Spectrum of organisms and outcome of neonatal infections in HIV-exposed and unexposed newborns at a tertiary care hospital in KwaZulu-Natal

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Declaration

I, Prasha Mahabeer, declare that:

(i) The research reported in this dissertation, except where otherwise indicated, is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

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Signed Date: 05 February 2021

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Dedication

I dedicate this work to my family - Nevon, Yashthi and Aruhi Ramsunder for their patience and support while I was writing up this thesis and for making a stressful journey Fun!

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List of Abbreviations

AGA:	Appropriate for gestational age
AMR:	Antimicrobial resistance
ART:	Antiretroviral therapy
BSI:	Bloodstream infection
CLSI:	Clinical and Laboratory Standards Institute
CoNS:	Coagulase-negative *Staphylococcus*
CPAP	Continuous positive airway pressure
CPE:	Carbapenemase-producing Enterobacteriaceae
CRP:	C-reactive protein
CSF:	Cerebrospinal fluid
EOS:	Early-onset sepsis
ESBL:	Extended-spectrum β-lactamase
ETA:	Endotracheal aspirate
GBS:	Group B *Streptococcus*
HAI:	Hospital-associated infection
HEU:	HIV-exposed uninfected
HIV:	Human Immunodeficiency Virus
HSV:	Herpes simplex virus
HU:	HIV unexposed
IPPV:	Intermittent positive pressure ventilation
KEH:	King Edward VIII Hosptial
KZN:	KwaZulu-Natal
LBW:	Low birth weight
LOS:	Late-onset sepsis
MDR:	Multidrug-resistant
MRSA:	Methicillin-resistant Staphylococcus aureus
MTCT:	Mother-to-Child Transmission
NEC:	Necrotising enterocolitis
NHLS:	National Health Laboratory Service
NICU	Neonatal intensive care unit
PCR:	Polymerase chain reaction
PMTCT:	Prevention of Mother-to-Child Transmission

SA:	South Africa
UVA:	Umbilical arterial catheter
UVC:	Umbilical venous catheter
VAP:	Ventilator-associated pneumonia
WHO:	World Health Organization
XDR:	Extensively drug-resistant
β-lactams:	Beta-lactam antibiotics

Abstract

Background:

The World Health Organisation's (WHO) recommendation of life-long antiretroviral prophylaxis to pregnant women who are positive for Human Immunodeficiency Virus (HIV) has increased HIV-exposed but uninfected infants. These infants are more likely to be born premature and small for their gestational age. They require prolonged hospitalisation, making them susceptible to nosocomial infections. The study aimed to determine the organisms causing infections in these HIV-exposed newborns, their susceptibility profiles, risk factors and outcome compared to their unexposed counterparts.

Methods:

This prospective descriptