaks

During the study, two outbreaks occurred at the EFSTH neonatal ward - first with *Burkholderia cepacia,* followed by K. *pneumoniae*. We proceeded to investigate these outbreaks in more detail.

Burkholderia cepacia

The first case of *B. cepacia* bacteraemia was reported during the pilot study on 1st July 2015. After the pilot study ended in August 2015, blood cultures were not performed until the initiation of the Main study in February 2016 following which five Cases were reported between March and May. The number of these isolates increased following commencement of routine blood cultures at the EFSTH Paediatrics laboratory from early June 2016 for neonates with severe clinical sepsis not enrolled in my PhD study. This laboratory is supported by the MRCG and all isolates are sent to the MRCG for confirmation. The total numbers of blood samples sent to the Paediatrics laboratory in June, July and August were 43, 30, and 38 and the proportion of *B. cepacia* isolated from these samples were 35%, 27%, and 58%, respectively. However, case fatalities in these three months were no different from those observed during the same months in the previous three years. There were 49 episodes of *B. cepacia* bacteraemia from 47 neonates admitted on the EFSTH neonatal ward from March to August 2016 (Figure 6.12) shows the monthly distribution of cases over the course of the outbreak. The characteristics and outcomes of *B. Cepacia* Cases are detailed in Table 6.28.

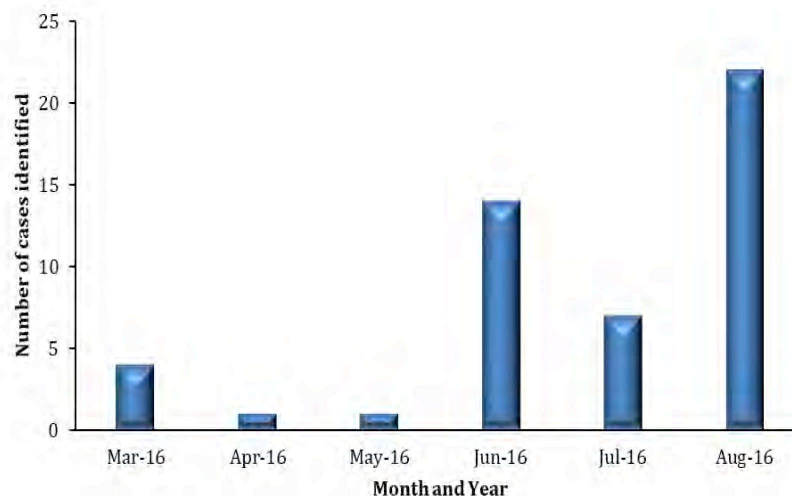


Figure 6.12 Monthly distribution of *Burkholderia cepacia* cases during an outbreak at the neonatal unit, EFSTH between March and August 2016

Table 6.28 Characteristics and outcome of neonates with *Burkholderia cepacia* bloodstream infection during an outbreak at the neonatal ward, EFSTH, March – August 2016

ID	Category	Date of birth	Date of admission	Date of culture	Age at culture	Sex	Place of delivery	Outcome
Case B 1	Study participant	20-Feb-16	07-Mar-16	08-Mar-16	17	M	ASB German Clinic	Discharged
Case B 2	Study participant	07-Mar-16	08-Mar-16	09-Mar-16	2	M	Kanifing General Hospital	Died
Case B 3	Study participant	09-Mar-16	09-Mar-16	10-Mar-16	1	F	Kanifing General Hospital	Died
Case B 4	Study participant	12-Apr-16	14-Apr-16	15-Apr-16	3	F	Westfield Clinic	Discharged
Case B 5	Study participant	14-May-16	16-May-16	16-May-16	2	F	Serekunda Health Centre	Died
Case B 6	Study participant	02-Jun-16	06-Jun-16	07-Jun-16	5	F	Sukuta Health Centre	Discharged
Case B 7	Other inpatient	14-Jun-16	NA	16-Jun-16	2	F	NA	Discharged
Case B 8	Other inpatient	12-Jun-16	14-Jun-16	16-Jun-16	4	F	Sukuta Health Centre	Died
Case B 9	Other inpatient	09-Jun-16	09-Jun-16	16-Jun-16	5	M	EFSTH	Discharged
Case B 10	Other inpatient	05-Jun-16	NA	16-Jun-16	11	M	EFSTH	Discharged
Case B 11	Other inpatient	17-Jun-16	17-Jun-16	20-Jun-16	3	M	EFSTH	Discharged
Case B 12	Other inpatient	12-Jun-16	14-Jun-16	20-Jun-16	8	F	Gunjur Health Centre	Died
Case B 13	Study participant	20-Jun-16	21-Jun-16	22-Jun-16	2	M	Sukuta Health Centre	Died
Case B 14	Other inpatient	22-Jun-16	22-Jun-16	23-Jun-16	1	M	NA	NA
Case B 15	Other inpatient	16-Jun-16	NA	23-Jun-16	7	M	NA	NA
Case B 16	Other inpatient	16-Jun-16	NA	24-Jun-16	8	F	NA	NA
Case B 17	Other inpatient	25-Jun-16	NA	29-Jun-16	4	F	NA	NA
Case B 18	Other inpatient	26-Jun-16	27-Jun-16	30-Jun-16	4	M	Bundung MCH Hospital	Discharged
Case B 19	Other inpatient	28-Jun-16	NA	30-Jun-16	2	M	NA	NA
Case B 20	Study participant	04-Jul-16	04-Jul-16	09-Jul-16	1	F	Sukuta Health Centre	Died
Case B 21	Other inpatient	29-Jun-16	30-Jun-16	11-Jul-16	3	M	Serekunda Health Centre	Discharged
Case B 22	Other inpatient	08-Jul-16	10-Jul-16	13-Jul-16	3	M	Essau Health Centre	Discharged
Case B 23	Other inpatient	12-Jul-16	13-Jul-16	13-Jul-16	1	M	Fajikunda Health Centre	Died
Case B 24	Other inpatient	25-Jun-16	NA	13-Jul-16	19	M	NA	NA
Case B 25	Other inpatient	13-Jul-16	13-Jul-16	14-Jul-16	1	F	MRC Keneba	Died
Case B 26	Other inpatient	23-Jul-16	25-Jul-16	27-Jul-16	4	F	Fajikunda Health Centre	Died
Case B 27	Other inpatient	24-Jul-16	24-Jul-16	27-Jul-16	3	M	Brikama Health Centre	Died
Case B 28	Other inpatient	27-Jul-16	28-Jul-16	02-Aug-16	6	M	Kanifing General Hospital	Died
Case B 29	Other inpatient	28-Jul-16	28-Jul-16	03-Aug-16	6	F	Kanifing General Hospital	Discharged
Case B 30[a]	Other inpatient	01-Aug-16	01-Aug-16	03-Aug-16	2	F	Bansang General Hospital	Died
Case B 31	Other inpatient	29-Jul-16	07-Mar-16	03-Aug-16	5	F		Discharged
Case B 32[b]	Other inpatient	01-Aug-16	02-Aug-16	03-Aug-16	2	F	NA	
				08-Aug-16	9	F		
				23-Aug-16	22	F		Died
Case B 33[c]	Other inpatient	03-Aug-16	03-Aug-16	04-Aug-16	1	M	Kanifing General Hospital	Died
Case B 34	Other inpatient	05-Aug-16	05-Aug-16	08-Aug-16	3	F	Kanifing General Hospital	Discharged
Case B 35	Other inpatient	03-Aug-16	04-Aug-16	08-Aug-16	5	F	Brikama Health Centre	NA
Case B 36	Other inpatient	03-Aug-16	NA	09-Aug-16	6	M	NA	NA
Case B 37	Other inpatient	25-Jul-16	NA	09-Aug-16	15	M	NA	NA
Case B 38[d]	Study participant	08-Aug-16	08-Aug-16	09-Aug-16	1	F	Kanifing General Hospital	Discharged
Case B 39	Other inpatient	03-Aug-16	NA	11-Aug-16	8	F	NA	Discharged
Case B 40	Other inpatient	05-Aug-16	05-Aug-16	12-Aug-16	7	F	Bundung MCH Hospital	Discharged
Case B 41	Other inpatient	06-Aug-16	06-Aug-16	12-Aug-16	6	M	Home	Died
Case B 42	Other inpatient	14-Aug-16	16-Aug-16	16-Aug-16	2	M	NA	Discharged
Case B 43	Other inpatient	09-Aug-16	NA	22-Aug-16	13	F	NA	NA
Case B 44[e]	Study participant	10-Aug-16	18-Aug-16	22-Aug-16	12	M	Brikama Health Centre	Died
Case B 45	Other inpatient	20-Aug-16	NA	22-Aug-16	2	F	NA	NA
Case B 46	Other inpatient	17-Aug-16	NA	23-Aug-16	6	M	NA	Died
Case B 47[f]	Other inpatient	15-Aug-16	NA	29-Aug-16	12	F	NA	Discharged

[a] This baby had mixed infection with *Acinetobacter baumanii*.
[b] This baby had 3 consecutively positive blood cultures with *Burkholderia cepacia*
[c] This baby had mixed infection with *Acinetobacter baumanii*.
[d] This study participant had a previous (admission) blood culture positive for *Acinetobacter baumanii*
[e] This study participant had a previous (admission) negative blood culture
[f] This baby had mixed infection with *Staphylococcus aureus*.

Outcome data from some of the non-study participants were not available to me. The CFR was 50% (18/36) for the 36 *B. Cepacia* cases with available outcome data. One of the Cases (Case B32) had three separate positive blood cultures and succumbed to infection.

All *B. cepacia* isolates were sensitive to co-trimoxazole, gentamicin, ciprofloxacin, ceftazidime, and chloramphenicol, but demonstrated resistance to tetracycline and amoxicillin-clavulanate.

Outbreak investigation

An outbreak investigation team comprising staff from the EFSTH and MRCG was set up on 1st September 2016 to detect the potential reservoir(s) of *B. cepacia* infection and the modes of transmission of the infection to the neonates in the neonatal ward. The team, led by an epidemiologist, consisted of the neonatologist, three paediatricians (including myself), two microbiologists, a molecular biologist and the head of the hospital Infection Control Unit. Fortnightly meetings were held where the members of the team discussed the progress of the investigation and took decisions on implementation of interventions.

An epidemiologic investigation was carried out and 90 environmental samples were collected from the neonatal ward between 5th – 8th September 2016. Table 6.29 lists all the different samples collected and the yield of *B. cepacia*. All clinical and environmental *Burkholderia* were sent to Imperial College London, for species level identification by whole genome sequencing, the results of which are not yet available.

Table 6.29 EFSTH neonatal ward samples and isolation of *Burkholderia cepacia*

Samples (number of sources/samples collected)	Type of sample	Date of collection	Culture result
IV fluid – 10% Dextrose with/without NaCl and KCl [In use]	Fluid	05/09/2016	*B. cepacia* isolated
Injection - Gentamicin - diluted with Normal saline [In use]	Fluid	05/09/2016	*B. cepacia* isolated
IV fluid – 10% Dextrose with/without NaCl and KCl [In use]	Fluid	08/09/2016	*B. cepacia* isolated
IV fluid – 10% Dextrose with/without NaCl and KCl [In use]	Fluid	08/09/2016	*B. cepacia* isolated
Injection - Metronidazole [In use]	Fluid	08/09/2016	*B. cepacia* isolated
IV fluid – Normal saline [In use]	Fluid	08/09/2016	*B. cepacia* isolated
Water from O_2 humidifier (1)	Fluid	05/09/2016	Negative
Hand sanitizer (2)	Fluid	05/09/2016	Negative
Tap water from main ward (1)	Fluid	05/09/2016	Negative
Tap water from critical care ward (1)	Fluid	08/09/2016	Negative
Water from O_2 humidifier (2)	Fluid	08/09/2016	Negative
IV fluid – Ringer lactate [In use] (1)	Fluid	08/09/2016	Negative
IV fluid – 10% dextrose [Stock] (2)	Fluid	08/09/2016	Negative
IV fluid – Normal saline [In use] (1)	Fluid	08/09/2016	Negative
IV fluid – Norman saline [Stock] (1)	Fluid	08/09/2016	Negative
IV fluid – Ringer lactate [Stock] (2)	Fluid	08/09/2016	Negative
Injection - Gentamicin [In use] (1)	Fluid	08/09/2016	Negative
Injection - Ciprofloxacin [In use] (2)	Fluid	08/09/2016	Negative
Injection - Ceftriaxone [In use] (1)	Fluid	08/09/2016	Negative
Injection - Metronidazole [In use] (1)	Fluid	08/09/2016	Negative
Injection - Ceftazidime [In use] (1)	Fluid	08/09/2016	Negative
Injection - Gentamicin [Stock] (2)	Fluid	08/09/2016	Negative
Injection - Vitamin K (1)	Fluid	08/09/2016	Negative
Injection - 5 % glucose [Stock] (2)	Fluid	08/09/2016	Negative
Injection - Metronidazole [Stock] (1)	Fluid	08/09/2016	Negative
Water for injection (1)	Fluid	08/09/2016	Negative
Povidone iodine (1)	Fluid	08/09/2016	Negative
Clean magic [Cleaning fluid; concentrated & diluted] (1)	Fluid	08/09/2016	Negative
Feeding pot (2)	Swab	05/09/2016	Negative
Incubator surface (1)	Swab	05/09/2016	Negative
Kidney dish (2)	Swab	05/09/2016	Negative
Sink surface (2)	Swab	05/09/2016	Negative
Bed surface (4 beds)	Swab	05/09/2016	Negative
BACTEC Peds blood culture vials (1)	Swab	05/09/2016	Negative
BACTEC 9050 Instrument surface (1)	Swab	05/09/2016	Negative
Umbilicus (3 babies)	Swab	05/09/2016	Negative
Nasal prong [In use] (4)	Swab	05/09/2016	Negative
Stethoscope (2)	Swab	05/09/2016	Negative
Radiant heater (3)	Swab	05/09/2016	Negative
IV cannula (3)	Swab	05/09/2016	Negative
Suction tube (1)	Swab	05/09/2016	Negative
Thermometer (1)	Swab	05/09/2016	Negative
Weighing scale (1)	Swab	05/09/2016	Negative
Staff hands (2 staff)	Swab	05/09/2016	Negative

All neonates on admission are given a bed-bath 2-3 times a week (personal communication with ward Matron). This is meant to be carried out by the nursing staff however, due to shortage of nursing staff, it is done by the mothers who use foam sponges. During the outbreak investigation, these sponges were noticed to be lying about the ward (often soaking wet in plastic containers on the floor or window sill) and 10 samples of these sponges were randomly collected for culture. Although none grew *B. cepacia*, all 10 samples grew multiple pathogenic bacteria (Table 6.30)

Table 6.30 Pathogens isolated from randomly selected foam sponges used by mothers to bath babies admitted on the EFSTH neonatal ward during an investigation of a *Burkholderia cepacia* outbreak

Sample	Location	Date of collection	Culture result
Sponge 1	Critical care ward	05/09/2016	*Acinetobacter baumanii, Escherichia coli, Rhizobium radiobacter*
Sponge 2	Critical care ward	05/09/2016	*Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii*
Sponge 3	Critical care ward	05/09/2016	*Acinetobacter baumanii, Vibrio metselinlavii, Chryseobacterium intogenes*
Sponge 4	Critical care ward	05/09/2016	*Klebsiella pneumoniae, Acinetobacter baumanii*
Sponge 5	Stable ward	05/09/2016	*Acinetobacter baumanii, Chryseobacterium intogenes*
Sponge 6	Stable ward	05/09/2016	*Chryseobacterium intogenes, Klebsiella pneumoniae, CoNS*
Sponge 7	Stable ward	05/09/2016	*Pseudomonas luteola, Acinetobacter baumanii*
Sponge 8	Stable ward	05/09/2016	*Acinetobacter baumanii, CoNS*
Sponge 9	Sepsis ward	05/09/2016	*Klebsiella pneumoniae, Pseudomonas luteola*
Sponge 10	Sepsis ward	05/09/2016	*Acinetobacter haematolyticus, Escherichia coli,*

As part of the epidemiological investigation, clinical procedures and practices by the staff were observed in order to identify potential sources of infection. The infection control review revealed lapses in nursing procedures (Figure 6.13) and suboptimal adherence to hand hygiene. Practices observed included the use of a single bottle of normal saline to dissolve/dilute all antibiotics with repeated insertions of a single syringe into the infusion bottle; storage of injections and intravenous fluids in use on an open medicine trolley next to the sink; and failure to wash and dry hands properly after contact with a newborn.

A) Intravenous fluids mixing area with used and unused bottles of intravenous fluids and repeated-use syringes (Kidney dish)

B) The medication trolley during the adminsitration drug rounds.

C) Drug and consumables cupboard

D) Hand washing and sterilisation area. Containers underneath the sink contain water (red-capped can, blue bucket), and re-useable devices soaked in disinfectant solution (transparent and yellow buckets)

Figure 6.13 Potential sources of infection on the EFSTH neonatal ward – intravenous fluids, parenteral injections, drug storage and hand washing facilities

Infection control interventions

Several infection control measures were initiated in phases after detection of an increase in the number of cases, and before the outbreak was formally investigated (Table 6.31). Although no further Cases of *B. cepacia* were identified in September and

October, infection surveillance continued until the end of the year with environmental sampling every 2 months. One further Case was identified in November but with a different susceptibility profile from those isolated during the initial outbreak.

Table 6.31 Phased infection control measures instituted following outbreak of *Burkholderia cepacia* on the EFSTH neonatal ward

Infection control measures	Implementation date
Reduction in the course of antibiotics to 72 hours in cases classified as high risk of sepsis	March 2016
Established the changes in cannulas at 5 days or when signs of phlebitis are present.	March 2016
Implemented daily disinfection of incubators and cribs done by nurses and nurse attendances	April 2016
Established changing incubators and cribs at five days of admission	April 2016
Instruct mothers not to use a sponge when bathing the newborn and institute the use of two washcloths for babies' bath: one for the face and the second for rest of the body.	August 2016
Established storage of all drugs and IV fluids, both used and unused, in a refrigerator located in the neonatal unit.	September 2016
Stop diluting gentamicin with dextrose or normal saline. Water for injection to be used for dilution if needed. One-ml syringes to be used for small neonates.	September 2016
Water for injection to be used for re-constituting antibiotics	September 2016
Hand washing – fully implemented using WHO guidelines	September 2016
Use different syringes for mixing different fluids	September 2016
Move the medicine tray away from the sink	September 2016
Use hand towels	September 2016
Educate mothers to dry towel in a proper place	September 2016
Health education – emphasize on infection control using health talks	September 2016
Visitor control improved	September 2016
Restricted access to mothers of critically ill babies during ward rounds.	October 2016

Multi-drug resistant extended-spectrum beta lactamase (ESBL) K. pneumoniae

On 26th October 2016, two months after the last case of B. cepacia, multi-drug resistant extended-spectrum beta lactamase (ESBL) *K. pneumoniae* was isolated from the blood of a six-day old neonate on admission at the EFSTH neonatal ward. Between October and December, 25 neonatal blood culture samples from non-study participants were

sent to the MRCG, 18 of which were positive for *K. pneumoniae* with the same antibiotic susceptibility profile indicating a common source. The characteristics and outcomes of the ESBL *K. pneumonia*-positive cases are summarised in Table 6.32 (study participants in bold red). Outcome data from the non-study participants were not available to me.

Table 6.32 Characteristics and outcome of neonates with *Klebsiella pneumoniae* infection during an outbreak at the neonatal ward, EFSTH, October – December 2016

ID	Category	Date of blood culture	Age(days)	Sex	Place of delivery	Outcome
Case K 1	Other inpatient	26-Oct-16	6	F	NA	NA
Case K 2	Other inpatient	07-Nov-16	2	M	NA	NA
Case K 3	Other inpatient	07-Nov-16	18	M	NA	NA
Case K 4	Other inpatient	07-Nov-16	7	M	NA	NA
Case K 5	**Study participant**	**08-Nov-16**	**5**	**F**	**EFSTH**	**Discharged**
Case K 6	Other inpatient	10-Nov-16	6	M	NA	NA
Case K 7	Other inpatient	10-Nov-16	39	M	NA	NA
Case K 8	Other inpatient	10-Nov-16	8	F	NA	NA
Case K 9	Other inpatient	12-Nov-16	4	M	NA	NA
Case K 10	**Study participant**	**15-Nov-16**	**1**	**F**	**EFSTH**	**Died**
Case K 11	Other inpatient	16-Nov-16	5	M	NA	NA
Case K 12	Other inpatient	16-Nov-16	15	F	NA	NA
Case K 13	Other inpatient	16-Nov-16	6	F	NA	NA
Case K 14	Other inpatient	25-Nov-16	3	M	NA	NA
Case K 15	Other inpatient	25-Nov-16	5	F	NA	NA
Case K 16	Other inpatient	28-Nov-16	14	F	NA	NA
Case K 17	Other inpatient	30-Nov-16	57	F	NA	NA
Case K 18	Other inpatient	30-Nov-16	1	M	NA	NA
Case K 19	Other inpatient	30-Nov-16	1	M	NA	NA
Case K 20	Other inpatient	30-Nov-16	6	NA	NA	NA
Case K 21	Other inpatient	30-Nov-16	27	M	NA	NA
Case K 22	Other inpatient	05-Dec-16	NA	M	NA	NA
Case K 23	Other inpatient	05-Dec-16	NA	M	NA	NA
Case K 24	Other inpatient	05-Dec-16	NA	M	NA	NA
Case K 25	Other inpatient	07-Dec-16	3	M	NA	NA
Case K 26	Other inpatient	07-Dec-16	9	M	NA	NA
Case K 27	Other inpatient	08-Dec-16	NA	M	NA	NA
Case K 28	**Study participant**	**19-Dec-16**	**2**	**F**	**Mbowen Clinic**	**Discharged**
Case K 29	**Study participant**	**19-Dec-16**	**2**	**M**	**EFSTH**	**Died**
Case K 30	**Study participant**	**19-Dec-16**	**1**	**M**	**Mbowen Clinic**	**Died**

Case-Control study participants presented in bold red
NA = information not available

Only study participants had rectal swabs; three of the study participants (Cases K5, K28 and K29) were also rectally colonised with *K. pneumoniae*. Eight samples of intravenous fluids and parenteral antibiotics were collected to determine the potential source, and three were positive with multi-drug resistant *K. pneumoniae.* All clinical and environmental *K. pneumoniae* were also sent to Imperial College London, for whole genome sequencing along with the *B. cepacia* isolates.

CHAPTER 7. DISCUSSION

A happy mother and her newborn - recovered from sepsis and ready for discharge

Overview of main findings

This PhD set out to describe the aetiology of neonatal infections in The Gambia and to evaluate the potential role of maternal bacterial colonisation in the acquisition of neonatal infections with a focus on inpatient care and sick neonates admitted with pSBI.

In Chapter 3, the systematic review of neonatal infection aetiology studies in sSA, showed *Klebsiella* species, *Escherichia coli*, *Staphylococcus aureus*, Group B *Streptococci*, and *Enterococcus* to be the top five reported bacterial pathogens across all regions. Application of the Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) checklist highlighted wide variation in clarity and completeness of reporting, impeding comparability and utility.

In Chapter 4, it was shown that despite significant improvements in child survival in The Gambia over a 25-year period between 1990 and 2016, neonatal mortality had remained stagnant. Over the same period, there had not been any formal study on the aetiology of neonatal infections. Pathogens associated with infection in Gambian newborns had only been described within the context of four published studies investigating infections in infants and children, and one unpublished laboratory surveillance study.

In chapter 5, The four-year audit of neonatal admissions and quality of care at The Gambia's largest referral hospital showed that possible serious bacterial infection (pSBI) accounted for 44% (2166/4944) of admissions. There was a striking mismatch of high antibiotic use (95%) and low microbiological investigation for infection was evident (1% of babies had a blood culture and 2% underwent a lumbar puncture).

In chapter 6, a hospital-based study was undertaken in three major urban/periurban health facilities in The Gambia to describe neonatal infection aetiology, and evaluate the role of maternal bacterial colonisation. This was a case-control study carried out over 17 months involving 203 sick newborn-mother case pairs admitted at three major urban/peri-urban health facilities in The Gambia, and 203 healthy newborn-mother control pairs recruited from the same communities in which the families of the cases were resident. Colonisation parameters were compared with healthy newborns and their mothers in the community There were 6 key findings from this case control study: (a) infection is a significant problem among hospitalised newborns in The Gambia with pathogenic bacteria isolated from 45% of 203 blood cultures; (b) results of blood cultures and speciation were available before discharge or death in only 38% of Cases; (c) *S. aureus* is the predominant clinically-significant organism associated with neonatal infection; (d) potential mother-to-newborn rectovaginal transmission of infection occurred in 14 Cases with positive blood cultures; (e) a high prevalence of neonatal infection associated with nosocomial outbreaks of *B. cepacia* and extended spectrum beta lactamase (ESBL) multi-drug resistant *K. pneumoniae* was documented in the main tertiary referral hospital; and (f) *Klebsiella* isolates demonstrated high resistance to third-generation cephalosporins.

The rest of this chapter presents an in-depth discussion of the findings from the case-control study in relation to the results and conclusions from earlier chapters.

7.1 Aetiology of infections

7.1.1 Conventional microbiology

Although 45% of blood cultures were positive, the overall prevalence of clinically-significant culture-positive infection was 25% (52/202). This is less than the pooled

prevalence of 30% from 73 previous studies identified in my systematic review of neonatal infection aetiology in sub-Saharan Africa (irrespective of culture method).[120, 125, 127-129, 131, 135-139, 144, 145, 148, 149, 151, 152, 154, 157-164, 173, 176, 177, 179, 180, 183, 187, 191, 195, 201, 207, 208, 213-219, 221-223, 225, 226, 229, 231-235, 238-248, 250-252, 256]

Blood cultures appeared to have limited clinical benefit on patient management as the median blood culture reporting time on the study was 6 days (IQR: 5 – 7), and only 38% (35/91) of cases with a positive blood culture received the result before discharge or death. Although still representing a significant delay, this is quicker than reported in a similar neonatal infection prevalence and aetiology study in Zambia where the median time was 7 days (IQR: 5 – 9), and only 25% of Cases had blood culture results available before discharge or death.[191] The routine practice in the MRCG clinical microbiology laboratory is to report both species and drug-susceptibility testing data together. Even when an 'early' preliminary result is provided for a positive culture, it is just the Gram-stain report without speciation and antibiotic susceptibility information, as these take an extra couple of days. In all cases, antibiotics were started empirically while waiting for the diagnostic information, and therefore such preliminary results are not of great additional value for clinical decision-making.

Prompt identification of microorganisms grown in blood cultures has the potential to enable earlier targeted clinical intervention for patients with sepsis. Matrix assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectrometry is a rapid method for identification of cultured bacterial isolates.[354] It is also able to correctly identify up to 85% of bacteria directly from positive blood culture broths, further reducing the waiting time.[355-357] Faster identification using MALDI-ToF has been shown to assist the clinician in assessing the significance of a blood culture isolate as early as

day one of positivity.[358] This can allow earlier appropriate choice of antimicrobial agent, even in the absence of susceptibility testing. Unfortunately, this technology is not available in most low- and middle-income settings and was not available to us in The Gambia. More important however, is the fact that neonatal blood cultures are not routinely done in any of the study sites and were only available because of this study. Even with the donation of an incubator, a BACTEC machine to the EFSTH Paediatrics department's laboratory and refresher microbiological training for the laboratory staff, blood cultures could not be continued due to the lack of the BACTEC Peds plus culture bottles.

Staphylococcus aureus

S. aureus was the predominant [25% (13/52)] clinically significant blood culture isolate accounting for an equal proportion (8/32) of early-onset infection Cases and 37% (5/14) of late-onset infection Cases. Our finding of *S. aureus* predominance is in keeping with an earlier study of infection aetiology among young Gambian infants in the early 1990s (conducted at the now EFSTH and the MRCG) where Gram-positive bacteria were the leading cause of bacteraemia among neonates, with *S. aureus* as the major isolate.[254] Similarly, retrospective laboratory surveillance data from the MRCG Clinical microbiology laboratory for the period 2005 – 2015 showed *S. aureus* as the predominant isolate from neonatal blood cultures (Darboe *et al*; unpublished data; Chapter 4, Table 4.2).

S. aureus is also very highly prevalent across sSA where it has been reported among the top five pathogens associated with neonatal infections in hospital-based studies, and is the predominant pathogen in West Africa (Chapter 3; Figure 3.4). The role of *S. aureus* as a true neonatal pathogen in sSA remains unclear, and its consistent identification as a

possible pathogen might be due to several reasons. First is because of horizontal transmission in facility-based deliveries and person-to person transmission in home deliveries resulting in colonization and infection.[359] Lack of appropriate hygiene during labour (including repeated intrapartum vaginal examinations), and failure to use fastidious aseptic technique during delivery and initial care of the baby can lead to infections with very early onset, as well as transmission of pathogens from one mother in labour to another.[21] Second is possible contamination at the time of sampling due to inadequate site sterilization resulting in the inoculation of bacteria into the blood culture bottles particularly where there is *S. aureus* skin carriage.[360] In this study, stringent aseptic measures were taken to avoid contamination during phlebotomy; however, eliminating the access of skin flora and/or environmental bacteria into blood specimens during phlebotomy and inoculation of blood culture bottles is not possible.[260] Lastly, contamination can occur in the laboratory while processing the specimens and culture plates.[360] In developed countries, *S. aureus* infection is more commonly associated with late-onset nosocomial sepsis and neonatal unit outbreaks, the main source of infection being the hands of health-care providers.[82, 360]

The true burden of disease in this setting is likely underestimated since most neonatal staphylococcal disease develops 1 – 3 weeks after discharge from hospital.[361] Cases in this study were not followed up beyond the end of the neonatal period (Day 27), but among the culture-negative Cases discharged before 27 days postnatal age, one died at home with a history of widespread severe skin infection suggesting *S. aureus* infection.

Klebsiella species

Klebsiella species (*K. pneumoniae* and *K. oxytoca*) were the second most commonly isolated clinically significant pathogen in this study. *Klebsiella* is the second leading

reported pathogen responsible for neonatal infections across sSA after *S. aureus* (Chapter 3; Figure 3.4). *Klebsiella* was not previously identified by Mulholland *et al*[348] as a cause of neonatal bacteriaemia in The Gambia; however, MRCG laboratory surveillance data reveal it to be the second most commonly isolated pathogen from neonatal blood cultures (Darboe *et al*; unpublished data; Chapter 4, Table 4.2). As mentioned previously, the observed change may represent a methodological change rather than an epidemiologic difference. During the Main study, an outbreak of *K. pneumoniae* occurred in one of the study sites and is discussed below. Again, this outbreak may have skewed the results leading to an overestimation of bacteraemia in this study.

Pseudomonas species* and *Burkholderia cepacia

Pseudomonas species and *Burkholderia* (previously *Pseudomonas*) *cepacia* together accounted for 31% (16/52) of clinically significant blood culture isolates in this study (Table 6.11), and were both among the leading causes of early-onset infection (Table 6.12). These Gram-negative, aerobic bacteria are commonly found in soil and moist environments and are capable of surviving and growing in nutrient-poor water.[362] Both pathogens are known to cause common-source outbreaks because they thrive in multi-use containers of medications, liquid soaps, antiseptics and disinfectants, as well as on inadequately reprocessed equipment or hospital tap water; the so-called "water bugs".[21, 82, 363, 364 365 366, 367] Although long recognised as a neonatal pathogen,[368] *Burkholderia* is mostly associated with respiratory infections in persons with cystic fibrosis.[369] Neither pathogen had previously been identified as a cause of neonatal sepsis in The Gambia,[348] but *Pseudomonas* was the third most commonly reported neonatal blood culture isolate after *S. aureus* and *Klebsiella* species in the MRCG laboratory surveillance data. Although the MRCG was one of the sites for the study by

Mulholland *et al*,[348] the observed change may represent a methodological change rather than an epidemiologic difference, and is to be interpreted with caution. The choice of blood culture bottle and system are among the important factors in the ability of blood cultures to detect significant organisms.[370, 371] The earlier study employed manual culture methods using tryptic soy broth (TSB) and brain-heart infusion containing sodium polyanethol sulfonate (Roche Diagnostica, Basel, Switzerland). Since 2004, MRCG has used the BACTEC automated blood culture system. Secondly, sick neonates are not routinely admitted at the MRC Clinical services department except as participants of ongoing studies and only where referral is not possible. The laboratory surveillance data is therefore not representative of neonates in the same catchment area in which the earlier study was carried out.

The isolation of *B. cepacia* in a Case during the Pilot study was the first time it had been reported in The Gambia, and its subsequent isolation in increased numbers in one of the study sites during the main study led to an outbreak investigation discussed below. The outbreak may have skewed the results in this study leading to an over-estimation of the prevalence of neonatal infection.

Other Gram-positive bacteria

One of the striking findings of our study was the paucity of clinically-significant Gram-positive organisms other than *S. aureus*. This is again in keeping with the report by Mulholland *et al*[348] where one Case each of *S. pneumoniae*, Group A *Streptococci* (GAS) and GBS were reported from 19 neonatal blood cultures compared with 9 Cases of *S. aureus* over a 25-month period. The MRCG clinical laboratory surveillance data similarly showed fewer cases of *S. pneumoniae* (4) and GBS (3) compared with *S. aureus* (33) over

an 11-year period. Interestingly, none of these organisms were isolated in this study but rather three *Enterococci* and two Streptococcus species.

GBS is a leading neonatal sepsis pathogen globally, and the dominant pathogen in developed countries.[271] In high neonatal and infant mortality regions like sSA, whether GBS is as important a cause of neonatal disease remains a major question.[264, 266] In the previously discussed review of neonatal infection aetiology in sSA, there appear to be regional variations in disease burden, with the highest burden reported from Southern Africa and the lowest burden reported from West Africa (Chapter 3; Figure 3.4 and Table 3.5). Intra-regional variations in disease burden is also apparent: almost 60% of reported GBS cases in Southern Africa were reported by 15 studies from South Africa,[130, 171, 172, 174, 175, 177, 179-182, 184, 185, 187-189] where the incidence of invasive GBS disease has been shown to again vary markedly at provincial level.[261] These findings from South Africa may shed some light on some of the reasons for the apparent wide variation in neonatal GBS disease in sSA: widespread empiric antibiotic treatment before referral, operational factors such as sampling techniques, blood culturing practices by attending physicians, variability in laboratory capacity, and suboptimal laboratory methods.[99, 112, 261] Other possible explanations include fundamental differences in disease epidemiology due to differences in strain virulence, or antibody levels, under identification of early-onset cases especially among home births due to challenges in accessing healthcare, or use of delivery practices promoting Gram-negative infections in some settings.[22, 261]

It is difficult to ascertain which of these reasons is at play in The Gambia. The EFSTH is the only public-sector hospital in The Gambia with blood and CSF culture facilities. Variability in laboratory capacity between the EFSTH and the MRCG is well

recognised.[99] The MRCG uses state-of-the-art automated blood culture methods in accredited laboratories; the EFSTH main hospital laboratory uses older manual methods and is plagued by stock-outs of laboratory supplies (personal communication with Head of Microbiology Laboratory) which has partly contributed to the poor practice of undertaking microbiological tests, including blood and CSF culture, in neonates with suspected sepsis. In a review of studies reporting neonatal GBS disease from developing countries, neonatal GBS incidence rates were consistently higher among studies using automated culture methods (0.80–3.06 per 1000 live births automated vs 0–0.39 per 1000 live births manual).[261] The greatest risk for the development of GBS neonatal sepsis is within the first 2 days after birth and under-ascertainment of early-onset GBS cases is possible if a significant proportion of the population gives birth outside of the hospital and access to healthcare is challenging, as most neonates will die before diagnosis.[261] Under-ascertainment of home births may play a less significant role in this setting as 75% - 93% of women in the study area deliver in a health facility[279] and less than 10% of neonates admitted to the EFSTH neonatal unit are born at home.[99] Maternal colonisation is the leading risk factor for both early and late onset GBS disease.[372] Newborns of mothers with colonisation or chorioamnionitis have been found to develop sepsis faster and are at higher risk of developing sepsis compared with non-exposed newborns.[373] Maternal chorioamnionitis was not examined in this study although data was collected on reported maternal intrapartum fever which showed that Cases with clinically significant infection were significantly more likely to have been born to mothers with reported intrapartum fever (Table 6.13). Recent data from Gambia show that GBS colonisation is present in 34% of women at delivery [253] which is similar to the reported pooled prevalence of 30.4% in South African women.[374] However, unlike South Africa where serotypes III and Ia are

the dominant serotypes among vaginal and neonatalcolonising isolates [375]rectovaginal and neonatal colonisation among Gambian women and their infants is dominated by serotype V.[253] Globally, type III is the most virulent and common invasive serotype in neonates <7 days of age).[375, 376] Although data is lacking on GBS serotypes causing invasive neonatal disease in The Gambia, the rarity of GBS disease in Gambian newborns may be due to lower rates of maternal carriage with the more virulent GBS serotypes.

Other Gram-negative bacteria

Cases of infection due to other Gram-negative bacteria - *E. coli, Acinetobacter, Salmonella, Citrobacter* and *Enterobacter* - collectively accounted for 14% of all clinically-significant isolates in this study. This is different from the report by Mulholland *et al*,[348] in which *Salmonella* was identified as the main Gram-negative pathogen associated with neonatal bacteraemia in The Gambia. In the 10 years covered by the MRCG clinical laboratory surveillance, there were only three cases of neonatal bacteraemia due to *E. coli*, two due to *Enterobacter*, and one due to *Acinetobacter* (Darboe *et al*; unpublished data; Chapter 4, Table 4.2). Again, the observed change may represent a measurement change rather than an epidemiologic difference, and is to be interpreted with caution. *Enterobacter* and *Acinetobacter* are not major pathogens reported in sSA, while *E. coli* is more commonly reported in Central African studies compared with other regions.

Unusual bacteria

Two unusual pathogens were isolated from cases in this study. The first was *Achromobacter xylosoxidans* (formerly known as *Alcaligenes xylosoxidans*). This non-fermentative non-enteric Gram-negative bacillus inhabits aquatic environments, including well water, intravenous fluids, and water in humidifiers, and is considered one

of the "water bugs".[364, 377, 378] *A. xylosoxidans* can be confused with other non-fermenting Gram-negative bacteria, especially Pseudomonas and correct identification of this organism is of clinical importance as *A. xylosoxidans* is usually multi-drug resistant and the source of infection needs to be pursued.[379] In this study, the identity of non-enteric bacteria was done using the manual API 20NE identification system (bioMérieux) which is known to consistently identify *A. xylosoxidans* correctly.[380] Septicaemia caused by this organism is rare and occurs usually in immunocompromised hosts and neonates.[381-383] The mortality rate of neonatal infections ranges from 13% to 75%.[382, 383] Preterm or SGA term infants are at particular risk of acquiring severe *Achromobacter* infections. Although most neonatal infections are nosocomial, vertical transmission from mother to baby has been described.[382] The case of *A. xylosoxidans* in this study was an 8-day old male born at (and recruited from) Brikama Health Centre. He was discharged after 6 days on admission but his blood culture result only became available 2 days after he was discharged. Whilst on admission, he received ampicillin, cloxacillin and gentamicin but the antibiotic susceptibility profile showed that this isolate was resistant to all three antibiotics and susceptible only to ceftazidime. At follow-up on day 27, this neonate was found to be alive and well. The fact that this neonate recovered and remained well even weeks after discharge despite *A. xylosoxidans* being resistant to the antibiotics received strongly suggests that this isolate might have been a contaminant, the source of which is not readily explained.

The second unusual pathogen was *Pantoea* species, a genus of Gram-negative bacteria of the family Enterobacteriaceae, usually isolated from soil, fruit, and vegetables. Among the species of this genus, only *P. agglomerans*, formerly known as *Enterobacter agglomerans*, is most commonly isolated in hospitals.[384] It is an unusual cause for neonatal sepsis, and infections are usually associated with outbreaks traced to

contaminated parenteral nutrition, intravenous anaesthetics, or packed erythrocytes.[384-389] The index case was a term day-old female born through assisted breech delivery at the EFSTH and referred to the EFSTH neonatal ward from the postnatal ward. The blood culture result was received within 3 days of sampling. The isolate was resistant to the three antibiotics that were administered (ampicillin, cloxacillin, and gentamicin) and sensitive to the only third-generation cephalosporin available, ceftriaxone. However, the antibiotics were not changed based on the sensitivity report; the newborn improved after 7 days of antibiotics and was discharged in good health. She was however lost to follow-up and her outcome on day 27 could not be ascertained. The fact that she improved on her initial treatment despite the contrary antibiotic susceptibility profile, strongly suggests that this isolate might also have been a contaminant.

Coagulase-negative staphylococci

CoNS were the most abundant pathogen isolated in this study accounting for 35% (32/91) of all positive blood cultures, but are considered clinically non-significant based on the MRCG clinical laboratory protocol and therefore not subjected to antibiotic susceptibility testing. CoNS are common inhabitants of the skin and mucous membranes; although a small proportion of neonates acquire CoNS by vertical transmission, acquisition primarily occurs horizontally from the hospital environment, the hands of their parents and hospital staff.[390, 391] In developed countries, CoNS is a major pathogen involved in late-onset infection, particularly among preterm and VLBW (<1500g) neonates.[73, 79] Within the first week of life, neonates become rapidly colonised by microorganisms originating from the environment.[95, 390, 392] During this period and among those admitted on neonatal intensive care units (NICUs), the risk of CoNS infection increases substantially with the use of ventilator support, indwelling devices,

parenteral nutrition, and exposure to other invasive skin or mucosa-breaching procedures.[393-395]

The role of CoNS is less well defined in developing countries where the burden of preterm birth is high and associated mortality even higher.[396, 397] Facilities for supportive care are much more limited in these settings; consequently, indwelling devices and invasive ventilation are much less frequently used, and if used, are restricted to urban referral centres.[99, 398, 399] In this context, the extent to which CoNS acts as a neonatal pathogen is unclear. In sSA, some studies reported it as a pathogen[138, 139, 144, 145, 148, 152, 154, 156, 157, 160, 172, 177, 180, 181, 183, 184, 188, 191, 201, 204, 208, 214, 219, 221-223, 225, 226, 233, 234, 237, 240, 241] and others excluded it as a contaminant.[124, 159, 254]

Despite the high likelihood that CoNS usually represents a contaminant when isolated from blood cultures, determining the likelihood of true bacteraemia can be challenging for clinicians and cannot be decided solely on the identity of the organism.[360] There is no blanket approach to classifying CoNS as a true neonatal pathogen or contaminant. A careful examination of clinical and laboratory data (e.g. CRP levels) is necessary in order to determine the clinical relevance of the isolated strains.[400] In this study, no major differences in relation to gestational age or birth-weight or previous antibiotic use were identified between Cases with CoNS infection and those with clinically significant bacteria. CRP levels were not measured. It is therefore difficult to determine the clinical significance of Cons in this setting, as it is possible that in some Cases it is a true pathogen and in others a contaminant.

Neonatal meningitis

Notable in this study is the poor practice of performing diagnostic lumbar punctures at the time of admission for neonates that met the clinical criteria for the procedure, the

reason being that such neonates are deemed to be too ill and unstable to undergo the procedure at the time. Most diagnostic lumbar punctures were performed after more than 72 hours on admission by which time the neonates had received several doses of antibiotics. These CSF samples were all processed at the EFSTH main hospital laboratory and all were sterile on culture.

Antimicrobial resistance

The success of treatment for an infectious disease depends on the isolated pathogens being susceptible to treatment choices. For *Klebsiella*, there was near universal resistance to both first-line (gentamicin and penicillin/ampicillin) and second-line therapy (third-generation cephalosporins) driven by ESBLs. Pooled resistance data from neonatal infection aetiology studies in sSA show similar high levels of resistance (Chapter 3, Table 3.6), consistent with the high degree of antimicrobial resistance documented in other regions of the world.[115] In the light of these data, the current WHO guidelines appear to be inadequate for the treatment of drug-resistant infections in neonates in community and hospital settings, particularly outbreaks of ESBL *Enterobacteriaceae*. Alternative therapeutic options, such as carbapenems, are limited and expensive for most countries in sSA; although in those countries where they are used, resistance rates as high as 50% to carbapenems have been reported for *Klebsiella*, *E. coli* and *S. aureus*, and the prevalence of carbapenemase-producing Enterobacteriaceae is increasing.[401]

Antibiotic resistance arises from poor infection control practices and gross overuse or inappropriate or prolonged use of antibiotics.[402] In a review of antibiotic prescribing patterns among neonatal admissions at the EFSTH, 27% of newborns received three antibiotics or more during admission in the absence of guidance from culture and

sensitivity results; treatment lasted as long as 3 – 4 weeks in some.[99] Reductions in price and local clinical experience is leading to third-generation cephalosporins being commonly used as first-line treatment for severe sepsis in many developing countries raising concerns about the spread of bacteria that are dually resistant to both first and second-line treatment.[402] In this study, 14% (27/195) of Cases that were treated with antibiotics on admission received ceftriaxone as first-line treatment; 63% (17/27) of these (all recruited at EFSTH) had not previously been exposed to any antibiotic. It is not clear why ceftriaxone was the first-line treatment for these 17 neonates given that they had not been exposed to antibiotics prior to admission. The 'antibiotic guidelines' in use at the study sites recommend the use of ampicillin and gentamicin as first-line treatment for pSBI (with addition of cloxacillin where there is suspicion of *S. aureus* infection) and ceftriaxone used as second-line. The EFSTH neonatal ward has the most staff with one neonatologist, and several junior doctors (usually one1 - 2 medical officers and between 4 – 6 house officers); the neonatal wards in other sites are overseen by medical officers only. Sick newborns are usually admitted by the more junior doctors (at the EFSTH this is the house officer) and reviewed by the medical officer; prescription of medications is therefore subject to the clinical assessment of the admitting doctor who might judge the newborn 'too ill' for the recommended first-line medications.

Simple and scalable infection control interventions can reduce the burden of multidrug-resistant bloodstream infections in neonates. In a before-and-after study conducted in the neonatal unit of the Hôpital Principal de Dakar, Senegal, Landre-Peigne and colleagues demonstrated the efficacy of a multifaceted hospital infection control programme in decreasing in the rate of neonatal nosocomial bloodstream infections (especially for *K. pneumoniae* ESBL sepsis) and the prevalence of resistant strains

without additional expenditure or work for the staff.[252] The interventions included clustering of nursing care, a simple algorithm for empirical therapy of suspected early-onset sepsis, minimal invasive care, and promotion of early discharge of neonates. Clustering of nursing care procedure involved sharing patients rather than duties. Each nurse or attendant was supposed to perform all care for each newborn for which she was responsible, and to wash her hands before attending to another infant. Previously, the nursing care staff shared duties and performed them serially for all newborns. The clustering of nursing care procedures reduced the number of patient contacts and thus the risk of cross-contamination in this unit with limited physical space. Protocols to help support decision-making for common situations were established. Changing the antibiotic prescribing policy resulted in a dramatic reduction in antibiotic use in newborns at risk for infection. Limitations were placed on indications for, and duration of, invasive care such as peripheral venous catheters, nasogastric feeding tubes, and blood tests. Finally, prompt discharge was actively promoted. In principle, such a strategy would be applicable to our neonatal unit although current operational conditions, if not addressed, would impede the success of the strategy. One of the challenges of implementing such a programme would be the common problem of correct hand washing in the absence of running water. This could be combated by using alcohol-based hand solutions, which have proven effectiveness and safety in neonatal units.[403, 404] Other challenges include 200% bed occupancy with sharing of cots and overcrowding (which promote horizontal transmission of infection); non-availability of routine laboratory investigations in the context of sub-optimal local laboratory capacity.[99]

The findings from both the neonatal unit audit and this case-control study will serve as the basis for development of the first-ever neonatal unit antibiotic stewardship

programme in The Gambia, beginning with the EFSTH and later including all public facilities providing neonatal inpatient care. Antimicrobial stewardship refers to coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal antimicrobial drug regimen, dose, duration of therapy, and route of administration, thereby improving patient outcomes, reducing microbial resistance, and decreasing the spread of infections caused by multidrug-resistant organisms.[405] Such a programme would necessitate the development and implementation of policies to support optimal antibiotic prescribing including facility-specific treatment guidelines based on local susceptibility data, and documentation of the dose, duration and indication for all courses of antibiotics so that they are readily identifiable.[406]

Vertical transmission

Maternal recto-vaginal colonization by pathogenic microorganisms is an important step in early-onset neonatal infection and to a lesser extent late-onset infection. In this study, concordant clinically significant bacteria were recovered from blood and maternal vaginal cultures in the same newborn-mother pairs in 25% (8/32) of early-onset infection cases suggesting possible vertical transmission of infection. The risk of transmission was highest for neonates with early-onset *S. aureus* infection of whom 63% (5/8) had mothers with concordant *S. aureus* rectovaginal colonisation. This is the first study in sSA where concordance between neonatal blood organisms and maternal vaginal flora has been demonstrated, although further molecular evidence for vertical transmission will be provided by whole genome sequencing of paired isolates. In a similar study in Uganda, no concordant organisms were identified although a broadly similar range of organisms was recovered from neonatal blood and maternal vaginal specimens.[407]

The risk of vertical transmission of bacterial infection acquired through direct maternal-foetal contact via maternal colonisation has been most widely studied with GBS.[75] Maternal vaginal colonization with GBS is essentially a prerequisite for both early colonization of the newborn infant and early-onset GBS sepsis.[92] Rectovaginal colonization rates exceed vaginal colonization rates by at least 50% or more.[408, 409] GBS vaginal and rectal colonization in pregnant woman may be persistent, transient, or intermittent[410, 411] but not much is known about post-partum maternal flora and whether there are substantial changes in the days after birth. Colonisation with *S. aureus* has been reported in 4%–22% of pregnant women and is associated with an increased risk of post-partum infections in women but not in their infants.[412] Early-onset maternal-foetal infections have however been reported following antenatal invasive procedures performed within a day of delivery.[413]

Inadequate hand hygiene and glove use, and excessive vaginal examinations may have contributed to the high rate of maternal *S. aureus* colonisation in our setting, and a potentially increased risk of vertical transmission. The study was not designed to distinguish between maternal, other caregiver, and environmental sources; it is therefore possible that the concordant isolates are not genetically related. The next step is to confirm genetic relatedness of paired neonatal and maternal isolates by whole genome sequencing.

7.1.2 Molecular diagnostics

Bacteraemia

PCR was positive in 17% (4/24) of Cases with a negative blood culture, two of which were of GBS infection. One of the Cases (P0091) had a CoNS-positive blood culture and maternal RVS culture positive for GBS. The other (P0099) had a negative blood culture, and GBS was not isolated from maternal RVS. Both Cases were 1-day old at the time of

recruitment and sampling. The identification of GBS by PCR suggests that GBS might be a 'missed' cause of early-onset neonatal infection in this setting. This seems reasonable given that up to 30% of pregnant women in The Gambia are rectovaginally colonised by GBS (predominantly serotype V) [253]and that GBS has previously been identified as a cause of neonatal sepsis[348] and meningitis[253, 255, 348] in The Gambia. Colonising serotypes among Gambian newborns born to colonised mothers are mostly dependent on maternal serotypes; nevertheless, neonates are most likely to be colonised with serotype V irrespective of maternal colonisation status.[253] However, the serotypes responsible for invasive neonatal disease in The Gambia are not known, and will be difficult to determine retrospectively given that historical isolates are not available for serotyping and PCR does not provide serotype information.

Respiratory infection.

The performance of the respiratory TAC with regards to pathogen detection was better than the blood TAC; PCR was positive in 62% (21/34) of culture-positive, and 23% (3/13) of culture-negative NPS specimens. Furthermore, PCR detected four concordant bacteria. Although the same limitations apply to the respiratory TAC with regards to small sample volume, the sensitivity of TAC for respiratory pathogens is much higher.[54] This is evident in the number of viruses identified; several of which are mixed infections with bacteria, and therefore difficult to interpret. Indeed, three of the Cases with a diagnosis of pneumonia were PCR-positive for bacterial and viruses (Table 5.19).

Interpretation of results

Other than the detection of GBS, the added value of the TAC PCR as an adjunct test for the diagnosis of bacteraemia/sepsis in this study was limited given that PCR failed to identify concordant bacteria among the three Cases in whom both PCR and blood culture were positive. Apart from the Case whose PCR was positive for GBS but blood

culture positive for CoNS (considered clinically non-significant in this study, and also not a target on the TAC), it is unclear why there was no concordance between the blood culture and PCR results. In a recent randomised clinical trial, multiplex PCR systems performed better than blood culture by BACTEC in identifying the causative organism in newborns with suspected sepsis, and demonstrated 100 % concordance with blood culture results. Diagnosis was also made earlier and mortality reduced.[414] Interpreting the mostly discordant microbiology and molecular diagnostic data is challenging and presents two possibilities. The first possibility is that the microbiology results are wrong and the 'more sensitive' PCR results are correct. This is rather unlikely as it would suggest either gross contamination of specimens or inadequacy of the microbiology laboratory. This MRCG also has a track record of carrying out excellent research involving diverse specimen types. This is further supported by the fact that the MRCG Clinical Laboratories are GCLP compliant and therefore operate to a standard that assures the reliability, quality and integrity of the work and results generated.

The second possibility is that there are inherent limitations with the TACs in their current form that might hinder their clinical applicability. One limitation of PCR assays is the risk of contamination at various stages of the experiments. This is a particular problem with open systems like the Fast-Track Diagnostics (FTD; Malta). The use of an automated extraction platform in this study reduced the potential for contamination, and the closed system format of TAC further limits the possibility of cross-contamination.[53]

Molecular detection of pathogens in blood suffers from poor sensitivity and may be affected by exposure to antibiotics,[415] although only three of the Cases with positive blood culture and negative PCR were exposed to antibiotics. The requirement of small

volumes of specimen by TAC, an obvious advantage when working with neonatal blood samples, is also one of its limitations. Each TAC well takes only 1.0μl of specimen; the sensitivity of the reaction therefore might be less than a typical individual singleplex real-time PCR which utilises more specimen volume. The is particularly important with neonatal blood specimens which contain a low pathogen load.[48] TAC has also been shown to be 10-fold less sensitive than individual real-time PCR assays with the same primers and probes.[54] During optimization of the TAC for the ANISA study, detection of bacteria in blood was found to significantly impaired relative to saline, with complete lack of detection of the lowest concentration of bacteria.[53] The first evaluation of the performance of the TAC on human blood was with the ANISA study, the results of which are yet to be published. There is therefore no yardstick against which to compare the performance of TAC on blood specimens in this study. Currently, TAC appears to be well suited only to the detection of respiratory pathogens and has been demonstrated to perform as reliably as the FTD platform in this regard.[416]

The TAC did not add much to my study, and my experience using it in a resource-limited African setting has highlighted several challenges that would preclude expansion of this technology to a regional clinical setting. The first challenge is cost of using this method. It needs a special platform to run the cards as well as sophisticated laboratory equipment and expensive reagents, all of which are unlikely to be a priority for health systems struggling with more basic infrastructural needs. Second is the level of expertise required to process specimens. Although using the TAC is simple and straightforward, upstream procedures such as DNA extraction require skilled personnel. The laboratory staff that ran the TAC assays in this study all had to undergo training in the molecular diagnostics lab. The MRCG laboratories are research and regional reference laboratories with 70 years of investment in infrastructure and

training of personnel, an asset not easily replicable in resource-poor countries. A third challenge is in the analysis of the generated data. Analysis of data is complicated involving interpretation of the individual amplification curves for all 384 wells on each card, and requiring unique software provided by the CDC, Atlanta, Georgia, USA. Without having a laboratory background, learning about PCR reactions and interpretation of results was a steep learning curve for me, made enjoyable with the continuous guidance and support of Dr. Kirsty Le Doare and collaborators at CDC who originally developed the cards.

7.2 Outbreak of hospital acquired infections

The identification of the outbreaks of *B. cepacia* and *K. pneumoniae* on the EFSTH neonatal ward and subsequent investigation to determine the source was the first reported outbreak in the history of the hospital. *B. cepacia*, *K. pneumoniae,* and other Gram-negative bacilli are associated with common-sources outbreaks of nosocomial neonatal bloodstream infections. Both pathogens were isolated from intravenous fluids and parenteral antibiotics in use in the ward: plain 10% dextrose; normal saline, 10% Dextrose plus sodium, potassium and chloride; metronidazole infusion and gentamicin injection. However, neither was isolated from any of the unused intravenous fluids that were in stock or from surfaces swabs.

According to ward protocol, neonates requiring intravenous fluids for the first 24 hours after birth receive 10% Dextrose, which is usually available as a commercially prepared 500ml bottle. Some months before the outbreak occurred, there was a nationwide stock-out of 10% Dextrose. The nurses on the neonatal ward improvised by mixing pre-calculated volumes of normal saline and 50% Dextrose (or used 5% Dextrose where 50% Dextrose was unavailable). Starting at 24 hours of age, assuming that urine

production is adequate, supplemental electrolytes (sodium, potassium and chloride) are required. An appropriately constituted fluid is not locally available and so this is routinely prepared on the ward by mixing pre-calculated volumes of 10% Dextrose, Normal saline, and Potassium chloride. Antibiotic powders for parenteral use are dissolved with Normal saline (or water-for-injection infrequently), and other injections similarly diluted in order to be able to draw up the small neonatal doses. All these procedures require stringent aseptic techniques.

The investigation suggests contamination of the intravenous fluids at the point of preparation, and contamination of intravenous medications at the point of dilution with already contaminated intravenous fluid. However, the outbreak source can only be confirmed by determining the genetic relatedness of neonatal and environmental isolates.[417, 418]. Demonstrating strain relatedness will also provide insight as to whether the outbreaks were a single event or simultaneous independent outbreaks from several sources. All isolates neonatal and environmental isolates were shipped to the UK for whole genome sequencing.

Several risk factors have been associated with the development of nosocomial infection in newborns, the most important being birth weight, immaturity of the immune system, type and duration of invasive procedures, and colonization by bacteria from hospital environment.[392, 419, 420] In developing countries, substandard sterilisation and disinfection practices are common.[21] In our setting, single-use nasal-prongs and intravenous fluid burettes are routinely re-used and soaked in disinfectant solution and without re-used without adequate sterilisation in-between use; single-use antibiotic vials are pooled with inappropriate storage and use of multiple-dose vials and containers of medications (Chapter 5, Figure 5.6).

Gram-negative bacilli are most often transmitted among neonates from the hands of colonised staff,[421] although patient-to-patient spread of infection has been reported.[366] In this study, the hands of personnel did not appear to be implicated in the transmission of infection although this is inconclusive, as the full complement of staff was not swabbed. The outbreak investigation however revealed numerous lapses in hand-hygiene practices. Running water is not always available and water is stored in plastic containers beneath the sink accessed using a common cup (Chapter 6, Figure 6.13). Patient-to-patient transmission in this study is certainly possible due to overcrowding with sharing of cots.[99] Nosocomial infection due to Gram-negative bacilli usually occurs in neonates already colonised with Gram-negative bacilli in the pharynx or intestine, and the risk of colonisation with hospital strains of Gram-negative bacilli (which are often resistant to multiple antibiotics) increases dramatically the longer a baby stays in intensive care. Neonates in whom intestinal colonisation with Gram-negative bacilli develops are a particularly important reservoir of Gram-negative bacilli in the nursery; once colonised, infants may harbour antibiotic-resistant hospital strains of Gram-negative bacilli in their stool for up to a year or more.[392] Four out of the five study participants with suspected hospital-acquired *K. pneumoniae* bacteraemia were also rectally colonised with Gram-negative bacilli but only three were colonised with *K. pneumoniae*. The spread of *K. pneumoniae* among different hospitals and even across country borders through the transfer of infected or colonised patients has also been documented.[422] Although eligibility in this study was restricted to neonates whose mothers were resident in the study area, this is a potential cause for concern in The Gambia where the cultural practice of a woman staying with relatives after delivery is widespread. Many families have relatives in neighbouring countries (Senegal, Guinea-Bissau) and there is a lot of cross-border movement.

7.3 Strengths and limitations

This is the first study of neonatal infections in The Gambia where an attempt has been made to ascertain the role of maternal transmission at birth to neonatal infection by correlating neonatal blood organisms with maternal vaginal and perineal flora. It is also the first study in sSA to explore the use of the TaqMan Array Card in the diagnosis of neonatal bloodstream infection notably for culture-negative cases and possible viral causes. Another strength of this study is its global comparability resulting from the use of clear inclusion criteria, and the reporting of the findings according to the STROBE-NI guidelines. The use of ISO accredited laboratories with validated standard operating procedures and protocols ensure confidence in the results. The study however, had several limitations.

Firstly, being a hospital-based study, complete ascertainment of neonatal infection cases in the study area that did not make it to hospital was not possible. The majority of the population in the study area however, give birth in health facilities and home-born neonates have good access to referral-level care, thereby supporting a reasonable level of case ascertainment. The hospital environment is also a hot bed of infection transmission through unclean delivery and unhygienic practices. Given that over 90% of cases in this study were facility-born and that all the pathogens in this study are known to be associated with neonatal nosocomial bloodstream infections[177, 423] and outbreaks, infections in this study might all have been hospital-acquired and not just those associated with the outbreaks. However, for non-outbreak pathogens, this would be difficult to prove in the absence of environmental samples.

Secondly, modification of the WHO YICSSG criteria for temperature and respiratory rate may have led to the exclusion of some Cases especially neonates in the first few days

presenting with only increased respiratory rate. This might have affected capture of neonates with GBS, as neonatal GBS colonisation (a prerequisite for invasive disease) has been associated with neonatal respiratory distress;[424] although historical data suggest that the incidence of neonatal GBS disease in The Gambia might be low with only seven reported cases between 1990 and 2015 (Chapter 4, Table 4,2).

Thirdly, there was non-accurate measure of gestational age and deliberate selection bias against babies with lower birth weight, who were underrepresented in the sample. Obtaining specimens for analysis and maternal consent are more challenging for premature and LBW neonates, although the reasons for refusal of consent were not documented.

A fourth limitation was the post-partum timing of maternal swab collection. It is possible that the maternal flora changes substantially in the days following birth, progressively obscuring the source of causative pathogens with delayed bacterial culture, particularly among mothers of newborns with late-onset infections.

Lastly, was the inability to carry out PCR on all study isolates, as well as further sequencing studies to determine clonal relatedness of maternal and newborn isolates. These were beyond the funding and time available for this PhD but are planned for part my post-doctoral work.

Inasmuch as the study findings may not be generalisable to all health facilities in the entire country, the multisite recruitment across the levels of care (teaching hospital, general hospital and major health centers) ensured good representation. The findings of this study have also been reported along the STROBE-NI guidelines, thereby permitting comparability with future neonatal infection studies.

7.4 Conclusion

In summary, this study has shown that while S. *aureus* is an important cause of neonatal infection in hospitalised newborns in The Gambia, with the majority of cases possibly maternally acquired, GBS is an under-diagnosed cause of early-onset neonatal infection. *K. pneumoniae* and *B. cepacia* are also important causes of infection, but their association with separate nosocomial outbreaks in the neonatal ward strongly suggests that the hospital environment plays a very important role in the acquisition of infection among Gambian newborns. Infection is associated with high levels of mortality, and WHO-recommended first- and second-line antibiotics are redundant in the face of multi-drug resistant infections and inadequate infection control. In this setting, blood culture remains a valuable diagnostic tool despite being slow and offering modest tangible benefit for high mortality risk neonates, with results available before discharge or death for only 38% (35/91) of cases. An exploratory Pilot study of a novel molecular diagnostic tool, the TaqMan Array Card, demonstrated limited additional benefit of this technology over blood cultures. Sensitive point-of-care diagnostics for bacterial bloodstream infections similar to those developed for HIV and malaria are needed. There is a need for improved infection control and antimicrobial stewardship to preserve limited antibiotic choices.

CHAPTER 8. IMPLICATIONS FOR PROGRAMME, POLICY & RESEARCH

Nursing staff during a consultant ward round at the main neonatal ward, EFSTH

Overview

This chapter discusses the problem of neonatal infection in the Gambia in the context of implications for programme and health policy, and priority areas for neonatal infection research - The '3 Ps' (Box 8.1). Mention is also made of more general priority areas for neonatal infection research such as discovery of new biomarkers and development of more sensitive diagnostic tools. The chapter ends with an overall conclusion of this PhD.

Box 8.1 Neonatal infections in The Gambia: The 3 P's

Problem of neonatal infections

What is already known about neonatal infections

- Infections are the 3rd most common cause of the 2.7 million annual neonatal deaths globally
- Antimicrobial resistance is a growing global threat to the treatment of neonatal infections
- Newborns are at a higher risk of hospital-acquired infections with infection rates in developing countries 3-20 times higher than in high-income countries.
- There is limited regional and local data on the aetiology of neonatal infections and antibiotic susceptibility profiles in sSA

What the systematic review of neonatal infection aetiology in sub-Saharan Africa presented in this thesis adds

- The main causes of neonatal infection in sSA are *S. aureus*, *Klebsiella* species, Group B *Streptococcus*, *Escherichia coli* and *Enterococcus* species.
- Reported antimicrobial resistance to WHO-recommended first- and second-line antibiotics is a problem, with documented resistance to alternative regimens including carbapenens

What the audit of neonatal care in The Gambia's largest hospital presented in this thesis adds

- At the Gambia's largest and national referral hospital, 94% of neonates admitted over a 4-year period received antibiotics yet only 3% were investigated by culture of blood or CSF

What the Case-Control study adds

- The commonest cause of bacteraemia/sepsis among hospitalised newborns in The Gambia is *S. aureus*.
- GBS is an under-diagnosed case of early-onset infection
- Hospital outbreaks due to multidrug resistant Gram-negative bacteria –*K. pneumoniae* and *B. cepacia* - are responsible for a significant proportion of neonatal infections that would have gone unnoticed without microbiological investigation.
- Sub-optimal hygiene practices and poor infection control measures during preparation of intravenous fluids and parenteral injections are responsible for nosocomial infection outbreaks on the neonatal ward.
- WHO currently recommended first- and second-line antibiotics are redundant in the face of multi-drug resistant infections and inadequate infection control.

Programme Implications & Protocols (patient/community, hospital and national level)

- Infection prevention and control should encompass intrapartum care both in the hospital and at home.
- Routine hospital care of the sick newborn should include microbiological investigations to determine aetiology and antimicrobial susceptibility patterns.
- Infection control protocols for preventing and detecting hospital outbreaks are necessary.
- Infection treatment protocols should include guidelines for management of hospital-acquired infections.
- Antibiotic stewardship programmes

Priority areas for neonatal infection research

- Epidemiological research
- Health systems and policy research
- Implementation research
- Discovery research into diagnostic and prognostic host biomarkers of infection with sufficient sensitivity and specificity for potential use as point-of-care diagnostics

8.1 Problem of neonatal infections

Neonatal infection remains a big problem. With the global emphasis placed on improvement of referral pathways and promotion of institutional delivery for high-risk births, an estimated 50% of the 36 million births in sSA are now in facilities.[425] Most developing country hospitals are hotbeds of infection transmission because of poor intrapartum and postnatal infection-control practices, and during outbreaks of neonatal infection in nurseries. In South-East Asia and sSA, HAIs are responsible for 75% of all causes of death in the neonatal period.[426] In these settings, the expectations of improved neonatal outcomes in hospitals are consequently subverted by HAIs and their associated morbidity, mortality, and cost.[21] HAIs also have an indirect detrimental effect on neonatal survival; families, especially in poor communities, are less likely to seek facility-based care when hospitals are seen as institutions where children experience poor outcomes associated with high out-of-pocket expenses for antibiotics and prolonged hospital stay cost.[21] Although in the Gambia antibiotics are usually provided free of charge by the hospital and parents only buy them when they are out of stock; hospital stays place other burdens on families (including the problem of care for other children at home, transport and feeding costs etc.). In this setting, poor neonatal inpatient outcomes are also related to operational factors including, but not limited to, lack of laboratory capacity, lack of appropriate antibiotics, shortage of intravenous fluids, over-crowding of wards with sharing of cots and radiant warmer beds, as well as shortage of staff.[99]

8.2 Programme implications and protocols

The Gambia is yet to develop its own national newborn action plan. This is an important first step in setting national newborn health targets and identifying priority areas for action. Focus is necessary in facilities and at home. In spite of the increasing trend in

facility births in The Gambia, an estimated 37% of the 83, 000 annual births still take place at home with or without a skilled attendant. In this study, just over 90% of Cases were facility-born implying that many home-born babies do not make it to the hospital, and probably die at home unreported.[427] Improving data on where newborn deaths occur and understanding of delays in the receipt of care are a priority for design of context-specific community and health system strategies.[287] Improving the quality of neonatal care in hospital requires education and training of staff with the support of standardised protocols on all aspects of hospital-based newborn care.

Another area for priority action is the development strategies that strengthen standard infection-control practices including WASH conditions,[96] hand hygiene,[428] minimization of vaginal examination,[429] and cord care.[430] Handwashing or use of an alcohol-based antiseptic hand rub before and after contact with each woman or newborn and after contact with bodily fluids is critical for infection prevention. Clean birth practices at home with no skilled attendant could reduce neonatal sepsis deaths by as much as 15%.[429, 431] At the community level, handwashing has been shown to significantly reduce mortality[432] and risk of cord infection.[433] Compliance with handwashing is however challenging in the context of poor access to clean water. In such settings, alcohol-based hand gel may provide an alternative option that is bacteriocidal against a broad range of Gram-negative and Gram-positive aerobic bacteria, including those commonly associated with neonatal infections.[434] Recently concluded clinical trials in sSA have evaluated whether the provision of alcohol hand gel to pregnant women and postpartum mothers is a clinically- and cost-effective way of preventing neonatal infections in settings without running water.[435] For newborns who are born at home, and in settings with high neonatal mortality (30 or more neonatal deaths per 1,000 live births), the application of antiseptics to the cord may have additional benefit.[430, 436, 437]

HAIs can be attributed to failures in knowledge and training regarding basic infection control processes, inadequate infrastructure, systems of care, and resources.[21] Detailed quality-of-care protocols and 'core nursing skills sets' for all aspects of newborn care will improve quality and give more responsibility for care to nurses, particularly with regard to infection prevention, feeding support and use of intravenous fluids. A shift in the hospital-based management of sick newborns with suspected infection is urgently needed to include routine blood cultures. The comprehensive management 'package' should include routine microbiological surveillance (including HAIs and and AMR surveillance, as well as antibiotic stewardship.

8.3 Priority areas for research

Major data gaps remain for high-quality, locally available data that can be used for planning and decision making and tracking the impact of policies and programmes on improving newborn survival. Now that most births occur in facilities, a crucial opportunity to improve the collection of epidemiological data is through establishing a standardised facility-based perinatal minimal dataset that will promote comparability of data with other units and regions.[287] Creation of a national perinatal/neonatal database for stillbirths, preterm births, CAI and HAI, as well as other key neonatal health indicators, will provide data for improving outcomes and increasing the quality, safety and value of newborn care. Extension of such a database to include other countries in the West African sub-region will facilitate comparability of data and quality improvement collaborations.

Epidemiological data draw attention to priorities to maximise progress towards improving newborn survival, but targets and data alone do not save lives; only changes in health systems and coverage of effective interventions do.[287] Effective interventions

exist which can reduce neonatal mortality by as much as 70% if they reach all mothers and newborns; the greatest challenge is to increase coverage of these interventions.[438] [439] Research priorities for neonatal health in The Gambia should therefore include health systems and health policy research. Equally important is operations research and research that addresses political, economic, social, cultural, behavioural, and infrastructural issues involved in addressing mortality due to neonatal infections.[438] Such implementation research is critical for generating the new knowledge needed to achieve high coverage with life-saving interventions. Areas of focus relating to prevention and management of neonatal infections are listed in Table 8.1 below.

Table 8.1 Priority areas for neonatal infection research in The Gambia and beyond

	Problem of neonatal infections	Programme Implications & Protocols
Epidemiological research priorities [a]	Incidence of nosocomial neonatal infection in postnatal wards and neonatal nurseries Identification of sources of lethal Gram-negative rod infections that cause early-onset infection using conventional microbiological and molecular approaches.	
Health systems and health policy research priorities [a]	Feasibility, effectiveness, and cost of approaches to increase clean delivery practices in facilities and in homes Feasibility and effectiveness of approaches to improve aseptic practices in labour rooms, maternity and neonatal wards	Detailed assessment of the entire process of health care delivery including patient population and flow, referral systems, key health processes, information management, staff competence and organisation, supplies, facility design and infrastructure Development of strategies to improve error-prone processes that lead to HAI, including behaviour change. Feasibility, cost and effectiveness of setting up newborn care corners in first referral units and district hospitals Feasibility and effectiveness of approaches to increase quality of care in hospitals using standardised protocols for management of newborn conditions.
Implementation research priorities	Facility-based cluster-randomised trials evaluating the multi-faceted hand-hygiene promotion programmes (alcohol-based hand gel) Clinical trials of targeted interventions to interrupt contamination and transmission of pathogens. Evaluation of cord-care regimens to reduce colonisation and infection (e.g single application of 4% chlorhexidine vs. daily application for 7 days)[440]	
Discovery research priorities (Global)	Improved clinical syndrome identification More sensitive and discriminatory biomarkers Maternal GBS and S. pneumoniae vaccines Point-of-care diagnostics Microfluidic technologies	

[a] Source: adapted from Bahl *et al.*[438] ; Zaidi *et al.*[21] ; Edmond[45]

At the global level, more research is needed to identify and validate more sensitive and discriminatory biomarkers of infection that can be used as adjunct diagnostic tools with the potential for incorporation into point-of-care diagnostics. Well-designed clinical trials evaluating new clinical diagnostic algorithms are needed. Such clinical algorithms would be based on a combination of clinical signs and host biomarkers that would discriminate between the neonatal infection syndromes – sepsis, meningitis and pneumonia.

Microfluidics is the study of the behaviour, precise control, and manipulation of fluids geometrically constrained to submillimetre (nanolitre or picolitre) channels. [441] Microfluidic technology has also allowed sample preparation and a number of different assays to be combined in small, disposable, single-use diagnostic cartridges or cards that have been called a ''lab on-a-chip'' or LOC.[45] For the detection of various pathogens, microfluidics-based platforms offer many advantages, including speed, cost, portability, high throughput, and automation. LOCs have been reported to perform assays at sensitivity, specificity, and reproducibility levels similar to those of central laboratory analysers, but yet require little user input other than the insertion of the sample. Single drops of blood, faeces, and saliva have all been tested with encouraging results. In a study in Rwanda, the 'mChip assay' had excellent performance in the diagnosis of HIV using only 1 μl of unprocessed whole blood and an ability to simultaneously diagnose HIV and syphilis with sensitivities and specificities that rival those of reference benchtop assays. [442] There are however several challenges to practical implementation of this technology especially in developing countries.[443]

8.4 Overall Conclusion

The burden of neonatal infection in sSA and its contribution to morbidity and mortality remains great. Preventing infection depends on improving our understanding of maternal health, including maternal colonisation, as well as vertical transmission (HIV, malaria, other congenital infections, and ascending bacterial infections) and hospital-acquired infections, particularly with multi-resistant Gram-negative bacteria. Infection control management is important, and interventions, isolated or as part of care bundles, need detailed, prospective evaluation in outbreak situations.[444]

I have presented new data on size of the problem, and the pathogens associated with neonatal infection across sSA, and in three main referral health facilities in The Gambia. Although more population-based data are needed; over 60% of births in The Gambia occur in health facilities and high-quality surveillance data can provide important information on cause and AMR. Routine programmatic data require significant resources, including strengthening laboratory quality control and assurance measures, and use and appropriate interpretation of molecular diagnostics to detect pathogens (including viruses). More research is also essential and investing in research to better measure and reduce the burden of neonatal infection, especially in sSA should therefore be a priority.

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APPENDICES

Appendix 1: Strengthening the Reporting of Observational Studies in Epidemiology for Newborn Infection (STROBE-NI) Checklist

		TITLE AND ABSTRACT
	STROBE 1(a)	Indicate the study's design with a commonly used term in the title or abstract
	STROBE 1(b)	Provide in the abstract an informative and balanced summary of what was done and what was found
		INTRODUCTION
Background / rationale	STROBE 2	Explain the scientific background and rationale for the investigation being reported
Objectives	STROBE 3	State specific objectives, including any pre-specified hypotheses
		METHODS
Study design	STROBE 4	Present key elements of study design early in the paper
	STROBE-NI 4.1	Clearly state case ascertainment methods (e.g. physician diagnosis, clinical algorithm), documenting individual clinical signs used for diagnosis of possible serious bacterial infection. Give microbiological and/or laboratory and/or radiological criteria for other infectious syndromes (e.g. meningitis, sepsis, pneumonia). Include indications for clinical investigations (e.g. lumbar puncture)
	STROBE-NI 4.2	Give criteria used to differentiate between new infection episodes and relapses
	STROBE-NI 4.3	For facility-based studies, indicate if the study is of community and/or hospital acquired infections (HAI), defining HAI using an international standard and presenting specific HAI clinical syndromes separately
	STROBE-NI 4.4	State whether this is an outbreak study, and if so define an outbreak, with reference to an international standard
	STROBE-NI 4.5	Describe sampling strategy (e.g. clinical indication vs. routine surveillance) and sampling details, (e.g. minimum volumes; timing in relation to antimicrobial administration)
	STROBE-NI 4.6	Describe conventional and/or molecular microbiological methods used, with details (e.g. automation, enrichment steps), and the use of controls
	STROBE-NI 4.7	List pathogens that are likely to be identified by microbiological methods used, and criteria used to determine clinical significance
	STROBE-NI 4.8	Describe antimicrobial susceptibility tests and thresholds used, with reference to an international standard (e.g. CLSI or EUCAST)
Setting	STROBE 5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
	STROBE-NI 5.1	Describe the study context in terms of incidence of neonatal mortality, stillbirth and preterm birth.
	STROBE-NI 5.2	Describe the population included e.g. facility live births, referrals from home, referrals from another facility
	STROBE-NI 5.3	For community-based studies, describe care-seeking and adherence and time to referral
	STROBE-NI 5.4	For facility-based studies, describe obstetric care (basic or comprehensive), including proportion of births by caesarean section. Report annual number of live births per facility and state proportion of births in the study area that occur in hospital (vs. community)
	STROBE-NI 5.5	For facility-based studies, indicate if the facility is public or private, and give the number of health care staff and their training. Indicate the level of neonatal care available (e.g. ventilatory support, indwelling catheters) and investigations available (e.g. biochemistry, radiology). Report antimicrobial guidelines used for the empiric management of neonatal sepsis.
	STROBE-NI 5.6	State the laboratory location and capacity to process different sample types, and give quality control and assurance measures in place.

Participants	STROBE 6(a)	Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
	STROBE 6(b)	Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case
	STROBE-NI 6.1	State age of participants (e.g. 0-27 days defines neonates; 'day 0' as day of birth). Disaggregate neonatal data from that of older infants and from stillbirths
Variables	STROBE 7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
	STROBE-NI 7.1	State criteria used to define clinically significant organisms for each sample type
Data sources measurement	STROBE 8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	STROBE 9	Describe any efforts to address potential sources of bias
Study size	STROBE 10	Explain how the study size was arrived at
Quantitative variables	STROBE 11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	STROBE 12(a)	Describe all statistical methods, including those used to control for confounding
	STROBE 12(b)	Describe any methods used to examine subgroups and interactions
	STROBE 12(c)	Explain how missing data were addressed
	STROBE 12(d)	Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
	STROBE 12(e)	Describe any sensitivity analyses
		RESULTS
Participants	STROBE 13(a)	Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
	STROBE 13(b)	Give reasons for non-participation at each stage
	STROBE 13(c)	Consider use of a flow diagram
	STROBE-NI 13.1	See Figure 3 for suggested components of a flow diagram for neonatal infections
Descriptive data	STROBE 14(a)	Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders
	STROBE-NI 14.1	Describe maternal infections (clinical or on screening, e.g. GBS or HIV) or risk factors for infection (e.g. PROM, peripartum fever).
	STROBE-NI 14.2	Describe key neonatal characteristics, including sex, postnatal and gestational age categories (range and median), birth-weight categories (range and median), birth place, feeding (breast milk or other) and comorbidities

Descriptive data	STROBE-NI 14.3	Report data on occurrence of individual signs (e.g. fast breathing), according to case definitions
	STROBE-NI 14.4	Give proportion of mothers and neonates with peripartum antibiotic exposure (+/- pre-admission exposure for neonates). Report details of antimicrobials (or supportive care) given during the study
	STROBE 14(b)	Indicate number of participants with missing data for each variable of interest
	STROBE 14(c)	Cohort study—Summarise follow-up time (e.g., average and total amount)
Outcome data	STROBE-NI 15	Cohort study—Report numbers of outcome events or summary measures over time Case-control study—Report numbers in each exposure category, or summary measures of exposure Cross-sectional study—Report numbers of outcome events or summary measures
	STROBE-NI 15.1	Report the number (+/- proportion) of samples microbiologically tested (including lumbar punctures for meningitis cases); the number (+/-proportion) that were positive (including thresholds for detection, where applicable); all isolates obtained (including clinically significant and non-significant); and antimicrobial susceptibilities of pathogens, where done.
	STROBE-NI 15.2	Report number (+/- proportion) of babies with microbiologically proven infection (and number of infections per baby), and include this in the flow chart (see Figure 3).
	STROBE-NI 15.3	Report infections by day, for days 0-6. State age categories, if used, defining 'early-onset' and 'late-onset' infection (e.g. <72 hours and ≥ 72 hours respectively).
	STROBE-NI 15.4	Report deaths and any sub-analyses by risk groups
Main results	STROBE 16(a)	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included
	STROBE 16(b)	Report category boundaries when continuous variables were categorized
	STROBE 16(c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
	SPRING 16.1	For incidence, give risk per 1000 live births, or if alternative denominator used (e.g. total births or bed days), define this clearly
Other analyses	STROBE 17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses
	DISCUSSION	
Key results	STROBE 18	Summarise key results with reference to study objectives
Limitations	STROBE 19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
	STROBE-NI 19.1	Discuss sources of recruitment bias, particularly regarding the period of time shortly after birth. State source of denominator data and discuss possible related biases
Interpretation	STROBE 20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	STROBE 21	Discuss the generalisability (external validity) of the study results
	OTHER INFORMATION	
Funding	STROBE 22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based
Ethics	STROBE-NI 23.1	Report any ethical considerations, including the recruitment of young mothers (minors), and the consent process for early recruitment of neonates after delivery. Provide details of research ethics approval.

Appendix 2: Search Strategies for Systematic Review of Neonatal Infection Aetiology in sub-Saharan Africa

Search Terms for: Global Health <1910 to 2016 Week 23>, Embase Classic+Embase <1947 to 2016 June 17>, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>

1 Cameroon/ (15402)
2 Central African Republic/ (2324)
3 Chad/ (2458)
4 Congo/ or "Democratic Republic of the Congo"/ (18017)
5 Gabon/ (4145)
6 Burundi/ (1768)
7 Djibouti/ (749)
8 Eritrea/ or Ethiopia/ (28122)
9 Kenya/ (42174)
10 Rwanda/ (5557)
11 Somalia/ (4219)
12 Sudan/ or South Sudan/ (16774)
13 Tanzania/ (30655)
14 Uganda/ (32332)
15 Angola/ (2934)
16 Botswana/ (4603)
17 Lesotho/ (1259)
18 Malawi/ (12965)
19 Mozambique/ (6473)
20 Namibia/ (2638)
21 South Africa/ (98929)
22 Swaziland/ (1615)
23 Zambia/ (11936)
24 Zimbabwe/ (16127)
25 Benin/ (4985)
26 Burkina Faso/ (9486)
27 Cote d'Ivoire/ (9344)
28 Gambia/ (7220)
29 Ghana/ (20392)
30 Guinea/ or Equatorial Guinea/ or Guinea-Bissau/ (6983)
31 Liberia/ (3440)
32 Mali/ (7100)
33 Mauritania/ (1278)
34 Niger/ (3815)
35 Nigeria/ (81701)
36 Senegal/ (16235)
37 Sierra Leone/ (4128)
38 Togo/ (3367)
39 Comoros/ (741)
40 Madagascar/ (9438)
41 Mauritius/ (1982)
42 Seychelles/ (923)
43 "Sao Tome and Principe".mp. (451)
44 Cape Verde/ (622)
45 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 (502033)
46 sub-Saharan Africa.mp. or "Africa South of the Sahara"/ (209260)
47 45 or 46 (533218)

Search Terms for: Global Health <1910 to 2016 Week 23>, Embase Classic+Embase <1947 to 2016 June 17>, Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present> ***(continued from overleaf)***

48 exp Infant, Newborn/ or (newborn* or neonat*).tw. (1461719)
49 exp sepsis/ or exp infection/ or (infection* or pathogen* or organism* or bacter* or virus or viral or etiology).tw. (9570459)
50 limit 49 to "etiology(sensitivity)" [Limit not valid in Global Health,Embase; records were retained] (7014498)
51 (neonat* adj3 sepsis).tw. (10161)
52 48 and 50 (206620)
53 51 or 52 (209193)
54 47 and 53 (9030)
55 limit 54 to ("all infant (birth to 23 months)" or "newborn infant (birth to 1 month)") [Limit not valid in Global Health,Embase; records were retained] (8854)
56 limit 55 to yr="1980 - 2017" (8161)
57 limit 56 to ("diagnosis (maximizes sensitivity)" or "diagnosis (maximizes specificity)" or "diagnosis (best balance of sensitivity and specificity)" or "causation-etiology (maximizes sensitivity)" or "causation-etiology (maximizes specificity)" or "causation-etiology (best balance of sensitivity and specificity)") [Limit not valid in Global Health; records were retained] (7506)
58 limit 57 to humans [Limit not valid in Global Health; records were retained] (7394)
59 limit 58 to (cab reviews archive or cab reviews: perspectives or cabi full text or cabi full text bundle) [Limit not valid in Embase,Ovid MEDLINE(R),Ovid MEDLINE(R) Daily Update,Ovid MEDLINE(R) In-Process,Ovid MEDLINE(R) Publisher; records were retained] (5644)
60 remove duplicates from 59 (4693)

Search strategy for PubMed (Date 17th June 2016)

User query:	(((((Neonatal Sepsis) OR Neonatal bacteraemia) OR Neonatal infection)) AND etiology) AND (((((sub-Saharan Africa) OR West Africa) OR East Africa) OR Central Africa) OR Southern Africa)
Query Translation:	((((Neonatal[All Fields] AND ("sepsis"[MeSH Terms] OR "sepsis"[All Fields])) OR (Neonatal[All Fields] AND ("bacteraemia"[All Fields] OR "bacteremia"[MeSH Terms] OR "bacteremia"[All Fields]))) OR (Neonatal[All Fields] AND ("infection"[MeSH Terms] OR "infection"[All Fields]))) AND ("etiology"[Subheading] OR "etiology"[All Fields] OR "causality"[MeSH Terms] OR "causality"[All Fields])) AND ((((("africa south of the sahara"[MeSH Terms] OR ("africa"[All Fields] AND "south"[All Fields] AND "sahara"[All Fields]) OR "africa south of the sahara"[All Fields] OR ("sub"[All Fields] AND "saharan"[All Fields] AND "africa"[All Fields]) OR "sub saharan africa"[All Fields]) OR ("africa, western"[MeSH Terms] OR ("africa"[All Fields] AND "western"[All Fields]) OR "western africa"[All Fields] OR ("west"[All Fields] AND "africa"[All Fields]) OR "west africa"[All Fields])) OR ("africa, eastern"[MeSH Terms] OR ("africa"[All Fields] AND "eastern"[All Fields]) OR "eastern africa"[All Fields] OR ("east"[All Fields] AND "africa"[All Fields]) OR "east africa"[All Fields])) OR ("africa, central"[MeSH Terms] OR ("africa"[All Fields] AND "central"[All Fields]) OR "central africa"[All Fields] OR ("central"[All Fields] AND "africa"[All Fields]))) OR ("africa, southern"[MeSH Terms] OR ("africa"[All Fields] AND "southern"[All Fields]) OR "southern africa"[All Fields] OR ("southern"[All Fields] AND "africa"[All Fields])))
Result:	489
Translations:	
Sepsis	"sepsis"[MeSH Terms] OR "sepsis"[All Fields]
bacteraemia	"bacteraemia"[All Fields] OR "bacteremia"[MeSH Terms] OR "bacteremia"[All Fields]
infection	"infection"[MeSH Terms] OR "infection"[All Fields]
etiology	"etiology"[Subheading] OR "etiology"[All Fields] OR "causality"[MeSH Terms] OR "causality"[All Fields]
sub-Saharan Africa	"africa south of the sahara"[MeSH Terms] OR ("africa"[All Fields] AND "south"[All Fields] AND "sahara"[All Fields]) OR "africa south of the sahara"[All Fields] OR ("sub"[All Fields] AND "saharan"[All Fields] AND "africa"[All Fields]) OR "sub saharan africa"[All Fields]
West Africa	"africa, western"[MeSH Terms] OR ("africa"[All Fields] AND "western"[All Fields]) OR "western africa"[All Fields] OR ("west"[All Fields] AND "africa"[All Fields]) OR "west africa"[All Fields]
East Africa	"africa, eastern"[MeSH Terms] OR ("africa"[All Fields] AND "eastern"[All Fields]) OR "eastern africa"[All Fields] OR ("east"[All Fields] AND "africa"[All Fields]) OR "east africa"[All Fields]
Central Africa	"africa, central"[MeSH Terms] OR ("africa"[All Fields] AND "central"[All Fields]) OR "central africa"[All Fields] OR ("central"[All Fields] AND "africa"[All Fields])
Southern Africa	"africa, southern"[MeSH Terms] OR ("africa"[All Fields] AND "southern"[All Fields]) OR "southern africa"[All Fields] OR ("southern"[All Fields] AND "africa"[All Fields])

Search strategy for Africa-Wide Information Database (Date 17th June 2016)

Search ID#	Search Terms	Search Options	Results
S8	S6 AND S7	**Search modes** - Boolean/Phrase	3,826
S7	Cameroon or Central African Republic or Chad or Congo or "Democratic Republic of the Congo" or Gabon or Burundi or Djibouti or Eritrea or Ethiopia or Kenya or Rwanda or Somalia or Sudan or Tanzania or Uganda Angola or Botswana or Lesotho or Malawi or Mozambique or Namibia or South Africa or Swaziland or Zambia or Zimbabwe or Benin or Burkina Faso or Cape Verde or Cote d'Ivoire or Gambia or Ghana or Guinea or Guinea-Bissau or Liberia or Mali or Mauritania or Niger or Nigeria or Senegal or Sierra Leone …	**Search modes** - Boolean/Phrase	1,653,716
S6	S4 AND S5	**Search modes** - Boolean/Phrase	8,989
S5	S1 OR S2 OR S3	**Search modes** - Boolean/Phrase	167,149
S4	neonate or neonatal or newborn or infant	**Search modes** - Boolean/Phrase	39,578
S3	meningitis or pneumonia	**Search modes** - Boolean/Phrase	14,374
S2	bacteremia	**Search modes** - Boolean/Phrase	1,288
S1	sepsis or infection	**Search modes** - Boolean/Phrase	158,384

Neonatal admissions, quality of care and outcome: 4 years of inpatient audit data from The Gambia's teaching hospital

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Background: National facility-based neonatal mortality audits are an important source of data to identify areas for improvement of service delivery and outcome of care.
Objectives: To examine admissions to the neonatal unit, Edward Francis Small Teaching Hospital, Banjul, The Gambia and make recommendations for programme action to reduce mortality through improvements in the quality of care, particularly with respect to suspected neonatal infections.
Methods: Case notes were reviewed for all neonates admitted to the neonatal unit during a 5-year period (1 January 2009 to 31 December 2013) to assess outcome and quality of care. Data for 2009 were subsequently excluded because of the low proportion of records retrieved.
Results: Of the 4944 admissions between 1 January 2010 and 31 December 2013, 1734 infants (35%) died, with 57% of all deaths occurring within the first 48 hours of admission. There were 1267 early neonatal deaths (deaths occurring during the first 7 days of life), 67% of which occurred during the first 48 hours of life. Independent predictors of neonatal death in the multivariable analysis were; maternal lack of antenatal care, non-teaching hospital delivery, admission weight <1500g, abnormal blood glucose concentration (<2.6 mmol/L or >6.9 mmol/L) and hypothermia (axillary temperature <36.5°C). Forty-eight per cent of newborns had point-of-admission hypothermia. Possible severe bacterial infection (pSBI) accounted for 44% (2166/4944) of admissions, prematurity/low birthweight for 27% (1340/4944) and intrapartum-related conditions for 20%. Only 5% (104/2166) of pSBI cases had at least one supportive investigation; 41 had a chest radiograph, 26 had a blood culture and 43 had a lumbar puncture. Although 94% of the newborns received intravenous antibiotics, 55% of those who did lacked clinical evidence of pSBI and had no diagnostic work-up.
Conclusion: Priority areas for action include infection prevention and improved diagnosis and management. There is also scope to reduce hypothermia with feasible interventions particularly targeting preterm infants. Improved patient records and audit data with linked action and accountability are interventions which could prevent such deaths of newborns in The Gambia and other developing countries.

Keywords: Neonate, Newborn, Mortality, Infection, Audit, Quality of care, Antimicrobial

Introduction

The Millennium Development Goals (MDGs) era has witnessed a remarkable decline in child mortality worldwide but not in the neonatal period. With an estimated 2.9 million neonatal deaths each year, accounting for 44% of under-5 child deaths globally, neonatal survival is an 'unfinished agenda' of MDG 4 for child health and remains a priority for the post-2015 period.[1] The highest risk of neonatal deaths is in sub-Saharan Africa (SSA), with epicentres in West and Central Africa where the neonatal mortality rate (NMR) is estimated to be 35/1000 live births.[2] The risk of a West African infant dying in the first 28 days of life is almost ten times that of an infant born in a high-income country,

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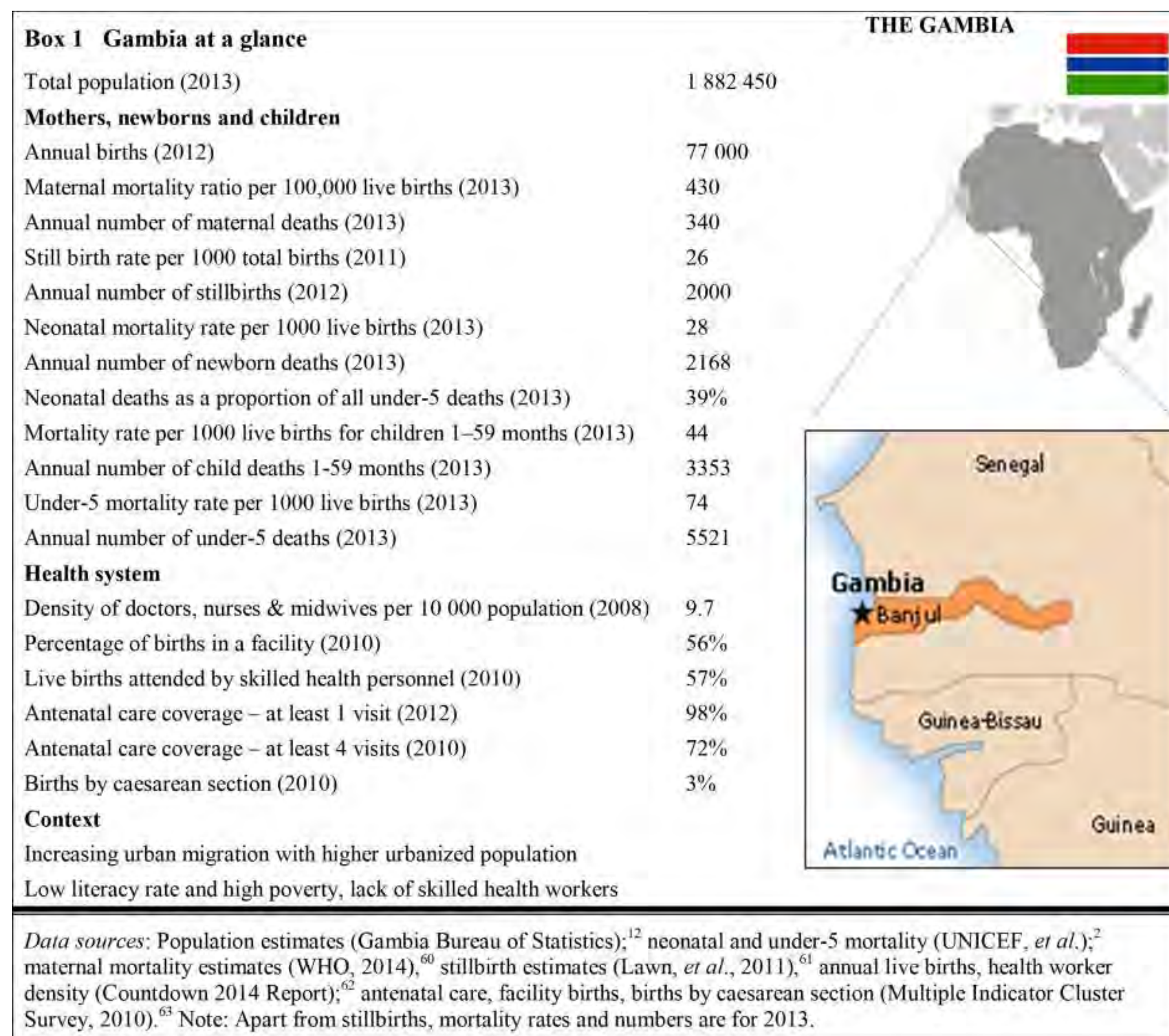
yet these countries are also making the slowest progress in reducing newborn mortality.[3] More than 80% of these deaths result from three preventable and treatable conditions: complications of prematurity, intrapartum-related deaths (including birth asphyxia) and neonatal infections.[4] Owing to limited laboratory resources and infrastructure, in most health facilities in SSA there are difficulties in diagnosing neonatal infection and in disaggregating it from other potential causes of death. In these settings, diagnosis depends on simplified clinical diagnostic algorithms that have high sensitivity and low specificity and often without laboratory confirmation.[5]

The Gambia is the smallest country in mainland Africa. In descending order, its neonatal mortality rate (NMR) (28/1000 live births) ranks 9th among the 15 countries of West Africa, and, globally, 30th of 194.[6] Data from the Farafenni Demographic Surveillance System located in a rural area on the North Bank region of The Gambia showed a 56% drop in under-5 mortality between 1992 and 2008, but the corresponding decline in neonatal mortality was only 38%.[7] Since 1990, there has been marked improvement in maternal and child health, despite high levels of poverty, limited resources and a shortage of adequately and appropriately trained staff (Box 1). However, The Gambia has not met the MDG4 target for child survival, and progress beyond 2015 now depends on reducing newborn deaths which account for 39% of under-5 mortality.[2] The public health sector comprises village health services (primary care), 38 minor and six major health centres, five general hospitals, a regional eye hospital and a university teaching hospital spread across eight local government areas and 43 districts.[8] Access to health facilities is relatively good, and over 85% of the population live within three kilometres of a primary health-care or outreach health post and 97% of the population within five kilometres.[9] The prevalence of HIV in adults aged 15–49 years was 1.2% in 2013,[10] and access to prevention of mother-to-child transmission (PMTCT) services is relatively high (80%). In 2011, the national prevalence of HIV1 and HIV2 infection in women attending antenatal clinics was estimated to be 1.65% and 0.07%,



Box 1 Gambia at a glance

Total population (2013)	1 882 450
Mothers, newborns and children	
Annual births (2012)	77 000
Maternal mortality ratio per 100,000 live births (2013)	430
Annual number of maternal deaths (2013)	340
Still birth rate per 1000 total births (2011)	26
Annual number of stillbirths (2012)	2000
Neonatal mortality rate per 1000 live births (2013)	28
Annual number of newborn deaths (2013)	2168
Neonatal deaths as a proportion of all under-5 deaths (2013)	39%
Mortality rate per 1000 live births for children 1–59 months (2013)	44
Annual number of child deaths 1-59 months (2013)	3353
Under-5 mortality rate per 1000 live births (2013)	74
Annual number of under-5 deaths (2013)	5521
Health system	
Density of doctors, nurses & midwives per 10 000 population (2008)	9.7
Percentage of births in a facility (2010)	56%
Live births attended by skilled health personnel (2010)	57%
Antenatal care coverage – at least 1 visit (2012)	98%
Antenatal care coverage – at least 4 visits (2010)	72%
Births by caesarean section (2010)	3%
Context	
Increasing urban migration with higher urbanized population	
Low literacy rate and high poverty, lack of skilled health workers	

Data sources: Population estimates (Gambia Bureau of Statistics);[12] neonatal and under-5 mortality (UNICEF, *et al.*);[2] maternal mortality estimates (WHO, 2014),[60] stillbirth estimates (Lawn, *et al.*, 2011),[61] annual live births, health worker density (Countdown 2014 Report);[62] antenatal care, facility births, births by caesarean section (Multiple Indicator Cluster Survey, 2010).[63] Note: Apart from stillbirths, mortality rates and numbers are for 2013.

respectively.[11] Among antenatal clinic attendees in Banjul, the prevalence of HIV1 was 1.9%; no case of HIV2 was detected.[11]

Patterns in newborn survival are a sensitive indicator of the functionality of a health system and its response to its most vulnerable population.[3] National facility-based neonatal mortality audits are an important source of data to identify areas for improvement in relation to service delivery and outcomes of care. In this paper, 4 years of neonatal inpatient audit data from the main national referral hospital in The Gambia between 2010 and 2013 are summarised. The aim was to examine admissions and outcome in newborns on the neonatal ward, and make recommendations for programme action to reduce mortality by improving the quality of care, particularly with respect to suspected neonatal infections.

Methods

Setting

The Edward Francis Small Teaching Hospital (EFSTH) in Banjul is the national teaching hospital and tertiary government referral hospital in The Gambia, to which patients come from all over the country.[12] It is the largest hospital in The Gambia, and the maternity unit delivers an average of 6000 newborns each year. In the labour ward, a sink, clean running water, soap and alcohol hand rubs are usually available. The midwife or attending medical officer mainly perform resuscitation of newborns as trained paediatricians or anaesthetists are not routinely available. Asphyxiated newborns receive oxygen by face-mask from a cylinder but on arrival at the neonatal unit, receive oxygen by nasal prongs from an oxygen concentrator. Bag-mask ventilation is not routinely performed because appropriately sized bags and masks are not always available. This is because they are often locked up (to prevent being stolen) and therefore not accessible when needed (personal communication with the Head of the Department of Obstetrics and Gynaecology). Endotracheal intubation of severely depressed infants is also not available.

The Edward Francis Small Teaching Hospital the only health facility in the country with a dedicated neonatal with 31 cots, separated into high- and low-risk areas. Cot occupancy is often more than 200% with neonates sharing cots and incubators during peak admission periods. Care is mostly supportive, consisting of intravenous fluids, antibiotics, nasogastric tube feeding, phototherapy, phenobarbitone, aminophylline and oxygen if indicated. Oxygen is administered from electricity-operated oxygen concentrators. During the period under review, there were only two oxygen concentrators available in the ward; using a splitter, up to ten infants could receive oxygen at one time from each concentrator. Skin-to-skin contact (kangaroo mother care, KMC) is not practiced. Neonatal intensive care facilities and major surgery are not available. A neonatologist, two medical officers and house officers staff the unit. In-house departmental training is occasionally conducted for medical and house officers as well as midwives, sometimes in conjunction with visiting foreign medical personnel. Trained nurses assisted by nurse attendants provide nursing care. There are usually between two and four trained nurses with an equal number of nurse attendants during the day (morning and afternoon shifts), and only one trained nurse and one nurse attendant during the night. This distribution is usually maintained during weekdays and weekends. Haematology and chemical pathology services are provided by the paediatric department's laboratory which is supported by the Medical Research Council (MRC) Unit in The Gambia. Microbiology is performed in the main hospital laboratory but blood culture samples are mostly sent to the MRC for processing. Full blood count and blood film for malaria parasites are the only laboratory investigations available during weekends. Blood glucose is measured on the ward using a glucometer; however, the glucometer strips are frequently out of stock. Laboratory sticks for protein measurement are not available. Only limited radiological services are available during weekends in the hospital.

Data collection

Data were collected retrospectively for the period 1 January 2009 to 31 December 2013. The medical records of neonates admitted during this period were retrieved from the Records Department. To determine the completeness of record retrieval, the ward admission books were reviewed to establish the total number of admissions for the period, and an exhaustive search was made for all missing records. Data were extracted from available records detailing dates of birth, admission and outcome; gender; birth and/or admission weight; estimated gestation; mode and place of delivery; antenatal care, obstetric complications; maternal HIV status; anti-retroviral administration for PMTCT; tetanus toxoid immunization and intermittent preventive treatment for malaria during pregnancy (ITPp); investigations and treatment; admission and final diagnoses. Where available, gestational age was copied from the antenatal card (this was usually calculated from the fundal height, and infrequently from an ultrasound scan), and few neonates were assessed for clinical maturity using the New Ballard Score.

Definitions and outcome

Diagnoses were mostly clinical. Sepsis, meningitis and pneumonia (possible severe bacterial infection, pSBI)

were diagnosed according to the diagnostic algorithms defined by the WHO Young Infants Clinical Signs Study Group.[13] Prematurity was defined as a gestational age of less than 37 completed weeks or a birthweight of <1.5 kg when the gestational age was not available; 1.5 kg (very low birthweight, VLBW) was used as the cut-off as preterm birth is the most likely cause of VLBW. Late preterm was defined as a gestational age of 34–36 completed weeks. For neonates admitted on the day of birth for whom no birthweight had been documented, the admission weight was used as the birthweight. Where no final diagnoses were provided, the admission diagnoses were used.

The primary outcome was death in the hospital. Age at admission and at death and length of hospital stay were calculated from the raw data. Times of admission and of death were examined using two different exposure categories. The first was based on whether admission occurred at the weekend (commencing on Friday at 2.00 p.m., when weekday work ends, until Monday at 7.59 a.m.) or during the week (Monday 8.00 a.m. until Friday 1.59 p.m.). The second exposure category was based on an aggregation of the day of the week (weekday *vs* weekend) and time of the day, defined as on-call duty hours (4.00 p.m. to 7.59 a.m. Monday to Thursday or at the weekend) and regular working hours (8.00 a.m. to 4.00 p.m. weekdays except Friday when it is 8.00 a.m. to 1.59 p.m.).

Statistical analysis

All statistical analyses were performed with STATA version 13 (Stata Corp., College Station, TX, USA) statistical significance defined as alpha 0.05 (two-sided). Categorical and continuous variables were summarised, respectively, as percentages and median (inter-quartile range). Cross-tabulations with outcome were performed using the χ^2 statistic for categorical variables. Neonates whose outcome could not be ascertained from their hospital records and those who were removed against medical advice (or absconded from care) were excluded from further analysis.

Neonatal characteristics of interest were assessed as risk factors for death using logistic regression, adjusting for age, sex and admission weight as potential confounders. Odds ratios with accompanying 95% confidence intervals and Wald test *P*-values (two-tailed) for the univariate and multivariable analyses are reported.

Ethical considerations

The Gambia Government/Medical Research Council Unit's Joint Ethics Committee approved the audit. Approval was also obtained from the Publications and Research Committee of the University of The Gambia School of Medicine and Allied Health Sciences.

Results

Between 1 January 2009 and 31 December 2013, there were 7161 admissions to the neonatal ward. There were on average 1432 admissions each year, with the fewest admissions (1307) recorded in 2011 and the highest (1502) in 2013. A total of 5285 neonatal medical records were recovered from the Records Department, representing 74% of all admissions for the period under review: 24% of records were retrieved for 2009, 77% for 2010, 80% for 2011, 98% for 2012 and 90% for 2013. Owing to low records capture, data from 2009 were subsequently excluded from analysis, and the results for 4944 admissions during 2010–2013 are presented.

Seasonality of admissions

The highest number of admissions (169) was recorded in October 2012, and the lowest (37) in February 2011 (Fig. 1). Between November and May, there is uninterrupted dry weather in The Gambia, and hot, humid weather predominates during the rest of the year, with a rainy season from June to October. A seasonal pattern of admissions was observed with increased admissions between September and December (peaking in October) each year. No seasonality was observed by place of delivery or admission weight.

Characteristics of neonates and mothers

The characteristics of admitted neonates are shown in Table 1. The majority (64%, 3142/4944) of births were at a health facility; however, birthweight was recorded in 67% (3336/4944) of all case notes. Gestational age records were missing for nearly two-thirds of neonates; of the 1289 neonates born at <37 completed weeks of gestation, 30% (387/1289) were between 34 and 36 weeks of gestation.

Respiratory rate was the most commonly recorded vital sign, found in 4525 (92%) of case notes; 38% (1729/4525) of newborns had a respiratory rate >60 breaths/minute. Forty-eight per cent (2282/4413) of newborns whose axillary temperature was documented at admission had hypothermia (temperature <36.5°C) and 28% (1310/4413) had fever (temperature ≥37.5°C). Hypothermia was more prevalent on admission in neonates born at EFSTH than in those born outside (69% *vs* 46%, $P<0.001$), and 80% of neonates admitted on the day of birth were hypothermic. Nearly two-thirds (62%) of those admitted with hypothermia weighed <2500g, and half of these were very small (<1500 g). The prevalence of hypothermia decreased significantly with increasing admission weight: 83% <1500 g, 51% 1500–2499 g and 36% ≥2500 g ($P<0.001$). Of the 3455 neonates with an

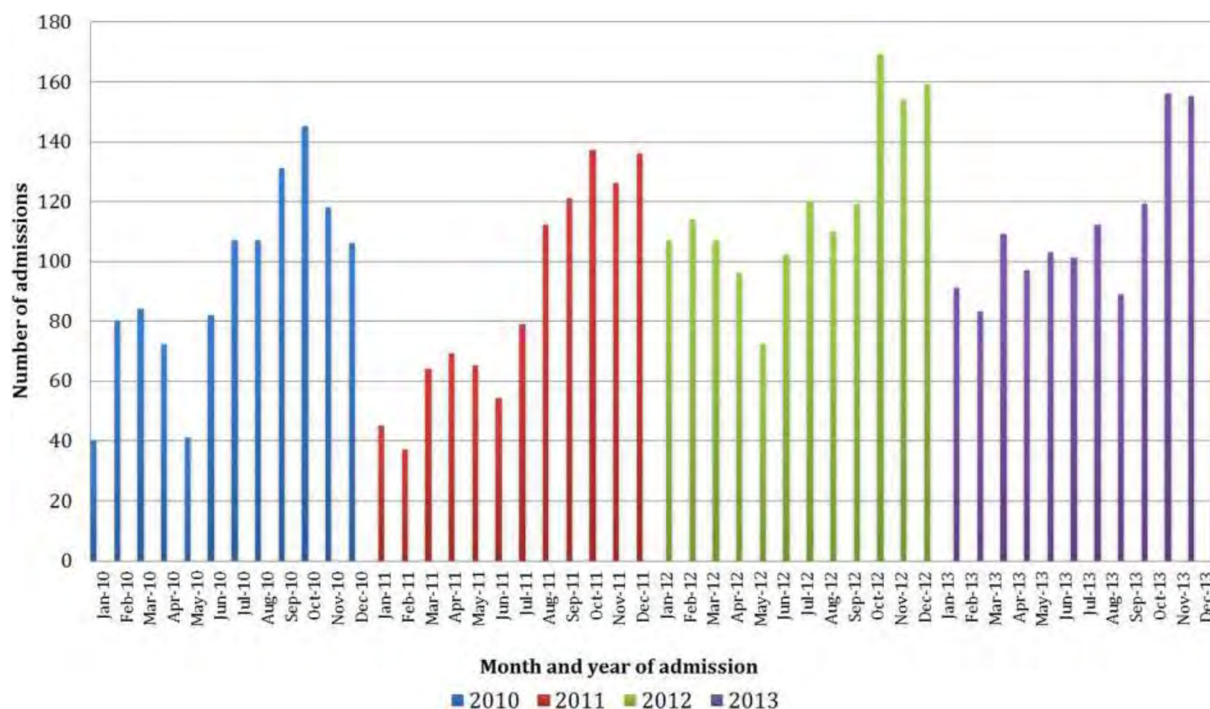


Figure 1 Seasonal pattern of admissions to the neonatal ward, EFSTH, Banjul, The Gambia between 1 January 2010 and 31 December 2013

Table 1 Characteristics of 4944 neonatal inpatients at EFSTH, Banjul, 2010–2013

Categories	*n* (%)
Male	2782 (56.3)
Unknown/missing	142 (2.9)
Age on admission, days	
Day of birth	2242 (45.4)
2–7	1550 (31.3)
≥8	907 (18.3)
Unknown/missing	245 (5.0)
Gestational age, wks	
Pre-term, <37	1289 (26.1)
Term, 37–42	500 (10.1)
Post-term, >42	17 (0.3)
Unknown	3138 (63.5)
Weight on admission, g	
<1500	942 (19.1)
1500–2499	1411 (28.5)
2500–3999	2347 (47.5)
≥4000	148 (3.0)
Unknown/missing	96 (1.9)
Place of delivery	
Home/TBA*	437 (8.8)
EFSTH	1590 (32.2)
Other health facility	1552 (31.4)
BBA†	14 (0.3)
Unknown/missing	1351 (27.3)
Mode of delivery	
Vaginal	3452 (69.8)
Caesarean	724 (14.7)
Unknown/missing	768 (15.5)
Maternal age, yrs	
<18	219 (4.4)
18–35	2924 (59.2)
>35	328 (6.6)
Unknown/missing	1473 (29.8)

* Traditional birth attendant; † born before arrival at a health facility

admission blood glucose measurement, hypoglycaemia (<2.6 mmol/L) was detected in 666 (19%) and hyperglycaemia (>6.9 mmol/L) in 608 (18%).

Information on maternal antenatal care was documented for 83% (4106) of newborns; the majority (62%, 2565/4106) of mothers received antenatal care, of whom 51% (1316/2565) attended at least four times. Evidence of maternal HIV screening was recorded for 132 (3%) newborns. Of the 132 mothers who were documented to have received counselling and testing, and/or PMTCT services, 30 (23%) were HIV-positive (mostly HIV-1, with only one case of HIV-1 and 2 dual infection). Only 11 (37%) of the 30 HIV-exposed newborns received a PMTCT drug (nevirapine). The presence or absence of obstetric complications was indicated in 59% (2937/4944) of records, most (70%, 2047/2973) of whom had at least one recorded complication.

Outcome

Overall, more than one-third (35%, 1734/4944) of neonates died during admission. There was evidence of a trend of increasing case-fatality from 33% in 2010 to 39% in 2013 (P=0.03). Outcome could not be ascertained for 17 neonates, and 38 neonates were taken home against medical advice. These newborns represented 2% of the dataset and did not differ significantly from newborns alive and well at discharge with regard to the variables of interest (data not shown); they were excluded from analysis of risk factors for mortality. The main causes of death in the unit were complications of pre-term birth, severe infections and intra-partum related events (Fig. 2A). Newborns admitted at weekends were more likely to die than those admitted during the remainder of the week (38% *vs* 35%, P=0.03);

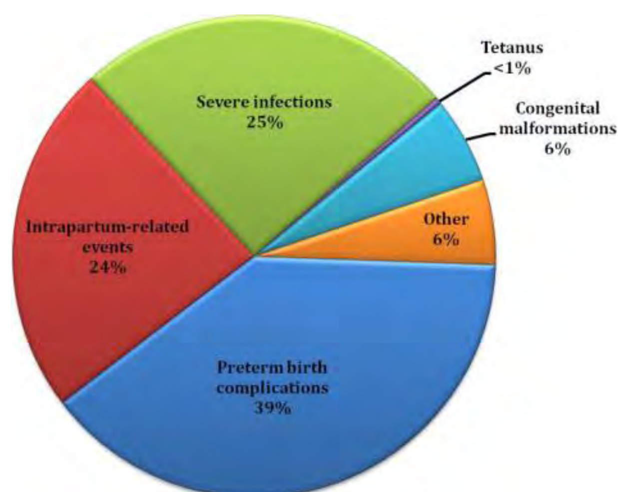


Figure 2 Distribution of causes of neonatal death in the neonatal ward, Edward Francis Small Teaching Hospital, 2010–2013 (Severe infections includes sepsis, meningitis and pneumonia)

similarly, the risk of death was greater for those admitted during on-call duty than for those admitted during regular working hours (38% *vs* 33%, $P=0.004$)

More than half of all deaths (57%, 989/1734) occurred during the first 48 hours of admission. Fifty-four per cent (482/897) of preterm neonates died compared with 38% (186/494) of term and 41% (7/17) of post-term neonates ($P<0.001$). Case fatality was associated with lower weight on admission; 58% (539/932) of neonates weighing <1500 g died compared with 34% (469/1399) of those weighing 1500–2499 g, 29% (674/2320) of those weighing 2500–3999 g, and 15% (22/144) of those weighing ≥4000 g (test for trend $P<0.001$).

Of the 1673 deaths with known age at death, 1267 (76%) were early neonatal deaths (defined as deaths occurring during the first 7 days of life), 67% (853/1267) of which occurred during the first 48 hours of life. Only 5% (83/1267) of neonates died after 21 days of age.

On multivariable analysis (Table 2), risk factors for neonatal death were weight <1500 g on admission (OR 1.61, 95% CI 1.15–2.26), delivery outside EFSTH [at home or by a traditional birth attendant (OR 2.17, 95% CI 1.40–3.38), delivery at another health facility (OR 1.79, 95% CI 1.34–2.39)], lack of antenatal care (OR 1.68, 95% CI 1.17–2.41), hypothermia (OR 2.48, 95% CI 1.76–3.49), hypoglycaemia (OR 1.60, 95% CI 1.19–2.15) and hyperglycaemia (OR 1.62, 95% CI 1.19–2.19).

Clinical diagnoses

Possible severe bacterial infection (pSBI) accounted for 44% (2166/4944) of admissions, 27% (1340/4944) prematurity/low birthweight (LBW), and 20% of intrapartum-related conditions. Jaundice was documented in 5% (243/4944) of newborns, two of whom had kernicterus; however, all were treated as cases of pSBI. Characteristics of newborns with pSBI are described further in Table 3. Of the 2026 pSBI cases with known age on admission, nearly half (984, 49%) were early-onset infections (defined as infections within the first 72 hours of life)

Skin and/or soft tissue infections were observed in 9% (421/4944) newborns; the most common soft tissue infections were mastitis and omphalitis. Congenital malformations accounted for 5% (241/4944) of admissions; the most common were neural tube defects/central nervous system malformations (20%, 48/241), cardiac chamber malformations (10%, 40/421), malformations of the intestinal tract (7%, 30/421) and musculo-skeletal malformations (5%, 21/421). Over the study period, there were 48 cases of ophthalmia neonatorum, 17 cases of tetanus and seven cases of diarrhoea, each accounting for less than 1% of admissions.

Investigations

The full blood count was recorded in 841 (17%) of the case notes, and haemoglobin concentration was documented in 1020 (21%). Serum bilirubin had been estimated in 36 (15%) of the 243 neonates with documented jaundice. Only 5% (104/2166) of pSBI cases had had at least one microbiological or radiological investigation: 41 had a chest radiograph, 26 had a blood culture and 43 had a lumbar puncture. Two blood cultures were positive for *Staphylococcus aureus*, one for *Escherichia coli* and three for coagulase-negative staphylococci. All neonates with positive blood cultures recovered and were discharged. Only one infant, a 2-day-old home/TBA-born neonate, had a positive cerebrospinal fluid (CSF) culture (*Klebsiella* species); he received antibiotics for 25 days but finally died.

Prescription of oxygen, intravenous (IV) fluids and antibiotics

There were 2559 neonates who, according to their history and examination findings (respiratory distress, cyanosis), met the indicators for oxygen therapy, but only 1965 (77% of cases in whom it was indicated) were documented to have received it. Seventy per cent (3455/4944) of neonates had documentation of receiving IV fluids. Antibiotics were the most frequently administered medication, and 94% (4635/4944) of neonates received parenteral (intravenous) antibiotics during admission. The majority (65%, 2995/4635) of these neonates received two antibiotics during admission, 27% (1235/4635) received three drugs, 5% (161/4635) received just one, and <1% (14/4635) five or more. Antibiotics are usually provided free of charge by the hospital; however, when they are out of stock, parents have to provide them. Ampicillin and gentamicin were the most

Table 2 Risk of death among neonatal inpatients 2010–2013 (excluding newborns who absconded or were taken home against medical advice

Categories	Dead, *n*	Discharged, *n*	Unadjusted OR	*P*	Adjusted OR	*P*
Male	695	1304	1.04 (0.92–1.17)	0.57	1.06 (0.85–1.34)	0.60
Female	978	1772	1		1	
Age on admission, days						
Day of birth	996	1225	2.87 (2.40–3.43)	<0.001	1.51 (0.93–2.45)	0.09
2–7	480	1052	1.61 (1.33–1.95)	<0.001	1.27 (0.81–2.00)	0.30
≥8	198	699	1		1	
Admission weight, g						
<1500	539	393	3.35 (2.86–3.92)	<0.001	1.61 (1.15–2.26)	0.01
1500–2499	469	930	1.23 (1.07–1.42)	0.004	0.86 (0.64–1.14)	0.30
2500–3999	674	1646	1		1	
≥4000	22	122	0.41 (0.37–0.48)	0.001	0.75 (0.38–1.47)	0.40
Place of birth						
Inborn (EFSTH)	529	1046	1		1	
Other health facility	640	899	1.41 (1.22–1.63)	<0.001	1.79 (1.34–2.39)	<0.001
Home/TBA	180	254	1.40 (1.13–1.74)	0.002	2.17 (1.40–3.38)	0.001
Mode of birth						
Vaginal	1325	2081	1		1	
Caesarean	196	525	0.60 (0.49–0.70)	<0.001	0.66 (0.48–0.91)	0.01
Resuscitation at birth						
Resuscitated	535	717	1.52 (1.33–1.73)	<0.001	1.33 (1.00–1.76)	0.05
Not resuscitated	1199	2438	1		1	
Maternal age, yrs						
<18	99	119	1.45 (1.10–1.91)	0.01	1.35 (0.85–2.15)	0.21
18–35	1059	1840	1		1	
>35	110	212	0.90 (0.70–1.15)	0.40	1.08 (0.73–1.58)	0.71
Maternal ANC						
None	535	978	1.30 (1.07–1.56)	0.01	1.68 (1.17–2.41)	0.01
1–4 visits	691	1084	1.51 (1.26–1.81)	<0.001	1.15 (0.87–1.51)	0.34
>4	228	540	1		1	
Temperature, °C						
<36.5	966	1178	2.99 (2.50–3.56)	<0.001	2.48 (1.76–3.49)	<0.001
36.5–37.4	203	739	1		1	
≥37.5	341	942	1.32 (1.08–1.61)	0.01	1.17 (0.79–1.73)	0.42
Blood glucose, mmol/L						
<2.6	325	336	2.14 (1.79–2.56)	<0.001	1.60 (1.19–2.15)	0.002
2.6–6.9	673	1490	1		1	
>6.9	281	320	1.94 (1.62–2.34)	<0.001	1.62 (1.19–2.19)	0.002
Time of day						
Regular work hours	508	1013	1		1	
On-call hours	1095	1805	1.21 (1.06–1.38)	0.004	0.97 (0.76–1.25)	0.84

Table 3 Characteristics and clinical assessment of neonates with possible severe bacterial infection (pSBI)

Characteristics	Total no.	pSBI *n* (%)	Non-pSBI *n* (%)	*P*
Place of birth, *n*=3593				
Inborn (EFSTH)	1590	379 (28)	1212 (54)	
Other hospital facility	1556	715 (53)	851 (38)	
Home/TBA	437	249 (19)	188 (8)	<0.001
Mode of delivery, *n*=4167				
Vaginal	3452	1522 (89)	1930 (78)	
Caesarean section	724	193 (11)	531 (22)	<0.001
Age at admission, days, *n*=4699				
1–3	2242	984 (49)	2313 (87)	
4–7	1550	413 (20)	83 (3)	
≥8	907	629 (31)	278 (10)	<0.001
Admission weight, g, *n*=4848				
<1500	942	187 (9)	756 (28)	
1500–2499	1411	643 (31)	768 (28)	
≥2500	2495	1280 (60)	1215 (44)	<0.001
Symptoms/signs*				
History of convulsions	393	264 (67)	129 (33)	<0.001
History of difficulty feeding	2192	1079 (49)	1113 (51)	<0.001
Fever, temp. ≥37.5°C	1259	1052 (81)	243 (19)	<0.001
Hypothermia, temp. <36.5°C	2165	455 (21)	1711 (79)	<0.001
Restlessness/irritability	474	359 (76)	115 (24)	<0.001
Difficulty breathing	2351	917 (39)	1434 (61)	<0.001
Lethargy/reduced movement	238	134 (56)	104 (44)	<0.001

*Row percentages

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frequently used medications (Fig. 3); among neonates who received a single antibiotic, gentamicin was the most commonly administered (122/161), followed by ceftriaxone (28/161). Fifty-five per cent (2533/4635) of neonates who received antibiotics, lacked clinical evidence of pSBI and did not have any diagnostic work-up. The median duration of treatment was 4 days (IQR 2–7, range 0–30) for both pSBI and non-pSBI neonates. Twenty-eight newborns received antibiotics for more than 21 days, of whom 50% (14/28) had a diagnosis of pSBI, and 25% (7/28) had congenital malformations. Ten (36%) of these newborns were also preterm and of low birthweight. Owing to a high case load and limited cot availability, most neonates were discharged once clinical improvement was obvious with oral antibiotics and advice on danger signs. Whether choice of fluid, fluid volumes, antibiotics and antibiotic dosage complied with WHO guidelines was not assessed.

Discussion

This study provides the first comprehensive description of morbidity and mortality and quality of care of sick newborns in a tertiary centre in The Gambia. As far as we know, this is the largest neonatal inpatient audit published from West Africa, and it provides a baseline from which to improve to clinical care and data collection.

Case fatality in the neonatal unit is higher than reported from similar tertiary referral hospitals in West Africa: 7% in Senegal,[14] 13–20% in Nigeria[15–18] and 13–15% in Burkina Faso.[19,20] The three most common causes of neonatal deaths in the unit are preventable, and are similar to the global and national WHO/Child Health Epidemiology Reference Group (CHERG) estimates for The Gambia for the same period[21] (Fig. 4), but rank differently. Whereas complications of preterm birth were the major cause of death in our unit, the WHO/CHERG estimates show intrapartum-related events to be the major cause of death in the country. This difference might be explained by the fact that most of the neonates admitted were facility-born and therefore more likely to reflect a selected population with obstetric complications, especially preterm birth. The risk of death in the unit was greatest on the day of birth and among very small infants. Small size at birth – owing to preterm birth or small-for-gestational-age (SGA), or both – is the greatest risk factor for most neonatal deaths.[3] Globally, antenatal corticosteroids have been of great benefit in preventing complications of preterm birth;[22] however, their use in preterm newborns admitted in the unit was not assessed. The majority of deaths occurred within 48 hours of admission. The inability of health-care facilities to provide timely, good-quality care on arrival at hospital is reported to be a leading[23] or secondary[24] cause of in-hospital newborn deaths. Although an increased odd of deaths of newborns admitted at the weekend (as well as among those admitted during on-call hours, data not shown) was noted, there was a lack of a weekend effect on death in the adjusted analysis. Consequently, improved survival of newborns requires fast interventions at any time of day or night as the time to death can range from a few minutes for the inadequately resuscitated neonate who does not breathe at birth, to an hour for the infant suffering a severe hypoxic event, and a few hours for severe early-onset sepsis.[3] All facilities which provide maternity services

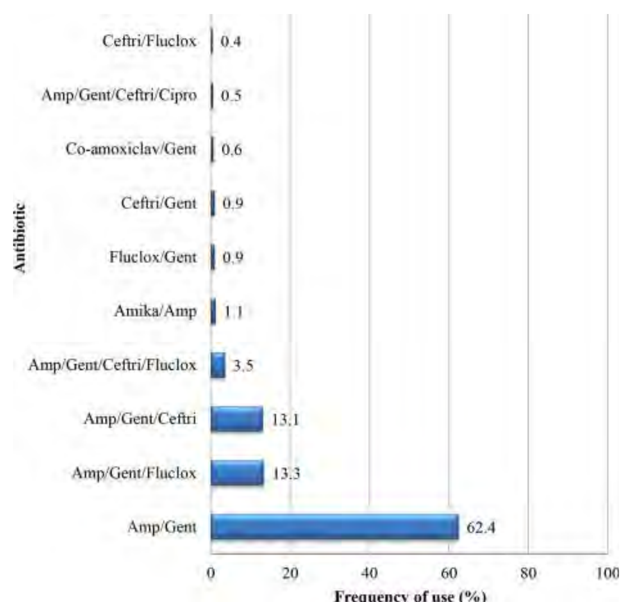

Figure 3 Parenteral antibiotic prescription patterns among 4635 neonatal inpatients at the EFSTH, Banjul, 2010–2013. (Amp, ampicillin; Gent, gentamicin; Fluclox, flucloxacillin; Ceftri, ceftriaxone; Amika, amikacin; Cipro, ciprofloxacin; Co-amoxiclav, amoxicillin and clavulanic aci)

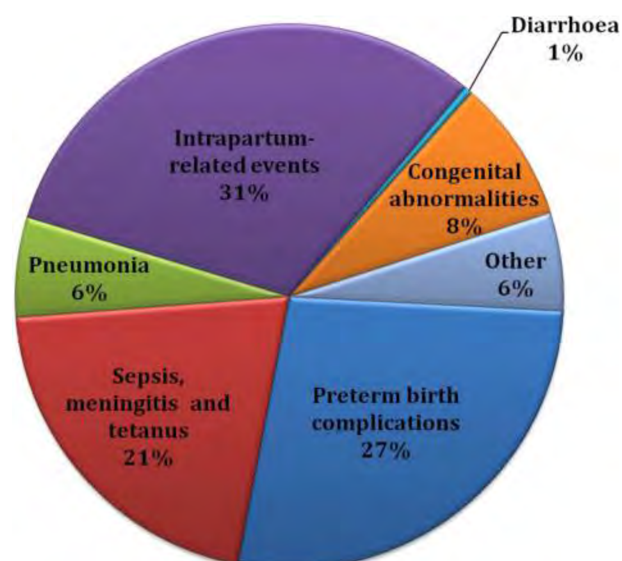

Figure 4 WHO/CHERG estimates for causes of neonatal death distribution in The Gambia, 2010–2013

should at least have a functioning mechanical suctioning device and a self-inflating bag and mask. Helping Babies Breathe (HBB) is an evidence-based educational programme to teach neonatal resuscitation techniques in resource-limited areas, with the goal of having at least one person who is skilled in neonatal resuscitation at every birth. Pre-service and in-service training curricula should be revised to support implementation of the HBB curriculum. As a tertiary referral hospital, EFSTH should in addition ensure that the appropriate equipment and expertise for endotracheal intubation are available for severely depressed infants.

The seasonal variation in admissions in our unit mirrors seasonal variations in births in EFSTH and nationwide (data not shown). Although seasonal variation is a cardinal feature of paediatric diseases in West Africa,[25] and accentuates the vulnerability of children in poor families, the reasons for it are not known. No seasonal variation was observed when stratified by admission weight; however, divergent patterns of seasonality have previously been reported in The Gambia for preterm birth and SGA.[26] In this study, the peaks in prematurity closely paralleled increases in agricultural labour (July) and malaria infections (October). The incidence of SGA was highest at the end of the annual hungry season, from August to December (peaking in November), and has been attributed to seasonal deterioration of nutritional status owing to food shortages, and to an increase in agricultural labour which often coincides with seasonal epidemics of infectious and parasitic diseases.[27]

Almost half of the neonates were admitted with clinical signs compatible with pSBI, and half of these presented as 'early-onset sepsis' (within the first 3 days of birth) as against 'late-onset sepsis' (after the first 3 days of birth until the end of the neonatal period). Early-onset sepsis is usually associated with vertically acquired infection from the birth canal, while late-onset sepsis is associated with acquisition from the home or hospital environment, particularly through the umbilical cord.[28] In developing countries, unclean delivery practices and initial care of the infant in hospital contribute to very early-onset infections.[29] The very high cot occupancy in the unit makes it difficult to isolate infected patients. Being the only neonatal referral centre, there is a strict policy not to turn patients away, leading to sharing of cots and incubators during peak admission periods. Consequently, infection control practices would have been sub-optimal, making it difficult to ascribe 'maternally acquired' or 'hospital-acquired' status to those presenting with early-onset neonatal infections.

There was a striking mismatch of high antibiotic usage and low laboratory investigations. Sick neonates do not routinely undergo microbiological investigations, mainly owing to an absence of ward protocols, unreliable diagnostic laboratory facilities with delayed results, and the general expectation of a 'negative' result by attending clinicians. The majority of newborns received only 'first-line' empirical treatment with the WHO-recommended ampicillin and gentamicin; however, many received additional antibiotics or other regimens including third-generation cephalosporins, and some were on treatment for as long as 30 days. In the absence of clinical guidelines, one major reason for the widespread use of ampicillin and gentamicin is the fact that the pharmacy does not dispense cephalosporins if a consultant has not signed the prescription. Unlike culture-proven sepsis, which is treated with a full course of antibiotics on the basis of antimicrobial sensitivity, the appropriate duration of treatment for suspected neonatal sepsis when cultures are negative or not available is a challenge.[30] Prolonged initial empirical antibiotic treatment (defined as ≥ 5 days of initial empirical antibiotic with sterile culture results) is associated with an increased risk of an adverse outcome, including invasive candidiasis and death, particularly in premature and extremely small newborns.[31,32] Moreover, antibiotic costs are not cheap and represent a significant proportion of the hospital drug budget.

Point-of-admission hypothermia was present in 48% of neonates. The prevalence of hypothermia on admission among newborns in SSA ranges from 22 to 85%.[33–35] Newborns must be kept warm at birth (at home or in hospital) and, especially, if ill and being transferred from home or between hospitals or within a hospital to a neonatal care unit. Preterm and small newborns are especially vulnerable, and can rapidly become hypothermic, increasing the risk of respiratory distress, hypoglycaemia, infections and death. About one-third of the newborns with hypothermia in the unit weighed <1500 g, and most were intra-hospital transfers from the maternity unit. Despite taking place within the hospital environment, that the distances involved are relatively short and the apparent risk of complications is low, intra-hospital transfer can constitute an additional risk, especially to pre-term infants.[36] Even in high-income settings, hospitals without a neonatal transport team and incubator may have significantly more deaths or adverse events in low-birth-weight infants with respiratory disease than do comparable hospitals with neonatal transport facilities.[37] Multi-disciplinary transport teams are, however, a luxury in low-income settings. Prevention of hypothermia should commence before delivery by ensuring that the place of delivery is warm, and maintaining a 'warm chain' of procedures at birth and during the hours and days that follow.[38]

These simple, cost-effective procedures include skin-to-skin contact with the mother (KMC) during transfer, wearing of hats, and the use of plastic bags/wraps particularly for very small and low-birthweight (LBW) newborns, and should be encouraged. KMC is particularly suitable when there is a lack of sophisticated equipment such as radiant heaters and incubators, and has been shown to substantially reduce mortality among clinically stable preterm/LBW infants in hospital.[39] It has also been shown to reduce the workload on the ward, thereby allowing nursing staff to focus attention on more unstable infants.[40] Minimal instruction is required and it can also be used in the community or at home. The current Gambian health policy framework does not include implementation of KMC;[8] there is therefore an urgent need to address barriers to scaling-up facility-based initiation of KMC in line with the post-2015 newborn health research priorities.[41]

Hypoglycaemia is an important contributor to hypothermia, particularly among those at risk (preterm or term SGA, large, infant of a diabetic),[42] and vice-versa: it maintains a vicious circle, which leads to weak sucking, weight loss and finally increased mortality. Nearly one-fifth of newborns in the unit were hypoglycaemic on admission, compared with one-third in a similar facility in Nigeria.[43] Breastfeeding prevents hypothermia by warming through the mother, especially by skin-to-skin contact, and also by replenishing a newborn's glucose levels. The Gambian national health policy subscribes to the principles of the Baby-friendly Hospital Initiative to implement practices which protect, promote and support breastfeeding.[44] Initiation of early breast-feeding is, however, difficult in newborns requiring admission, particularly if a mother is recovering from anaesthesia following caesarean delivery or is too ill with other post-partum complications. Delays in referral and transfer to the neonatal unit and establishing intravenous access further contribute to hypoglycaemia in those at risk.

There are several possible reasons for the sub-optimal neonatal care in The Gambia. One is the lack of appropriate equipment and trained staff. The level of laboratory support in the unit falls short of essential newborn care standards for a district hospital, let alone a referral-level hospital.[45] Although this deficiency highlights health system challenges in the provision of appropriate equipment (e.g. lack of blood culture bottles, laboratory reagents and equipment) and appropriately trained personnel (e.g. microbiologists and laboratory technicians), it also draws attention to the capabilities of the clinicians managing sick newborns as investigations were not requested for most of the neonates in whom there was clinical suspicion of infection.

Poor knowledge and training of neonatal care-providers and a shortage of qualified staff are associated with sub-optimal care in developing countries.[23,24] As is the case in most other countries with very high maternal and neonatal mortality, The Gambia lacks the minimum requirement of 23 doctors, midwives and nurses per 10,000 population to provide a basic package of care.[46] There are currently 157 Gambian and 479 non-Gambian medical doctors registered with the Medical and Dental Council. There are also non-registered Cuban, Egyptian and Syrian doctors providing medical care as part of bilateral agreements with their respective governments. Since its inception in 1999, 111 indigenous doctors have graduated from the University of The Gambia. Upon completion of a 2-year internship programme, the medical officers are usually retained at the EFSTH; since January 2014 however, the Ministry of Health has begun posting medical officers to district hospitals and health centres to serve communities. The goal is for 80% of all hospitals and major health facilities to be managed by indigenous doctors by 2016. Unfortunately, postgraduate medical training is not available in The Gambia and so locally trained doctors lack affordable avenues to acquire specialized skills and competencies for neonatal care; the provision of specialist neonatal care depends solely on expatriate doctors who also work in EFSTH. Nurses are the most accessible health-care providers in The Gambia with more nurses than doctors per 1000 people. Basic training for nurses follows either a 2-year track to the level of state-enrolled nurse (SEN) or a 3-year track to the level of state-registered nurse (SRN). SENs wishing to become SRNs have to undergo an additional 2 years of training. Most nurses undergo additional training in midwifery but there is no formal training in paediatric or neonatal nursing, and competence is acquired through experience. The mix of nursing staff is not regulated, and, in health centres, care-givers might not have the necessary competence to provide neonatal care, and may not even be nurses but attendants with no clinical training at all. Uncompetitive salaries and benefits have resulted in high attrition rates, staff shortages and low motivation which have further contributed to the poor quality of care. Urgent systematic attention is required, non-rotation of nurses with skills in neonatal care, and, where appropriate, the development of a neonatal nurse cadre, as well as rewarding those who work against the odds in hard-to-serve areas.[47]

In developed countries, detailed quality-of-care protocols and 'core nursing skills sets' for almost every aspect of newborn care have improved quality and given more responsibility for care to skilled neonatal nurses, particularly with regard to infection prevention, feeding support and use of intravenous

fluids.[48] Hospitals providing neonatal care have service standards that take account of the nurse-to-patient ratio (maximum number of patients who may be assigned to a nurse during one shift) as well as nursing skills, training and development depending on the category of newborn care.[49,50] The British Association of Perinatal Medicine's (BAPM) recommendations for neonate-to-nurse ratios for intensive care (IC), high dependency care (HDC) and special care (SC) in England are currently 1:1, 2:1 and 4:1, respectively.[49] Similar recommendations and service standards for essential neonatal care are lacking in The Gambia and West Africa, despite studies in Ghana and Nigeria which have shown that levels of nursing staff in a neonatal care unit affect patient outcomes (mortality and adverse events), patient experience, quality of care and the efficiency of care delivery.[51,52] Appropriate nurse-to-patient ratios cannot be generalized because of factors such as patient load and characteristics, availability of nurses and work environment; the ratio that is sufficient for one unit might be insufficient for another. A nurse-to-patient ratio of 1:12.5–1:25 has been reported in the neonatal intensive care unit of a Ghanaian teaching hospital.[51] WHO has developed a tool for calculating optimal health worker levels, known as the Workload Indicators of Staffing Need (WISN). The software calculates the number of health workers per cadre, based on health facility workload, and provides two indicators to assess staffing: the gap/excess between the current and required number of staff, and the WISN ratio, a measure of workload pressure. WISN has been used extensively in East and Southern Africa but there are no case studies of its use in West Africa.[42–44,53] Owing to a lack of essential information, the staffing requirements for the EFSTH neonatal unit could not be calculated.

Given the strong emphasis on improving referral pathways and promoting institutional delivery for high-risk births as a means of improving neonatal survival, more needs to be done to ensure that the EFSTH neonatal unit is suitably equipped and staffed. With just over 70,000 births annually, nearly 60% of which take place in health facilities, the 30-cot EFSTH neonatal unit is grossly inadequate to meet the national need for referral-level hospital care of sick newborns. As the proportion of neonates delivered in health facilities increases, it is necessary to accelerate coverage of essential newborn care. Urgent attention needs to be focused on scaling up the provision of appropriate packages of newborn care at district hospitals, and possibly major health centres, as recommended by WHO.[45] The packages of care with the greatest impact on preventing neonatal deaths and stillbirths include care during labour, childbirth and the first week of life, and care for the small and sick newborn.[54] These packages, the focus of WHO's Every Newborn action plan to end preventable deaths, include management of preterm births (including the use of antenatal corticosteroids), essential newborn care (hygienic care, thermal control, support for breastfeeding and newborn resuscitation), interventions to deal with complications arising from preterm birth and/or SGA, and neonatal infections (sepsis, meningitis, pneumonia and diarrhoea).[54] Appropriate management of small sick newborns includes extra thermal care and support for feeding small or pre-term newborns, including KMC, antibiotic treatment for infections and full supportive facility care. Since endorsement of the newborn health action plan in 2014, only two West African countries, Ghana and Nigeria, have hosted national newborn events and are in the process of developing their own national newborn action plans.[55]

Insufficient government funding of the health service, lack of an integrated maternal and newborn health policy, and greater investment on addressing maternal rather than fetal and newborn outcome are further reasons for sub-optimal neonatal care in The Gambia. In 2001, The Gambia and other African Union governments pledged to commit at least 15% of their annual budgets to improve the health sector.[56] At the end of 2013, only two West African countries, Togo and Liberia, had achieved this target.[57] In The Gambia, priority areas for child health have been scaling-up immunization coverage and prevention of deaths from malaria, pneumonia and diarrhoea. It is only in the last decade that attention has turned to the contribution of neonatal deaths to child mortality.[58,59] The Gambian national health policy for 2012–2020 is focused on 'acceleration of quality health services and universal coverage';[8] however, newborn survival and health are not specifically addressed. There is therefore an urgent need to strengthen newborn health components in existing health sector plans and strategies, especially those which relate to reproductive, maternal and child health, as outlined in the Every Newborn action plan.

The study has some limitations. Although data presented are from the main national tertiary referral hospital, it cannot be assumed that the findings represent neonatal morbidity and mortality in The Gambia as a whole. Furthermore, the results should be interpreted in the light of the following. Firstly, this is a retrospective study with data abstracted from routine medical records. About 26% of records for the period under review could not be located and data were missing for nearly all variables. Secondly, poor documentation of medical histories and examination findings, lack of systematic

assessment of gestation and possible ascertainment bias at the point of data abstraction from written records might have led to misclassification of diagnoses. Furthermore, diagnoses were based almost entirely on clinical assessment without laboratory support. A report on microbiological aetiology could not be presented as only six newborns had positive blood cultures and only one had a positive CSF culture. Lastly, poor documentation of care and the implicit assumption that written records reflect actual practice might have resulted in bias regarding quality of care.

This study has provided a comprehensive overview of inpatient care for newborns in The Gambia, showing that over one-third of them die, even in the country's teaching hospital. Further operational and research data are required on infections, notably regarding aetiologies and antimicrobial resistance. Education and training of health-care workers, development of guidelines and standards of care, and regular audit are necessary for the provision of high-quality neonatal services. Creation of national and regional perinatal/neonatal databases for stillbirths, pre-term and very low-birthweight infants, as well as those meeting other eligibility requirements, will provide data for improving outcomes and increasing the quality, safety and value of newborn care through quality improvement collaborations with other countries in the sub-region.

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Disclaimer Statements

Contributors UO designed the study, analysed and interpreted the data, and drafted the manuscript. KB and BK advised on the study design and contributed to the development of the questionnaire as well as the manuscript. SMAZ, JEL and SRCH proofed the data analysis and contributed to the manuscript. TB and KK collected most of the data and contributed to the manuscript.

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Conflicts of interest None.

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Appendix 4: Definition of Clinical Signs Of Possible Serious Bacterial Infection

Fever	If the newborn's axillary temperature is found ≥38.0°C, the infant is unwrapped and hydrated (breast feed for breastfed infants) during a 30 minutes period. A second temperature reading is taken 30 minutes after unwrapping. The baby is considered as having fever only if the second reading after 30 minutes is ≥38.0°C
Hypothermia	If the newborn's axillary temperature is found ≤35.5°C, the infant is wrapped with warm dry clothes including head and extremities (using e.g. a woollen cap, woollen sweater or woollen socks) and then wrapped in a blanket for 30 minutes. A second temperature reading is taken 30 minutes after unwrapping. The baby is considered as having fever only if the second reading after 30 minutes is ≤35.5°C
Lethargy	The newborn only moves (e.g. limb movement or eye opening) on tactile stimulation, but is not otherwise moving **OR** wakes up but does not stay awake after a disturbance.
Convulsions	During an episode of convulsion, the child's arms and legs stiffen because the muscles are contracting. The child may lose consciousness or not be able to respond to any other external stimulus. There may also be random or roving eye movements, eyelid blinking or fluttering, eyes rolling up, eye opening, staring, sucking, smacking, chewing and protruding tongue, unusual bicycling or pedalling movements of the legs, thrashing or struggling movements; long pauses in breathing (apnea)
Fast breathing	If when quiet, at rest or sleeping, the newborn breathes 60 times or more in one minute, count again. The baby is considered as having fast breathing only if the second count is ≥60 breaths/minute.
Severe chest indrawing	Lower chest indrawing present each and every breath observed for one minute
Poor feed with poor suck	According to the mother or care giver, the newborn (without any congenital malformation of the mouth) is not sucking the breast well; in a non-nutritive suck, the infant is reluctant to turn the head towards the stimulus; even after touching the roof of the mouth, he initiates only few sucks then becomes reluctant to suck again; not adequate for breastfeeding. If the infant has not fed in the previous hour, the mother is asked to put her infant to the breast and breastfeeding observed for 4 minutes. If the infant was fed during the last hour, the mother is asked to inform the study nurse when the infant is willing to feed so that the feeding can be observed. During feeding assessment, the baby is placed in comfortable position, well attached and the mother helped to keep the baby in the correct position. Feeding difficulty is then measured. The newborn is considered to have feeding difficulty if there is a complete suspension of sucking motion and if the infant is consistently refusing the mother's breast or other liquid.

Appendix 5: Staff Training Manual for Collection of Study Samples

Aetiology of Serious Neonatal Infections in The Gambia

Study Specific Procedure (SSP)

SSP Title:	BLOOD, NASOPHARYNGEAL SWAB, RECTAL, GENITAL (RECTO-VAGINAL) SWAB & CEREBROSPINAL FLUID COLLECTION			
SSP Number:	01	Version and date:	1.0	25 January 2016
Category:	Clinical			
SCC/Protocol Number	1384			
Prepared by: (Name and job title)	Dr. Uduak Okomo, MRC Clinical Research Fellow			
Signature and date*	----------			----------
Reviewed by:	Muhammed Camara, Junior Clinical Trials Monitor			
Signature and date*	----------			----------
Approved by: (Name of Principal Investigator)	Dr. Uduak Okomo			
Signature and date*	----------			----------
Date effective:	25 January 2016			

* to be hand-written to indicate approval.

1. Abbreviations, contractions and definitions

ANC	Assistant Nurse Coordinator
BC	Blood Culture
BHC	Brikama Health Centre
CRF	Case Report Form
CSF	Cerebrospinal fluid
DM	Data Manager
EFSTH	Edward Francis Small Teaching Hospital
FHC	Fajikunda Health Centre
FW	Field Worker
JFPH	Jammeh Foundation for Peace Hospital
LT	Laboratory Team
Lab Tech	Laboratory Technician
MRC	Medical Research Council; representing MRC Unit, The Gambia
NPS	Nasopharyngeal Swab
NFW	Nurse Field Worker
PI	Principal Investigator
PCB	Polyclinic Banjul
SKGH	Serrekunda General Hospital
SHC	Serrekunda Health Centre
SukHC	Sukuta Health Centre
SO	Scientific Officer
SOP	Standard Operating Procedure
SSP	Study Specific Procedure
STTG	Skim-milk tryptone glucose glycerol
RC	Research Clinician
RP	Research Paediatrician
RS	Rectal Swab
RVS	Recto-vaginal Swab

2. Background

2.1. This is an observational study aiming to describe the aetiology of serious neonatal infections at the Edward Francis Small Teaching Hospital, Banjul and examine the role of maternal colonisation in these infections by examining the recto-vaginal flora in mothers of neonates with confirmed bacterial infection, comparing isolates from mother–newborn pairs in order to understand the extent to which the same isolates are found in both mother and newborn, and also compare the recto-vaginal flora in mothers of these sick neonates with that of mothers who have healthy newborns.

2.2. According to section B3 of the study SCC proposal the following samples will be collected from consented participants at enrollment (Appendix 1):

Participant	**Samples to be collected**
Case	1. Venous blood (for culture & molecular studies) 2. Nasopharyngeal swab (NPS) for culture and PCR 3. Rectal swab (RS) for culture and PCR 4. Cerebrospinal fluid (CSF) for culture, biochemistry and PCR
Healthy Control	1. Venous blood (for molecular studies only) 2. Nasopharyngeal swab (NPS) for culture and PCR 3. Rectal swab (RS) for culture and PCR
Mother of infant	1. Nasopharyngeal swab (NPS) for culture and PCR 2. Recto-vaginal swab (RVS) for culture and PCR

2.3. Collected samples will be transported to the following locations at MRC, Fajara as appropriate:

- The Clinical Microbiology Lab, Kuyateh building **AND/OR**
- The Biobank (Freezer Room), Himsworth building

3. Purpose & Scope

3.1. This SSP describes the collection of blood, NPS, RVS, RS and CSF samples from study participants and the transportation of these samples to the relevant laboratories at MRC Fajara.

3.2. The SSP applies to research clinicians (RC), nurses, fieldworkers (FW), who will be involved in the collection, transportation and receipt of blood samples.

4. Safety Precautions

Infection can be transmitted from patient to staff and from staff to patient during collection of blood and other body fluids. Viral agents are the greatest hazard and in some instances, are potentially lethal. Of particular importance are the viruses causing hepatitis and acquired immune deficiency syndrome (AIDS).

All study staff MUST follow appropriate safety precautions detailed below when drawing, handling and transporting blood samples and other body fluids to different laboratory units at Fajara.

4.1 Venepuncture (for collection of venous blood specimen)

4.1.1. Ensure all study staff follow body fluid precaution according to the MRC safety manual when collecting, handling and transporting samples to the lab.

4.1.2. Wear disposable latex gloves that are impermeable to liquids whilst performing the procedure and used gloves will be disposed of in waste bags (yellow colour) for biological waste.

4.1.3. Change gloves between patients;

4.1.4. The used needle(s) must be discarded in the "sharpsafe" box after the procedure.

4.1.5. Used syringes will be disposed of in waste bags (yellow colour) for biological waste.

4.1.6. Use new needles and a new syringe(s) for each participant;

4.1.7. Maintain a clean, clutter-free and disinfected work space at all times.

4.2 Lumbar Puncture (LP)

4.2.1. Ensure all study staff follow body fluid precaution according to the MRC safety manual when collecting, handling and transporting samples to the lab.

4.2.2. Wear sterile latex gloves that are impermeable to liquids whilst performing the procedure and used gloves will be disposed of in waste bags (yellow colour) for biological waste.

4.2.3. The used needle(s) must be discarded in the "sharpsafe" box after the procedure.

4.2.4. Use new needles for each participant;

4.2.5. Maintain a clean, clutter-free and disinfected work space at all times.

4.3 Nasopharyngeal swab (NPS)

4.3.1. Ensure all study staff follow body fluid precaution according to the MRC safety manual when collecting, handling and transporting samples to the lab.

4.3.2. Wear disposable latex gloves that are impermeable to liquids whilst performing the procedure and used gloves will be disposed of in waste bags (yellow colour) for biological waste.

4.3.3. The swab stick must be discarded in the "sharpsafe" box after the Floqswab tip has been cut off.

4.4 Recto-vaginal swab (RVS) and newborn Rectal swab (RS)

4.4.1. Ensure all study staff follow body fluid precaution according to the MRC safety manual when collecting, handling and transporting samples to the lab.

4.4.2. Wear disposable latex gloves that are impermeable to liquids whilst performing the procedure and used gloves will be disposed of in waste bags (yellow colour) for biological waste.
The swab stick must be discarded in the "sharpsafe" box after the Floq swab tip has been cut off.

4.4.3. Use new needles and a new syringe(s) for each participant;

4.4.4. Maintain a clean, clutter-free and disinfected work space at all times.

5. Equipment / Materials / Supplies / Reagents

5.1 Nasopharyngeal swab

- Tray
- Gloves
- Swab stick (Floq swab)
- Scissors
- STGG cryovials
- Sticker labels sheet
- Leak-proof specimen transportation bags
- "Sharpsafe" box
- Ice packs
- Cold box
- Waste disposal bag (yellow)

5.2 Genital (Recto-vaginal) swab

- Tray
- Gloves
- Swab stick (Floq swab)
- Scissors
- STGG cryovials
- Sticker labels sheet
- Leak-proof specimen transportation bags
- "Sharpsafe" box
- Ice packs
- Cold box
- Waste disposal bag (yellow)

5.3 Rectal swab

- Tray
- Gloves
- Swab stick (Floq swab)
- Scissors
- STGG cryovials
- Sticker labels sheet
- Leak-proof specimen transportation bags
- "Sharpsafe" box
- Ice packs
- Cold box
- Waste disposal bag (yellow)

5.4 Blood (Venepuncture)

- Tray
- Tourniquet;
- Well-fitting non-sterile gloves;
- 70% alcohol swab pads for skin disinfection;
- 10% povidone-iodine swab pads for skin disinfection;
- Sterile no. 23 butterfly needle (blue);
- Sterile 1ml or 2 ml syringe,
- Sterile 21G (green) needle
- Bactec peds plus/F blood culture bottle (pink top);
- EDTA microtainer tube (lavender top)
- Sterile cotton ball to be applied over the puncture site;
- Stella strip or adhesive plaster
- Laboratory specimen labels;
- Laboratory forms
- Leak-proof specimen transportation bags;
- "Sharpsafe" box
- Waste disposal bag (yellow)

5.5 Lumbar puncture

- Well-fitting sterile gloves;
- Masks – for person performing procedure and assistant
- 70% alcohol swab pads for skin disinfection
- 10% Povidone-Iodine swab pads for skin disinfection
- 1% Lignocaine
- 1 ml syringe
- Tuberculin (25G) needle
- Neonatal spinal needle (Size 25G) OR Size 23G needle
- 2 Sterile Bijou bottles
- Glucometer
- Pulse oximeter
- Aprons
- NaF tubes for glucose and protein estimation (grey top)
- Sterile gauze or cotton ball to be applied over the puncture site
- Sterile drapes
- Adhesive plaster
- Laboratory specimen labels
- Laboratory forms
- Leak-proof specimen transportation bags;
- "Sharpsafe" box
- Waste disposal bag (yellow)

6. Responsible Persons

6.1 The principal investigator (PI) has the overall responsibility of ensuring that this SSP is appropriately followed to enable timely collection and transportation of study samples to the appropriate laboratories at MRC Fajara.

6.2 The PI and The Nurse coordinator (NC) are responsible for ensuring that:

- Staff are appropriately trained on these procedures
- Accurate documentation/records are maintained

6.3 The nurse coordinator (NC) supported by the assistant nurse coordinator (ANC) are responsible for ensuring that:

- All the necessary supplies are available at the EFSTH and other sites (SKGH, BHC, FHC, PCB, JFPH, SHC), as appropriate;
- Samples are collected, labeled and transported in a timely manner;
- Accurate and complete sampling logs are maintained at the EFSTH and other sites as appropriate;
- "Sharpsafe" containers are transported to Fajara for incineration.
- All samples (except CSF) are transported back to the laboratories at Fajara within three hours. ***Note – CSF samples must be in the labs in Fajara within one hour of collection.***

6.4 The NC and study nurses are responsible for collecting required blood, NPS, RVS and RS samples from study subjects.

6.5 Only Clinicians will collect CSF samples.

6.6 The NC is responsible for establishing a rota for the transportation of samples to MRC Fajara when required.

6.7 The Field worker (FW) or designated Nurse is responsible for transporting samples in a timely manner to the Clinical Microbiology Laboratory (when collected at the site) and Biobank (Freezer room) units at MRC, Fajara and for ensuring the samples are handed over to a member of the laboratory team and that this is documented

6.8 Scientific officer (SO) and laboratory technicians (LT) are responsible for the receipt of blood samples in the laboratories at MRC Fajara and for subsequent processing of these samples

7. Procedures

7.1 Collection of materials & preparation of work spaces

	Description	**Person(s) responsible**
7.1.1.	Ensure that the materials and equipment listed in section 5 are available.	NCs
7.1.2.	Make sure that materials to be used have not reached their expiry date	NCs & Nurses
7.1.3.	Deliver materials listed in section 5 to the site(s) and monitor stock levels	NCs
7.1.5.	Ensure that the sample collection room is fully equipped daily before participants are brought for sample collection.	NCs
7.1.6.	Maintain an up-to-date inventory of study supplies	NCs

7.1.7.	Ensure that the sample collection room and work space are clean and free from clutter.	NCs & Nurses
7.1.8.	Inspect the materials for each procedure to confirm that they have not reached their expiry dates.	Nurse
7.1.9.	Arrange the material required for the procedure on a tray and ensure that this is visible, accessible and safe.	Nurse
7.1.10.	Ensure that the rack for sample collection is close by, but away for the patient to avoid accidental knocking over.	Nurse
7.1.11.	Two nurses must be present during procedures. The role of the second staff member is to assist in the procedure, to cross-check the identity of the subject from whom samples will be obtained, complete/cross-check relevant documentation	Nurse
7.1.12.	Cross check the subject's identity against the CRF folder to ensure that these match. This step must be confirmed by a second person. This is to ensure that the samples are collected from the right subject.	Nurse
7.1.13.	Affix the pre-printed barcoded labels on their corresponding tubes immediately after the sample is collected and dispensed into the sample container, ensuring that these labels contain the correct information (study number and sample type).	Nurse

7.2 Collection of samples

Procedure(s)	Responsible person(s)
NPS, RVS, RS	All study nurses
Venepuncture	All study nurses /Clinician
LP (CSF collection)	Clinician assisted by any study nurse

The NC/Nurse will telephone the designated laboratory staff (Lab Tech/SO) at the Clinical Microbiology and notify that samples being transported to the lab. This advance telephone notification is necessary to enable laboratory staff to prepare and receive the samples.

7.2.1 Nasopharyngeal swab in newborns

7.2.1.1. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. Use required swab sticks for NPS (small tip).

7.2.1.2. Explain the procedure to the mother. Ensure that informed consent has been given

7.2.1.3. Use clean dry cotton swab to clean off mucous from the nose of the newborn.

7.2.1.4. Estimate the distance to the nasopharynx before insertion of the swab and whilst it is still in the package, measure the distance from the corner of the nose to the front of the ear.

7.2.1.5. Peel off the protective wrapping around the swab stick and remove the swab stick. Do not let your fingers touch the cotton end of the swab

7.2.1.6. When swabbing the baby, ask your assistant (the mother or another study nurse) to help support the baby's head. Tip the baby's head slightly backward and pass the swab directly

backwards, parallel to the floor of the nasopharynx keeping close to the midline/nasal septum. The swab should pass without resistance until it reaches the posterior pharynx, which is about half the distance from the nostril to the ear lobe (**Figure 1**).

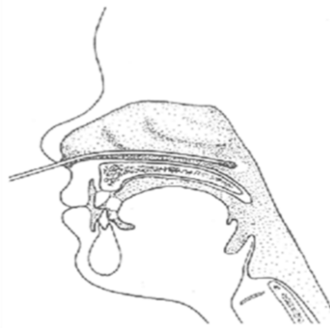

Figure 1: Nasopharyngeal swab collection in a baby

7.2.1.7. If resistance is encountered, remove the swab and dispose safely into the “sharpsafe” box. Use a new swab and try the other nostril.

7.2.1.8. Once the swab is in place, leave in position for five seconds to saturate the tip, and then rotate the swab to the left and right before removing it slowly.

7.2.1.9. Remove the cap from the sterile vial containing 1.5ml of STGG transport media and place the tip of the swab into the tube.

7.2.1.10. Using a pair of scissors wiped with alcohol, cut off the excess swab stick handle, leaving the tip in the transport media. Ensure the cap fits firmly. Put the vial containing the swab into the transport bag and place the bag on wet ice in the cold box.

7.2.1.11. Dispose what remains of the swab stick handle into the yellow waste bag for safe disposal.

7.2.1.12. Stick the appropriate labels on the vial containing the swab tip, the laboratory form and the corresponding CRF section.

7.2.1.13. Repeat the procedure with a second swab stick.

7.2.1.14. Complete the ***sample collection log***.

7.2.1.15. Ensure that both specimens and are transported to the MRC Fajara within six hours of collection.

- The swab labelled “**Baby NPS culture**” should be given to the designated staff (study Lab Tech) at the microbiology laboratory, Clinical services department.
- The swab labelled “**Baby NPS PCR**” should be taken to the Biobank (Freezer Room) and stored in the designated Freezer.

7.2.1.16. Complete the appropriate ***sample receipt logs*** that are kept at the laboratory and Freezer room respectively.

7.2.2 Nasopharyngeal swab in mothers

7.2.2.1. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. Use required swab sticks for NPS (large tip).

7.2.2.2. Explain the procedure to the mother

7.2.2.3. Estimate the distance to the nasopharynx before insertion of the swab and whilst it is still in the package, measure the distance from the corner of the nose to the front of the ear.

7.2.2.4. Peel off the protective wrapping around the swab stick and remove the swab stick. Do not let your fingers touch the cotton end of the swab

7.2.2.5. Tip the mother's head slightly backward and pass the swab directly backwards, parallel to the floor of the nasopharynx keeping close to the midline/nasal septum (**Figure 2**). The swab should pass without resistance until it reaches the posterior pharynx, which is about half the distance from the nostril to the ear lobe.

Figure 2: Nasopharyngeal swab collection in adult

7.2.2.6. If resistance is encountered, remove the swab and dispose safely into the "sharpsafe" box. Use a new swab and try the other nostril.

7.2.2.7. Once the swab is in place, leave in position for five seconds to saturate the tip, and then rotate the swab to the left and right before removing it slowly.

7.2.2.8. Remove the cap from the sterile vial containing 1.5ml of STGG transport media and place the tip of the swab into the tube.

7.2.2.9. Using a pair of scissors wiped with alcohol, cut off the excess swab stick handle, leaving the tip in the transport media. Ensure the cap fits firmly. Put the vial containing the swab into the transport bag and place the bag on wet ice in the cold box.

7.2.2.10. Dispose what remains of the swab stick handle into the yellow waste bag for safe disposal.

7.2.2.11. Stick the appropriate labels on the vial containing the swab tip, the laboratory form and the corresponding CRF section.

7.2.2.12. Repeat the procedure with a second swab stick.

7.2.2.13. Complete the ***sample collection log***.

7.2.2.14. Ensure that both specimens and are transported to the MRC Fajara within six hours of collection.

- The swab labelled "**Mother NPS culture**" should be given to the designated staff (Lab Technician) at the microbiology laboratory, Clinical services department.
- The swab labelled "**Mother NPS PCR**" should be taken to the Biobank (Freezer Room) and stored in the designated Freezer.

7.2.2.15. Complete the appropriate ***sample receipt logs*** that are kept at the laboratory and Freezer room respectively.

7.2.3 Genital (Recto-vaginal) swab in mothers

7.2.3.1. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. Use the required swab sticks for genital swab (large tip).

7.2.3.2. Explain the procedure to the mother.

7.2.3.3. Wipe the lower vagina (vaginal introitus) for 5 seconds with large enough sterile cotton swab. Do not let your gloved fingers touch the mother's skin.

7.2.3.4. Peel off the protective wrapping around the swab stick and remove the swab stick. Do not let your fingers touch the cotton end of the swab

7.2.3.5. Insert the swab a distance of 2cm into the lower vagina. Gently rotate the swab from left to right swabbing the sides and then remove. (**Figure 3A**)

7.2.3.6. Next, insert **the same swab** a distance of 1cm into the anus (**Figure 3B**)

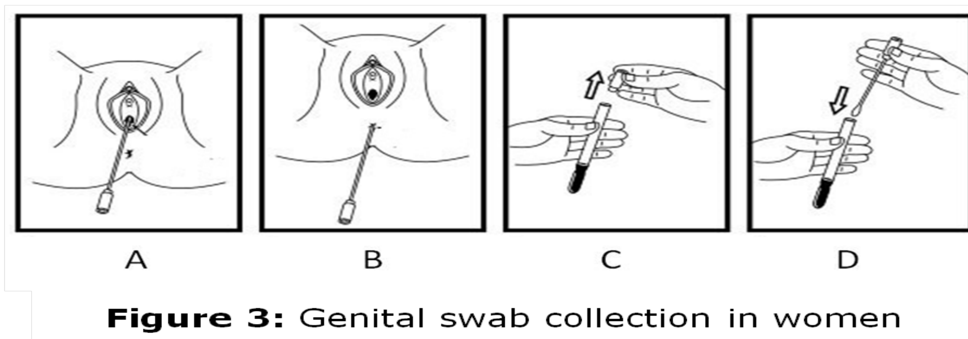

Figure 3: Genital swab collection in women

7.2.3.7. Remove the cap from the sterile vial containing 1.5ml of STGG transport media (**Figure 3C**), and place the tip of the swab into the tube (**Figure 3D**).

7.2.3.8. Using a pair of scissors wiped with alcohol, cut off the excess swab stick handle, leaving the tip in the transport media. Ensure the cap fits firmly. Put the vial containing the swab into the transport bag and place the bag on wet ice in the cold box.

7.2.3.9. Dispose what remains of the swab stick handle into the yellow waste bag for safe disposal.

7.2.3.10. Stick the appropriate labels on the vial containing the swab tip, the laboratory form and the corresponding CRF section.

7.2.3.11. Repeat the procedure with a second swab stick.

7.2.3.12. Complete the ***sample collection log***.

7.2.3.13. Ensure that both specimens and are transported to the MRC Fajara within six hours of collection.

- The swab labelled "**Mother RVS culture**" should be given to the designated staff (study Lab Tech) at the microbiology laboratory, Clinical services department.
- The swab labelled "**Mother RVS PCR**" should be taken to the Biobank (Freezer Room) and stored in the designated Freezer

7.2.3.14. Complete the appropriate ***sample receipt logs*** that are kept at the laboratory and Freezer room respectively.

7.2.4 Rectal swab in newborns

7.2.4.1. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. Use required swab sticks for RS (Small tip).

7.2.4.2. Explain procedure to mother

7.2.4.3. Ask your assistant (the mother or another study nurse) to help you hold the baby in position lying in bed with the legs lifted up.

7.2.4.4. Peel off the protective wrapping around the swab stick and remove the swab stick. Do not let your fingers touch the cotton end of the swab.

7.2.4.5. Insert the swab a distance of 1cm into the anus (**Figure 4**). Gently rotate the swab from left to right swabbing the sides and then remove.

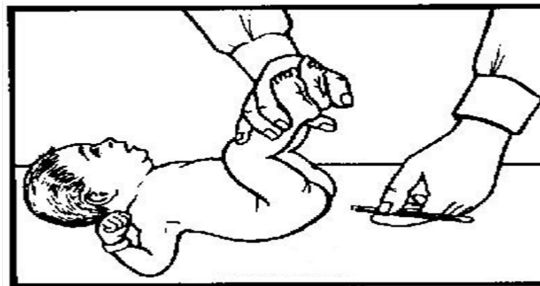

Figure 4: Rectal swab collection in the newborn

7.2.4.6. Remove the cap from the sterile vial containing 1.5ml of STGG transport media and place the tip of the swab into the tube.

7.2.4.7. Using a pair of scissors wiped with alcohol, cut off the excess swab stick handle, leaving the tip in the transport media. Ensure the cap fits firmly. Put the vial containing the swab into the transport bag and place the bag on wet ice in the cold box.

7.2.4.8. Dispose what remains of the swab stick handle into the yellow waste bag for safe disposal.

7.2.4.9. Put the appropriate labels on the vial containing the swab tip, the laboratory form and the corresponding CRF section.

7.2.4.10. Repeat the procedure with a second swab stick.

7.2.4.11. Complete the ***sample collection log***.

7.2.4.12. Ensure that both specimens and are transported to the MRC Fajara within six hours of collection.

- The swab labeled "**Baby Rectal Swab culture**" should be given to the designated staff (study Lab Tech) at the microbiology laboratory, Clinical services department.
- The swab labeled "**Baby Rectal Swab PCR**" should be taken to the Biobank (Freezer Room) and stored in the designated Freezer.

7.2.4.13. Complete the appropriate ***sample receipt logs*** that are kept at the laboratory and Freezer room respectively.

7.2.5 Venepuncture (Newborn)

7.2.5.1. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible.

7.2.5.2. Look carefully with a tourniquet for the most suitable vein. Remember that in newborns the best vein may not necessarily be palpable. Use a pocket pen light to display the veins. Choose a vein in the order of preference shown in **Figure 5**:

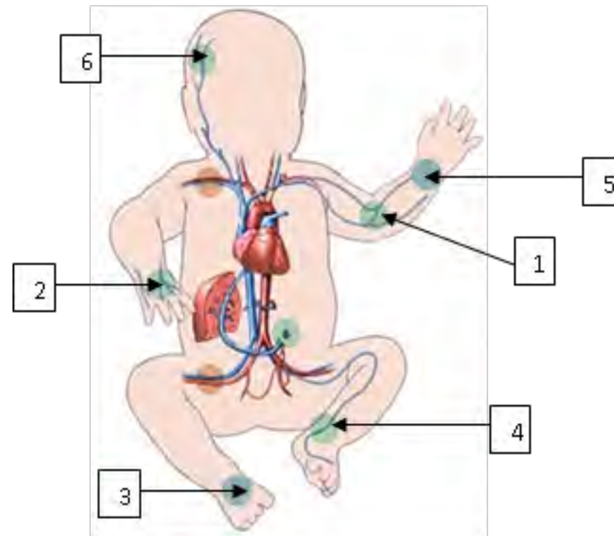

Figure 5: Preferred areas for venepuncture on a neonate's body.

(Numbers are described below)

1. Antecubital fossa
2. Dorsum (back) of hands
3. Dorsum (top) of feet
4. Greater saphenous vein at the ankle
5. Vein in the centre of the volar aspect of the wrist.
6. Scalp

7.2.5.3. Palpate the chosen vein, and to dilate the vein, apply a tourniquet to the limb proximal to (above) the intended site of puncture. Be careful of pinching skin or compressing artery.

7.2.5.4. Immobilise the vein by applying traction on the skin around the puncture site. Ask your assistant to stabilise the limb by holding joint above & joint below if necessary.

7.2.5.5. Vigorously wipe of the skin of the chosen area with a 70% alcohol swab, and wait for 30 seconds. ***DO NOT use chlorhexidine on children under 2 months of age.***

7.2.5.6. Next, swab the area with 10% povidone-iodine. Apply firm but gentle pressure starting from the centre of the venepuncture site and work downward and outwards to cover an area of 2 cm or more until the whole area is saturated with iodine. Allow the area to dry for 60 seconds. Failure to allow enough contact time increases the risk of contamination.

7.2.5.7. ***DO NOT*** touch the cleaned site; in particular, ***DO NOT*** place a finger over the vein to guide the shaft of the exposed needle. If the site is touched, repeat the disinfection.

7.2.5.8. When the skin is completely dry, proceed with the venipuncture.

7.2.5.9. Attach a sterile size 23G butterfly needle in at the tip of a sterile 1ml or 2ml syringe after the disinfectant has dried.

7.2.5.10. Insert the needle into the vein with the bevel of the needle face up (**Figure 6**). Puncture the skin 3–5 mm distal to (i.e., away from) the vein; this allows good access without pushing the vein away. If the needle enters alongside the vein rather than into it, withdraw the needle slightly without removing it completely, and angle it into the vessel.

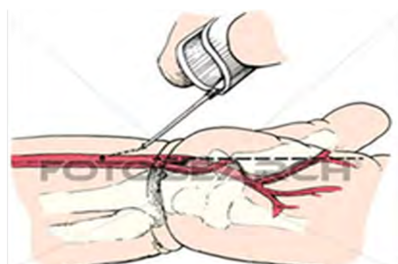

Figure 6: Venepuncture

7.2.5.11. Draw blood slowly and steadily. Target to draw 2mls of blood. (If using a 1ml syringe, remove the syringe once full, replace with another one and draw blood again.

7.2.5.12. After obtaining blood, release the tourniquet (if used) and place a sterile cotton-ball over the puncture site while holding the butterfly needle in place. Withdraw the butterfly needle and have your assistant hold the cotton-ball firmly in place until the wound has stopped bleeding.

7.2.5.13. Put a plaster/Stella strip on the puncture site.

7.2.5.14. Remove the butterfly needle and attach the 21G (green) needle to the syringe.

7.2.5.15. Remove the flip-off cap from BACTEC PEDS PLUS/F culture bottle top and inspect the bottle for cracks, contamination, excessive cloudiness and bulging or indented stoppers **(Cases Only)**.

7.2.5.16. Before inoculating blood into the culture bottle **(Cases Only)**, swab the top with the 70% alcohol swab (**DO NOT use povidone-iodine)**, and immediately (within 1 minute) aseptically inject appropriate volume of the blood in the culture bottle. (See Table 1.1) Dispense the rest of the blood (0.5ml) into the EDTA tube.

Table 4.1: Volume of blood to be dispensed in different vials

Blood volume drawn	**Blood in EDTA microtainer tube**	**Blood in BACTEC blood culture bottle**
≤1.0 ml	Nil	Everything
>1.0 ml to 2 ml	0.5 ml	The rest

7.2.5.17. On occasions it may not be possible to obtain the volume of blood required in a particular subject at a particular visit. Generally, up to three attempts may be made if a parent remains willing but judgment must be used with this regard to limit distress (and subsequent potential withdrawal of consent). Indicate on the CRF and inform the PI in the event that no blood sample could be collected.

7.2.5.18. Mix the contents of the EDTA microtainer and the culture bottle by eight gentle inversions. Wipe any residual blood from the outside of the bottle/microtainer before removing gloves.

7.2.5.19. Dispose of everything used for this procedure into the appropriate waste disposal bags.

7.2.5.20. Affix the appropriate labels to the Blood culture bottle and EDTA microtainer tube and the corresponding CRF section, and ensure that the appropriate laboratory forms are completed with the relevant information (study number; hospital number, sex, age, etc.).

7.2.5.21. Complete the *field sample log sheet* kept at the study office.

7.2.5.22. Put the Blood culture bottle in the incubator. Make sure that the incubator temperature is set at 37°C

7.2.5.23. Place the EDTA microtainer tube containing the blood sample on ice packs in the cold box.

7.2.5.24. If there is more than one eligible participant, repeat the above procedures. Use new needles and a new syringe(s) for each participant.

7.2.5.25. Ensure that specimens are transported to the MRC Fajara within six hours of collection. Transport the blood culture bottle in the field bag. **Do not place the blood culture bottle in the cold box**.

- The **Blood culture bottle(s)** should be given to the designated staff (study Lab Tech) at the microbiology laboratory, Clinical services department.
- EDTA microtainer tube labelled "**Blood PCR**" should be taken to the Biobank (Freezer Room) and stored in the designated Freezer.

7.2.5.26. Complete the appropriate *sample logs* that are kept at the laboratory and Freezer room respectively.

7.2.6 Lumbar puncture (Case ONLY)

7.2.6.1. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible.

7.2.6.2. The nurse should assess and document the temperature, pulse, respiratory rates and oxygen saturation in the observation chart.

7.2.6.3. The clinician must ensure that there are no contraindications to the procedure (Appendix 2). Ensure infant has not been fed in previous hour (aspirate infant's stomach if fed within the past hour).

7.2.6.4. A random glucose estimation should be taken using a glucometer at the bedside, **before** the lumbar puncture is performed.

7.2.6.5. The spinal cord in neonates extends further down the spinal canal than in older children. Lumbar punctures should be performed at or below the L4 level. Once the infant is in position, palpate the iliac crest and slide your finger centrally toward the L4 vertebral body. This created imaginary line will help identify the L4-L5 interspace as the preferred site of neonatal lumbar puncture (**Figure 7**).

Figure 7: Positioning a newborn for lumbar puncture

7.2.6.6. A reliable assistant should restrain the infant in a sitting or lateral decubitus position (the head and legs must be flexed - knee-chest position) without compromising the infant's cardio-respiratory status, and making sure airway patency is maintained at all times.

Some degree of flexion of the spine is helpful since it opens up the interspinous spaces and also stretches the skin over the processes, allowing better definition of landmarks. It is not necessary to flex the neck with compromise of the airway and increase in cerebral venous pressure. Infants may not tolerate the procedure well. This is usually because of excessive flexion of the infant.

Alternatively, term infants may be placed in a seated position on the edge of the table, with trunk flexed forwards, stabilised from the front by the assistant. The infant's shoulders and hips are held in order to maintain vertical alignment of the hips and shoulders during the procedure. This has been shown to be the best tolerated and to also have the best chance of obtaining CSF.

7.2.6.7. Apply face mask. Wash hands with soap and water. Put on sterile gloves.

7.2.6.8. Open the sterile pack, while ensuring the spinal needle and specimen bottles (2 bijou bottles and 1 NaF bottle) are within easy reach.

7.2.6.9. Clean the skin over the site with sterile cotton pad soaked in 70% alcohol in circular motion from the centre outwards. Allow the alcohol to evaporate. **Don't** touch the cleaned area.

7.2.6.10. Subcutaneous infiltration with 0.1ml/kg of 1% lignocaine using a 25G tuberculin needle over the L4-L5 space at least 2minutes before the procedure

7.2.6.11. Advancing the needle in the chosen intervertebral space, aiming towards the navel. Once through the skin, STOP. Wait for the infant to resettle.

7.2.6.12. Reorient yourself, making sure that you are still in the midline and advancing at the appropriate angle. The subsequent advance of the needle is less distressing than the initial insertion.

7.2.6.13. Advance needle about 0.5 cm. Remove stylet and observe for CSF flow. If negative, fully reinsert the stylet and advance a little further. Repeat this process until CSF is obtained.

7.2.6.14. A 'pop' or 'give' may be felt as the needle passes through the posterior ligaments and dura, but do not rely on this. The 'stop-start' approach is less likely to give a bloody tap.

7.2.6.15. Allow CSF to drip into each of the sample bottles **(See Figure 8)** as follows:

- Bijou bottle 1 - microscopy, culture & sensitivity (5 drops)
- Bijou bottle 2 - PCR (5 drops)
- NaF tube (grey top) - protein estimation (5 drops)

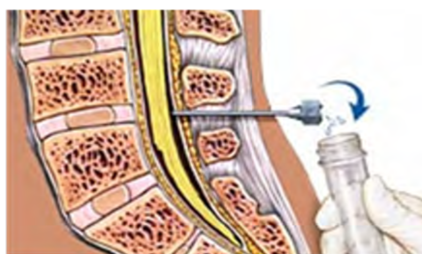

Figure 8 Collecting the cerebrospinal fluid

7.2.6.16. When adequate CSF has been obtained, replace the stylet and then remove the needle. Apply pressure to the puncture site with a sterile cotton wool ball or gauze to control ooze. When ooze has ceased, use a piece of plaster or Stella strip.

7.2.6.17. Place baby on its back for 60 minutes after the procedure to minimise the chance of headache.

7.2.6.18. Document the procedure, and CSF appearance in the case note stating the date and time.

7.2.6.19. Send all CSF samples to the MRC Fajara **immediately**.

- The sample labelled "**CSF culture**" should be given to the designated staff (study Lab Tech) at the microbiology laboratory, Clinical services department.
- The samples labelled "**CSF protein**" and "**CSF glucose**" should taken to the biochemistry laboratory, Clinical services department.
- The swab labelled "**CSF PCR**" should be taken to the Biobank (Freezer Room) and stored in the designated Freezer

7.2.6.20. Ensure the child is clinically stable after the procedure with the documentation of vital signs immediately after LP. Continue routine monitoring, including checking the temperature. Any clinical deterioration must be reported immediately to the clinician/neonatologist for urgent attention.

7.2.6.21. Discontinue cardio-respiratory and oxygen saturation monitoring 1 hour following procedure, unless ongoing monitoring is otherwise indicated.

7.2.6.22. Continue oxygen saturation monitoring for 4 hours post procedure if sedation with narcotics was administered prior to procedure. Sedated infants should remain nil orally for 2 hours post procedure.

7.2.6.23. Contraindications to lumbar puncture (LP) can be absolute or relative.

- Increased intracranial pressure (ICP) is an absolute contraindication.
- For patients with a bleeding diathesis or cardiopulmonary instability, the contraindications are relative to the importance of cerebrospinal fluid (CSF) results for immediate management decisions. (E.g. an unstable patient who may have bacterial meningitis should receive a blood culture and antibiotics. LP can be performed when the child's condition stabilizes)

7.2.6.24. Specific contraindications are as follows:

- **Increased intracranial pressure (ICP)** — Children with elevated ICP are at risk for cerebral herniation when LP is performed. Consequently, CT of the head should be obtained before LP for all patients with clinical suspicion for increased ICP, including those at risk for brain abscess (immunocompromise or congenital heart disease with a right-to-left shunt).
- **Bleeding diathesis** — Evidence regarding the safety of performing LP in patients with thrombocytopenia or coagulation factor deficiency is limited. Nevertheless, because of the risk of subdural or epidural hematoma formation, it is not advised to perform LP in patients with coagulation defects who are actively bleeding, have severe thrombocytopenia (eg, platelet counts <50,000/microL), or an INR >1.4, without correcting the underlying abnormalities. When LP is considered essential for a patient with an abnormal INR or platelet counts in whom the cause is not obvious, please consult with a haematologist to provide the best advice for safe correction of the coagulopathy prior to performing the LP. In all cases, the relative risk of performing LP

has to be weighed against the potential benefit (eg, diagnosing meningitis due to an unusual or difficult-to-treat pathogen).

- **Cardiopulmonary instability** — The position in which the patient must be placed in order to perform LP may result in further cardiopulmonary compromise. Administration of antibiotics must not be delayed for the child with suspected meningitis who cannot tolerate an LP.
- **Soft tissue infection at the puncture site**

8. References

- SCC 1384
- Aetiology of Neonatal Infection in South Asia (ANISA): Manual of Operation *Version 4.0_12.09.2011*
- Lumbar puncture (LP) for neonates. Neonatal ehandbook. http://www.health.vic.gov.au/neonatalhandbook/procedures/lumbar-puncture.htm [accessed 29 December 2015]
- Lumbar Punctures in Neonates: Improving Success Rates and Minimizing Pain. https://clinicaltrials.gov/ct2/show/record/NCT01606150 [accessed 29 December 2015]

Sampling summary table

Case (EFSTH, Kanifing General Hospital, Brikama Health Center)

Participant	Type of Sample	Comments	Responsibility
Newborn	• Blood culture • Blood PCR • 2 NPS (Culture & PCR) • 2 Rectal swabs (Culture & PCR) • CSF (Culture, protein, glucose, PCR)	**Collect at admission after consenting and recruitment** (as much as possible collect samples before antibiotics are given)	**Nurse** (and Clinician for CSF)
Mother	• 2 NPS (Culture & PCR) • 2 RVS (Culture & RVS)	Collect at the time of recruitment (as much as possible – if mother is at the postnatal ward, collect sample on the ward)	**Nurse**

Control (SKGH, PCB, BHC, FHC, JFPH, SHC)

Participant	Type of Sample	Comments	Responsibility
Newborn	• Blood PCR • 2 NPS (Culture & PCR) • 2 Rectal swabs (Culture & PCR)	Collect immediately after consenting and recruitment	**Nurse**
Mother	• 2 NPS (Culture & PCR) • 2 RVS (Culture & RVS)	Collect immediately after consenting and recruitment	**Nurse**

The Gambia Government/MRC Joint
ETHICS COMMITTEE

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1 August 2014

Dr Uduak Okomo
MRC Unit the Gambia
Fajara

Dear Dr Okomo

SCC 1384v2, Aetiological Agents and Risk Factors for Neonatal Sepsis in The Gambia

Thank you for submitting your proposal dated 14 July 2014 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 26 July 2014.

Members noted that you will conduct a pilot study of the first 50 cases and controls to test the feasibility and practicality of this approach and other proposed study procedures. In this regard, members further noted that taking virginal swabs from mothers of controls may be sensitive and would be interested to know your approach and its acceptability during the pilot phase.

For logistical reasons, it is understood that you will recruit controls from among healthy newborns brought for routine infant vaccination at major clinics closest to where the case lives.

The information sheet was considered long and technical and should be shortened and simplified.

We are pleased to approve the pilot study; however you are requested provide feedback on the outcome and request permission to proceed.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- SCC approval letter – 16 July 2014
- SCC response – 5 June 2014
- Response letter – 14 July 2014
- SCC application form, version 2.0 – 14 July 2014
- Informed Consent Document (cases), version 1.0 – 12 July 2014
- Informed Consent Document (controls), version 1.0 – 12 July 2014
- CRF (cases), version 1.1 – 28 April 2014
- CRF (controls), version 1.1 – 28 April 2014

The Gambia Government/MRC Joint Ethics Committee:

Mr Malamin Sonko, Chairman
Professor Ousman Nyan, Scientific Advisor
Ms Naffie Jobe, Secretary
Mrs Tulai Jawara-Ceesay
Dr Ahmadou Lamin Samateh
Dr Roddie Cole
Prof. Umberto D'Alessandro
Dr Stephen Howie
Dr Kalifa Bojang
Dr Ramatoulie Njie
Dr Momodou L. Waggeh
Dr Siga Fatima Jagne

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12 January 2016

Dr Uduak Okomo
Disease Control and Elimination Theme
MRC Unit, The Gambia
Fajara

Dear Dr Okomo

L2015.67v2, Re: SCC 1384v2: Aetiological Agents and Risk Factors for Neonatal Sepsis in The Gambia

Thank you for submitting your letters dated 19 October and 10 November 2015 for consideration by The Gambia Government/MRC Joint Ethics Committee at its meeting held on 18 December 2015.

The feedback on the outcome of the pilot case-control study is noted. The Committee is also pleased to approve your proposed modifications to the main study.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- Revised letter – 10 November 2015
- Request letter – 19 October 2015
- EC reply letter – 1 August 2014
- Informed Consent Document (controls), version 4.0 – 19 August 2015
- Informed Consent Document (cases), version 4.0 – 19 August 2015
- SCC application form for SCC 1384

The Gambia Government/MRC Joint Ethics Committee:

Mr Malamin Sonko, Chairman
Professor Ousman Nyan, Scientific Advisor
Ms Naffie Jobe, Secretary
Dr Roddie Cole
Dr Ahmadou Lamin Samateh
Mrs Tulai Jawara Ceesay
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17 August 2015

Dr Uduak Okomo
MRC Unit The Gambia
Fajara

Dear Dr Okomo

L2015.33, Re SCC 1384: Request inclusion of follow-up of participant by telephone

Thank you for submitting your letters dated 22 June and 24 July 2015 for consideration by the Gambia Government/MRC Joint Ethics Committee at its meeting held on 31 July 2015.

We are happy to approve your request.

With best wishes

Yours sincerely

Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- SCC approval letter – 9 July 2015
- Request letter – 22 June 2015
- Response letter – 24 July 2015
- Informed Consent Document (cases/controls), version 3.0 – 22 June 2015

The Gambia Government/MRC Joint Ethics Committee:

Mr Malamin Sonko, Chairman
Professor Ousman Nyan, Scientific Advisor
Ms Naffie Jobe, Secretary
Dr Roddie Cole
Dr Ahmadou Lamin Samateh
Mrs Tulai Jawara Ceesay
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Observational / Interventions Research Ethics Committee

Dr. Uduak Okomo
LSHTM

3 February 2015

Dear Dr. Okomo

Study Title: Aetiology and Risk Factors for Severe Neonatal Infections in The Gambia

LSHTM Ethics Ref: 8622

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Information Sheet	Participant Information Sheet _controls version 2_02 August 2014	02/08/0204	2
Protocol / Proposal	Case Report Forms_Cases.pdf	25/06/2014	1
Protocol / Proposal	Case Report Forms_Controls.pdf	25/06/2014	1
Protocol / Proposal	Okomo MRC Gambia SCC Proposal_12 July 2014.pdf	12/07/2014	2
Information Sheet	Information leaflet _cases 12 July 2014 version 2.pdf	12/07/2014	2
Information Sheet	Information leaflet _controls 12 July 2014 version 2.pdf	12/07/2014	2
Information Sheet	Consent form _cases 12 July 2014 version 2.pdf	12/07/2014	2
Information Sheet	Consent form _controls 12 July 2014 version 2.pdf	12/07/2014	2
Local Approval	Local Ethic s approval.pdf	01/08/2014	1
Information Sheet	Participant Information Sheet _cases version 2_02 August 2014	02/08/2014	2
Information Sheet	Consent form _cases version 2_02 August 2014	02/08/2014	2
Information Sheet	Consent form _controls version 2_02 August 2014	02/08/2014	2
Information Sheet	PIS and IC _cases version 2.pdf	02/08/2014	2
Information Sheet	PIS ans IC _controls version 2.pdf	02/08/2014	2
Investigator CV	CV_Okomo Uduak .pdf	29/10/2014	1
Investigator CV	Dr Howie_CV.pdf	29/10/2014	1
Protocol / Proposal	Case Report Forms_Cases_version 2.pdf	28/11/2014	2
Covering Letter	LSHTM Ethics Committee response covering letter	20/12/2014	1
Protocol / Proposal	Case Report Forms_Controls_version 2.pdf	28/12/2014	2
Covering Letter	Uduak Okomo LSHTM response letter 28 January 2015	28/01/2015	1

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

Professor John DH Porter
Chair

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Appendix 8: ANISA SOP for analysis of TaqMan Array Cards

Initial Data Analysis

1. After the run is complete, the software will automatically proceed to the "Analysis" option in the "Experiment Menu." The amplification plot will resemble Figure 1

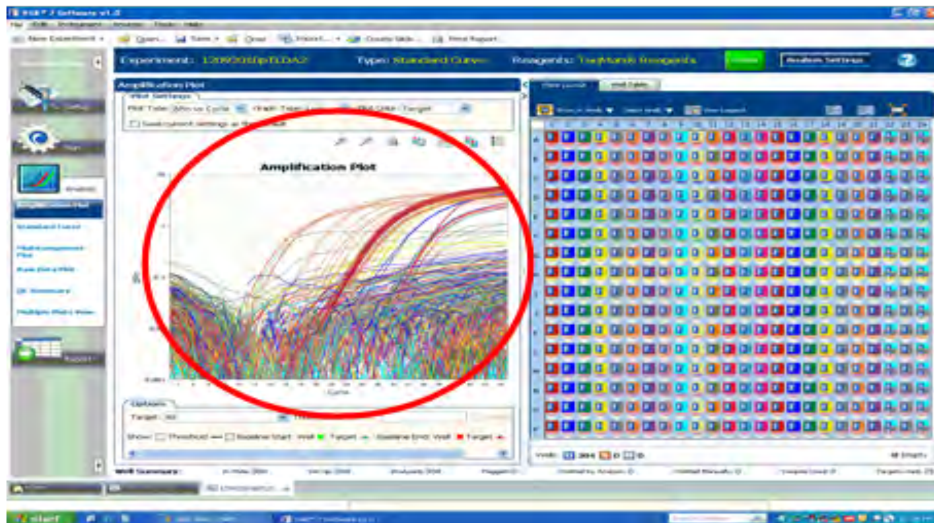

Figure 1: Amplification curves for all wells following run completion.

2. Click the green "Analysis" button near the top of the screen (Figure 2) to initiate automated analysis.

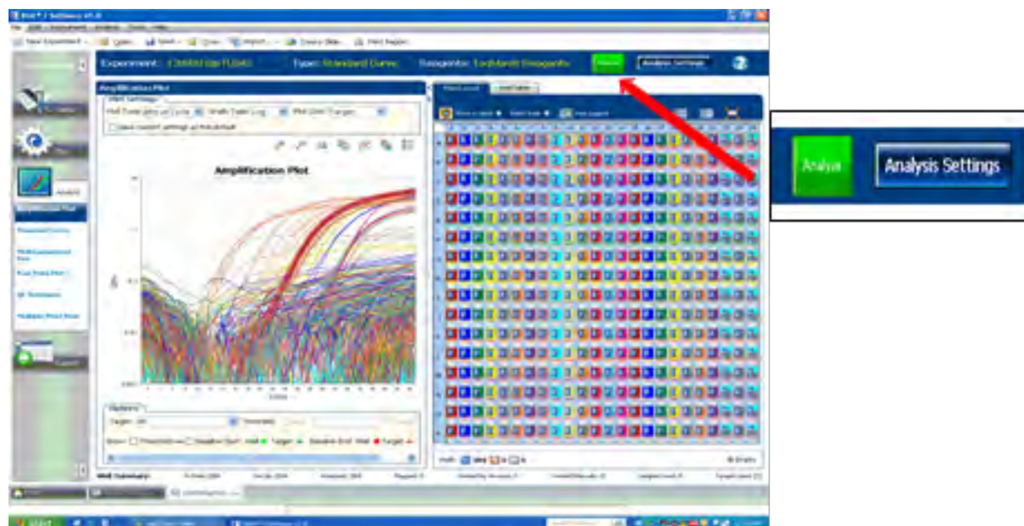

Figure 2: Initial analysis

3. The curves and thresholds for ALL assays will appear on the screen (Figure 3). Each threshold must be independently adjusted. The next section describes how to adjust individual thresholds.

Figure 3: Post-analysis data

Validating Run Results

Run results will be validated by analyzing performance of internal positive control (IPCO_1) reactions and all assay results for the NTC and PC.

1. Identify IPCO_1 wells:

   To assess results of IPCO_1 assay, highlight column 11 in the plate layout display on the right side of the screen (Figure 4). The manufacturing control assay (GADH_1) is present in alternating rows of column 11 (A, C, E, G, I, K, M, and O). GADH_1 results will not be analyzed for this study.

   a. Blood v2: On the blood TAC, IPCO_1 **and** RNaseP (RNP3_1) control are present in the same well (column 11: B, D, F, H, J, L, N, and P), but different fluorescent reporters are used for each assay. When these wells are selected, amplification curves for both assays (IPCO_1 (VIC) and RNP3_1 (FAM)) will appear on the amplification plot.

   b. Respiratory v2: The wells in column 11 (B, D, F, H, J, L, N, and P) correspond to internal positive control (IPCO_1) on the respiratory TAC.

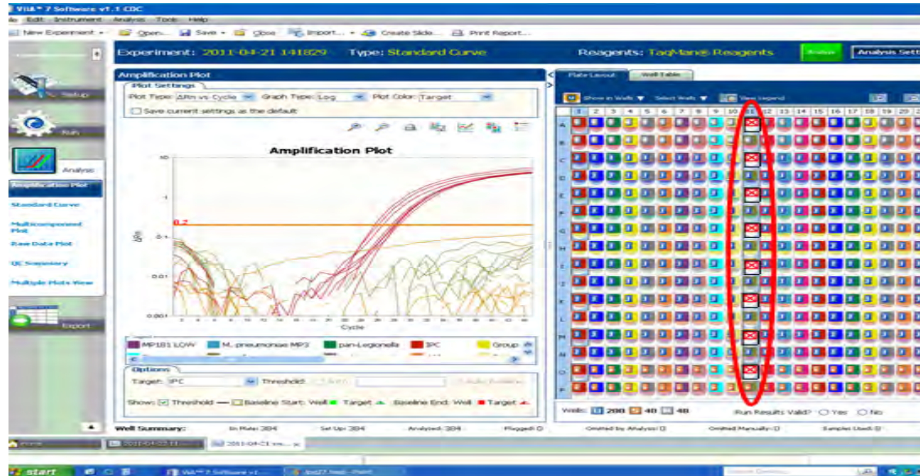

Figure 4: Identification of IPCO_1 assay

2. Select the IPCO_1 assay:

   Select "IPCO_1" from the "Target" drop-down menu on the "Options" tab under the amplification plot display. Only curves and threshold for IPCO_1 assay will be displayed on the plot (Figure 5).

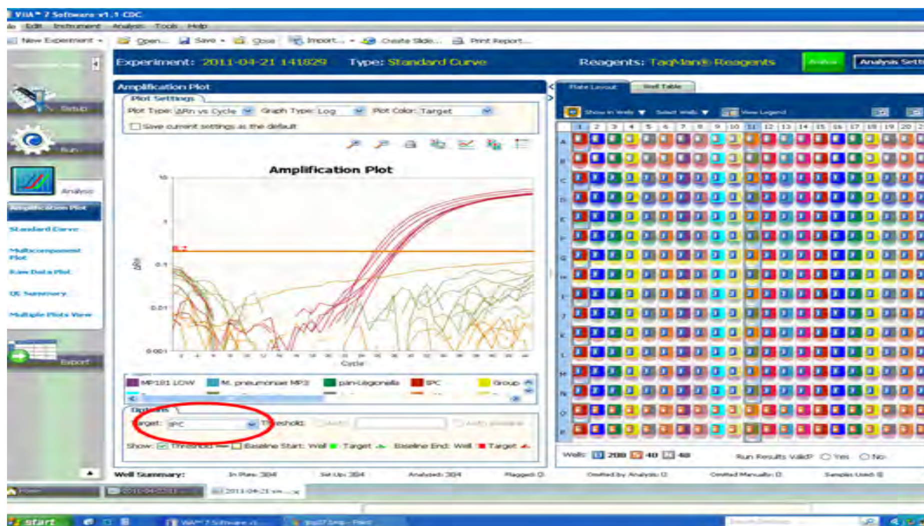

Figure 5: Selection of IPC amplification curves

3. Adjusting the threshold in log view:

   De-select the "Auto" checkmark under the options tab to freely move the threshold. Use the cursor to adjust the threshold line for this assay so that it is just above background fluorescence (Figure 6).

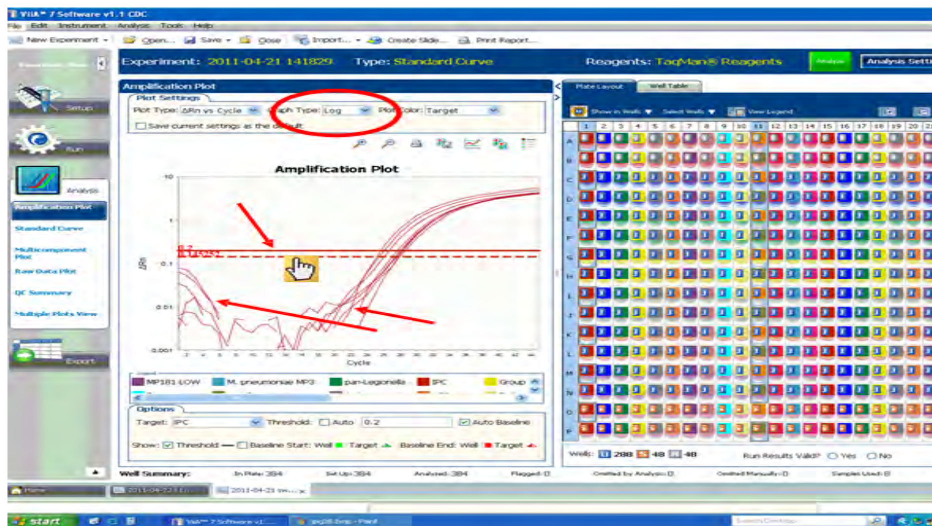

Figure 6: Adjustment of threshold line in log mode

4. Adjusting the threshold in linear view:

   Select "Linear" from the "Graph Type" field in the "Plot Settings" tab above the amplification plot display. The graph will update displaying the curves in linear format. Again you must de-select the "Auto" checkmark under the options tab to move the threshold with your cursor. Refer to Figures 7a and 7b for examples of "correct" and "incorrect" threshold settings.

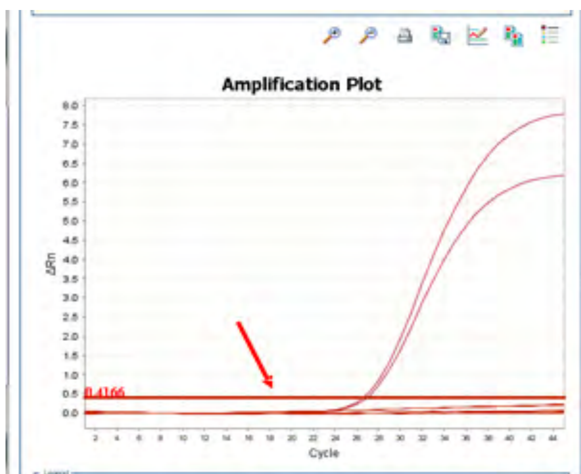

Figure 7a: Threshold set correctly

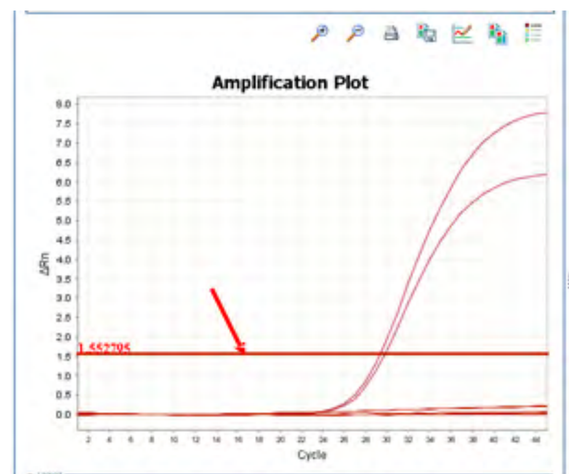

Figure 7b: Threshold set incorrectly.

5. Interpretation of amplification curve (IPCO_1):

In linear display mode, all specimens, including the NTC, should exhibit exponentially increasing (sigmoidal or "S"-shaped) amplification curves that cross the threshold for the IPCO_1 assay. Refer to "Interpretation of Results and Reporting" on pages 48-50 for additional guidance.

a. If **ALL** assays exhibit an amplification curve for the IPCO_1 assay, proceed to analyzing results for NTC and PC.

b. If **ALL** specimens fail to exhibit an amplification curve for the IPCO_1 assay, invalidate the run by selecting the checkbox corresponding to "No" next to "Run Results Valid?" Specimens from an invalid TAC run need to be repeated on a new TAC. Refer to the "Repeat Testing" section on page 50 for more guidance.

c. If an **individual** specimen(s) does not exhibit an exponential amplification curve that crosses the threshold line for the IPCO_1 assay, invalidate results for all assays for this specimen only. Refer to "Assigning Interpretation Comments for Each Reaction" section on page 38

Important! If a specimen is positive for one or more assays, even in the presence of a failed IPCO_1 reaction, the assay-specific result should be considered valid. A negative IPCO_1 reaction does not preclude a positive result in another reaction well. However, it is strongly recommended that the specimen be repeated.

6. Validating NTC reaction wells:

There should be no amplification in NTC wells, except for the IPCO_1 assay. Select rows corresponding to NTC sample (A and B). Ensure no amplification curves are observed in NTC wells (except IPCO_1). Refer to the section "Interpretation of Results and Reporting" for additional guidance.

7. Validating PC reaction wells:

All of the assays, except GADH_1, should display a sigmoidal amplification curve for the PC wells. Select the rows corresponding to PC sample (O and P). Ensure amplification curve is present in every well for PC reactions, except GADH_1. Refer to "Interpretation of Results and Reporting" on pages 48-50 for additional guidance.

8. Confirming validation:

If **ALL** controls have performed as expected, validate the run by selecting the radio button corresponding to "Yes" next to "Run results valid?" in the box below the plate layout on the right side of the screen (Figure 8).

Figure 8: Validation of TAC run

Analysing Other Assays

1. Highlight the assay to be analyzed by selecting the entire column. The target (assay) in the drop-down menu will automatically update when a new column is selected. Placing the cursor over an individual well will bring up a box displaying the information for that particular reaction well (target, sample, etc.) (Figure 9)

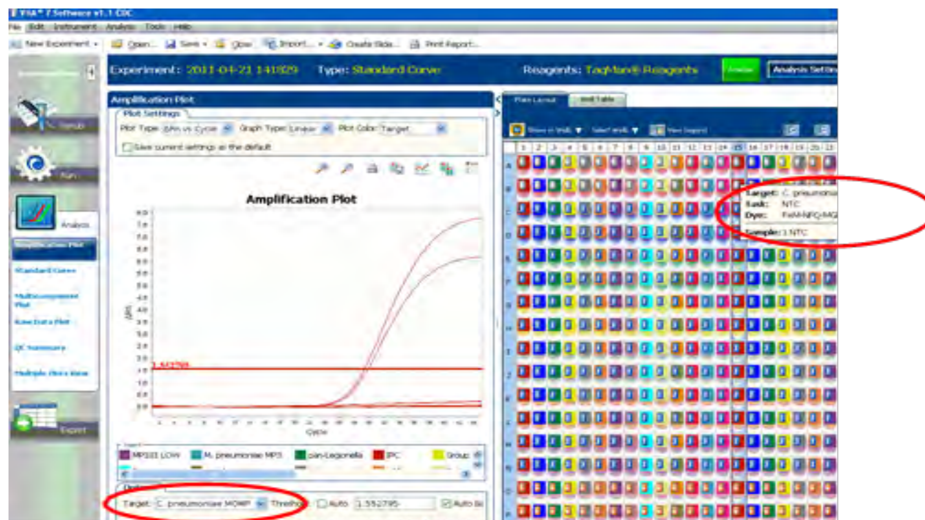

Figure 9: Assay selected in "Plate Layout" matches target in "Amplification Plot".

2. De-select the "Auto" checkbox under the "Options" tab (under Amplification Plot) to move the threshold by clicking and dragging with your cursor. Adjust the assay threshold so that it crosses the amplification curve for the positive control at the starting point of exponential increase. The threshold should be slightly above background fluorescence (Figure 10).

Figure 10: Proper adjustment of threshold

Note: The level of background fluorescence may vary between assays on a single TAC. In some cases, the background fluorescence may be high such that setting the threshold above this level would artificially increase the Ct value of the PC and any other positive results. In this case, the threshold should be set below background fluorescence; the threshold line should cross amplification curve for PC at the inflection point. Although the background will cross the threshold (and have a Ct value) in this case, results should be interpreted as “Negative” as described in the following section

3. Repeat this process for all remaining assays (columns).

4. After **ALL** thresholds have been adjusted individually, click on the “Analysis” button to allow the software to recalculate the Ct values based on the adjusted thresholds (Figure 11).

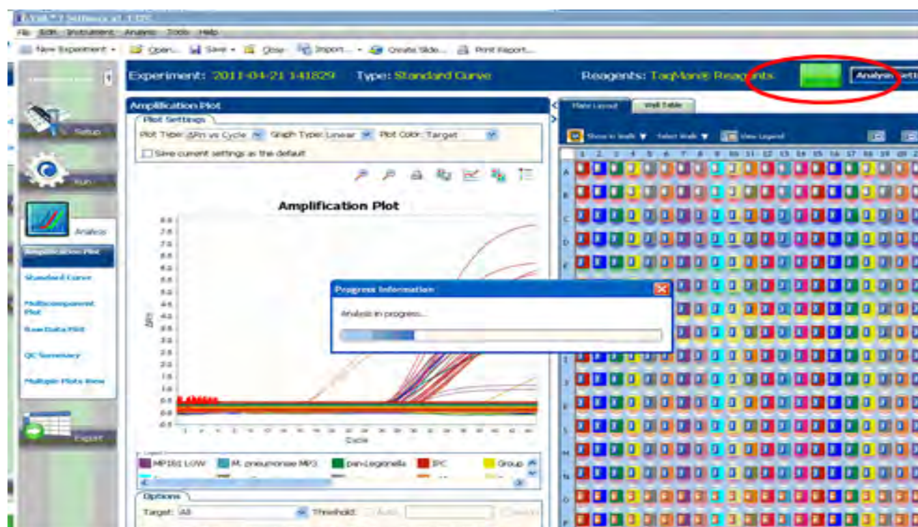

Figure 11: Analysis of all assays with thresholds adjusted

Assigning Interpretation Comments for Each Reaction

1. To assign an interpretation to an individual reaction (well), right click on the corresponding well position in the “Plate Layout” on the right side of the screen. Choose “Select Comments” from the menu. A box will open with a drop-down menu allowing three possible selections: “Positive,” “Negative,” or “Indeterminate” (Figure 12).

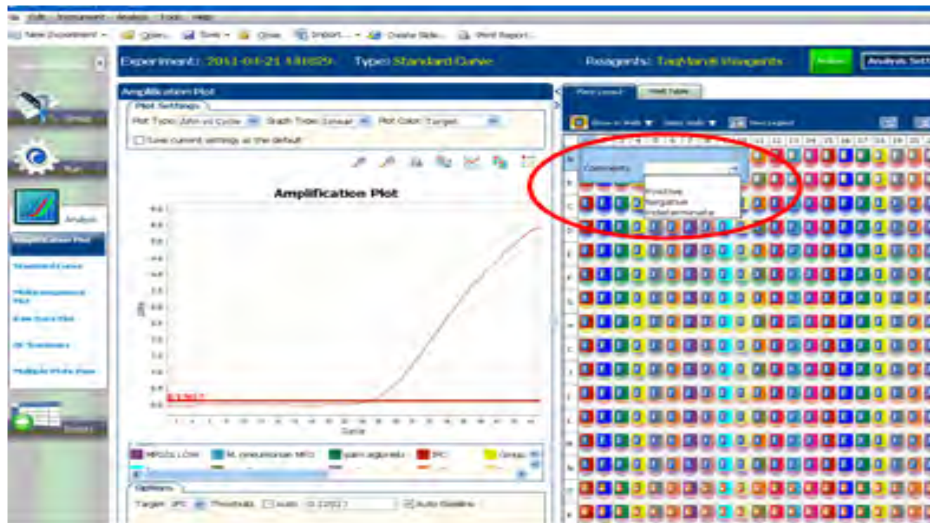

Figure 12: Assigning interpretation to a single well

2. If an amplification curve is observed and determined to be of the proper shape, select “Positive” from the drop-down menu (Figure 13).

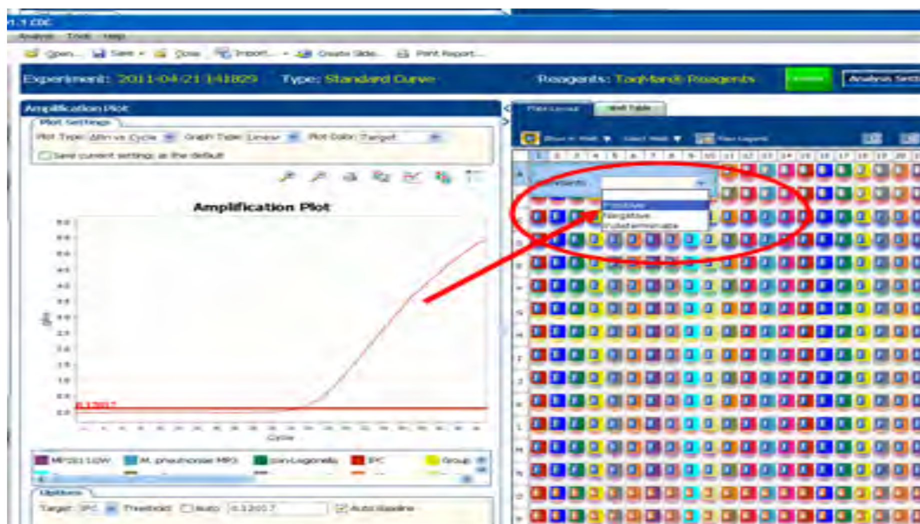

Figure 14: Selection of “Positive” interpretation

3. If no amplification curve is observed for an individual reaction (well), select “Negative” from the drop-down menu (Figure 15)

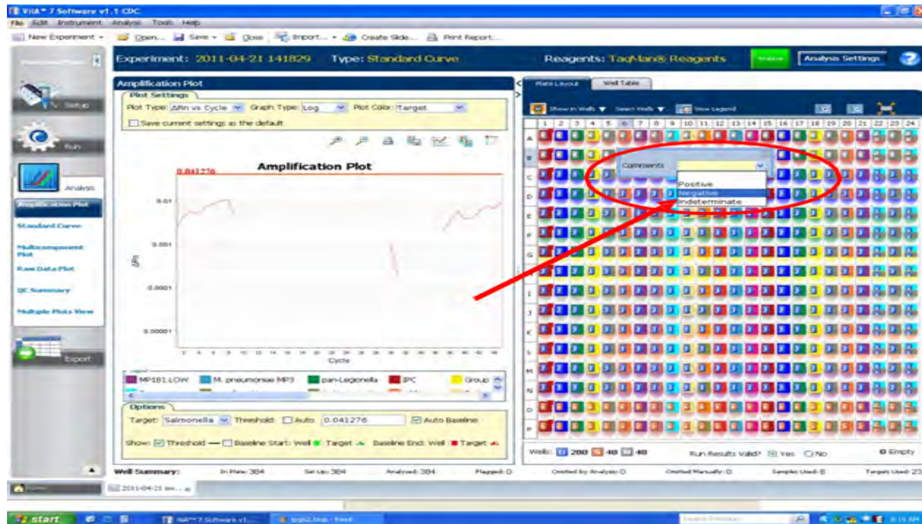

Figure 15: Selection of "Negative" interpretation

Note: All wells should be considered individually. Comments should reflect result in an individual well. An interpretation is required for ALL wells in the plate, except the wells containing GADH_1. The next section provides detailed methods to assign comments for each well.

Note: A red mark will appear in the corner of each well after it has been assigned an interpretation (Figure 16).

Important! Only one comment can be entered for each well. Therefore, for the wells containing both RNP3_1 (FAM-labeled) and IPCO_1 (VIC-labeled) on the ANISA Blood v2 TAC, comments should be assigned for the RNP3 target **ONLY**

Figure 16: Red corner marks indicating well has been interpreted

Assigning Interpretations to Multiple Wells Simultaneously

1. To assign interpretations for multiple wells, select the appropriate wells in the "Plate Layout" display. Use the "Shift" and "Ctrl" keys to select multiple rows, columns, or wells in order to assign the same comment to all selected wells (Figure 17).

Figure 17: Assigning the same comments to multiple wells.

2. Right-click, and choose "Select Comments" from the menu. Assign the correct interpretation, and click the "×" in the upper right corner of the box to exit (Figure 18)

Figure 18: Assigning the same comments to multiple wells.

3. All selected wells will now be labelled with the same interpretation (Figure 19).

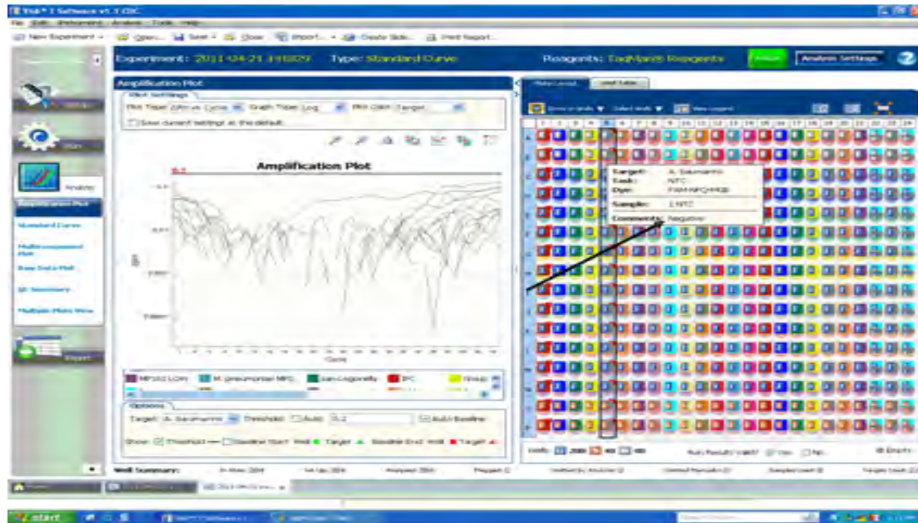

Figure 19: Same comments assigned to all selected wells.

Note: The same comment can be assigned to multiple wells by selecting multiple wells in the "Well Table" tab, then returning to the plate layout and assigning comments as described in steps 2-3 above

Saving Interpretations and exporting data

After all results have been analysed and interpretations have been assigned for each well,

select "Save" from the main tool bar

Click the "Export" button on the bottom left side of the screen. This will bring up the export portion of the software, which will allow you to transfer all data to a Microsoft Excel format

Interpretation of Results and Reporting

Internal Positive Control (IPCO_1)

All specimens, including the NTC, should exhibit exponentially increasing (sigmoidal or "S"-shaped) amplification curves that cross the threshold line for the IPCO_1 assay.

- If ALL specimens fail to exhibit an amplification curve for the IPCO_1 assay:
    - Invalidate the run by selecting the checkbox corresponding to "No" next to "Run results valid?"
    - Repeat testing is required for all specimens; refer to the "Repeat Testing" section for additional guidance.

- If an individual specimen or subset of specimens fail to exhibit an amplification curve for the IPCO_1 assay:
  - o Assign “Negative” comment for the well corresponding to the IPCO_1 assay for the individual specimen(s).
  - o Assign “Indeterminate” comment for all other assays corresponding to that specimen. See note below for exception to this convention.
  - o Repeat testing is required for the affected specimen(s); refer to the “Repeat Testing” section on page 50 for additional guidance.

Note: If a specimen is positive for one or more assays, even in the presence of a failed IPCO_1 reaction, the assay-specific result should be considered valid. A negative IPCO_1 reaction does not preclude a positive result in another reaction well. However, it is strongly recommended that the specimen be repeated.

No Template Control (NTC)

The NTC consists of using sterile nuclease-free water in the real-time RT-PCR reactions instead of TNA extract. The NTC reactions for all assays should not exhibit amplification curves that cross the threshold line; amplification in NTC wells, except IPCO_1 assay, indicates contamination may have occurred in the laboratory. All assays should be considered individually.

- If an amplification curve that crosses the threshold line occurs in the well(s)

  corresponding to the NTC for an individual assay:

  - o Assign “Positive” comment for the wells corresponding to the indicated assay.

  - o Assign “Indeterminate” comment for the wells corresponding to all other specimens for the indicated assay(s).

  - o Refer to “Precautions” section on page 9-10 for recommended decontamination procedures. After decontamination, repeat the assay with strict adherence to the instructions. Refer to the “Repeat Testing” section for additional guidance.

Note: Occasionally, amplification may occur in an NTC reaction for the human nucleic acid control assay (RNP3_1). This usually indicates contamination with human cells during the assay setup. In this case, the results for the affected specimen(s) should still be considered valid. Do NOT invalidate all results. Decontaminate work areas and adhere more closely to guidelines during future testing. See “Precautions” section for additional guidance on decontamination procedures.

Note: The *E.coli* (ECSH_1) and Rubella virus (RUBV_1) assays may show amplification with the

NTC. Refer to “Assay Specifications and Limitations” for additional guidance.

RNase P (Human Nucleic Acid Control) (RNP3_1)

All human clinical samples should exhibit fluorescence amplification curves in the RNP3_1 reaction that cross the threshold line within 38 cycles (Ct < 38). Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid from original specimen
- Absence of sufficient human cellular material due to poor specimen collection, handling, or storage
- Improper assay setup and performance
- Reagent or equipment malfunction

If the RNase P assay does not produce a positive result for a clinical specimen(s), interpret as follows:

- If a specimen is positive for one or more assays, even in the presence of a negative RNP3_1 result, the assay-specific result should be considered valid and the interpretation assigned as "Positive." A negative RNP3_1 result does not preclude the

  presence of a pathogen in a clinical specimen. However, it is strongly recommended that the specimen be repeated.
- If all markers AND RNase P are negative for the specimen, the assay results are "indeterminate" for that specimen.
  - Select "Negative" from the drop-down menu in the well table for the wells corresponding to the RNase P assay.
  - Select "Indeterminate" from the drop-down menu in the well table for the wells corresponding to all other assays for the indicated specimen(s).

o If residual specimen is available, repeat the extraction procedure and repeat the test on a new TAC. Refer to the "Repeat Testing" section for additional guidance.

***E. coli/Shigella* (ECSH_1) assay**

Amplification may occur in NTC or specimen extracts due to the presence of residual *E. coli* nucleic acid in the enzyme preparation. Therefore, this assay is only interpretable when Ct ≤ 30. Comments should be assigned as follows:

- Ct value ≤ 30: positive
- Ct value > 30: indeterminate
- No amplification: negative

Rubella virus (RUBV_1) assay

Non-specific amplification may occur in NTC or specimen extracts after 35 cycles. Therefore, this assay is only interpretable when Ct ≤ 35. Comments should be assigned as follows:

- Ct value ≤ 35: positive
- Ct value > 35 or no amplification: negative

Repeat Testing

Repeat testing using TAC is required for a specimen if any of the following apply:

- IPCO_1 failure for individual specimen
- RNP3_1 failure for individual specimen

  Note: If RNP3_1 failure occurs, original specimen should be re-extracted prior to repeat testing on TAC.

- Invalid TAC run results due to:
  - Failure of ALL IPCO_1 reactions
  - Complete failure of PC reactions (lack of amplification in all wells)
  - Complete failure of NTC reactions (amplification in all wells)
  - Instrument failure during run
  - Other run errors which result in invalid results

Repeat testing for individual assays may be required in some cases. These specimens will be considered on an individual basis.

All specimen extracts requiring repeat testing should be stored at ≤ -70°C until the end of the specimen collection and testing period

Assay Specifications and Limitations

Assay name	Organism(s) detected	Limitations
ADEV_1	Adenovirus	
BOP1_1	*Bordetella pertussis, Bordetella holmesii*	Cross-reactive with *B. holmesii* for a small proportion of isolates.
CHPN_1	*Chlamydophila pneumoniae*	
CHTR_1	*Chlamydia trachomatis*	
CYMV_1	Cytomegalovirus	
ECSH_1	*Escherichia coli* (all), *Shigella* spp. (except *S. dysenteriae* serotype I)	Interpret positive results when Ct < 30 only. Specimens exhibiting amplification after cycle 30 should be marked as “Indeterminate.” Sporadic amplification may occur after cycle 30 due to contaminating *E. coli* nucleic acid in enzyme mix.

ENTV_1	enterovirus	
FLUA_1	Influenza type A	
FLUB_1	Influenza type B	
GADH_1	Glyceraldehyde-3-phosphate dehydrogenase	Manufacturing control. Results from this assay should be disregarded.
GAST_1	*Streptococcus pyogenes*	
GBST_1	Group B *Streptococcus* (all serotypes: IA, IB, II, III, IV, V, VI, VII, VIII, IX)	
HIAT_1	*Haemophilus influenzae*	
HMPV_1	Human Metapneumovirus	
HPEV_1	Human Parechovirus	
IPCO_1	Internal Positive Control	Positive result should be observed for ALL specimens, NTC, and PC.
KLPN_1	*Klebsiella pneumoniae*	
MYPN_1	*Mycoplasma pneumoniae*	
NMEN_1	*Neisseria meningitidis*	
PIV1_1	Parainfluenza virus-1	
PIV2_1	Parainfluenza virus-2	
PIV3_1	Parainfluenza virus-3	
PSAE_1	*Pseudomonas aeruginosa*	
RESV_1	Respiratory Syncytial Virus	
RHIV_1	Rhinovirus	
RNP3_1	Human RNase P gene	
RUBV_1	Rubella virus	Interpret positive result when Ct ≤35. Specimens exhibiting amplification after cycle 35 should be marked as "Negative."
SALS_1	*Salmonella* spp.	

STAU_1	*Staphylococcus aureus*	
STPN_1	*Streptococcus pneumoniae*	
URUP_1	*Ureaplasma parvum* (biovar 1; serovars 1, 3, 6,14), *Ureaplasma urealyticum* (biovar 2; serovars 2, 4, 5, 7-13)	URUP_1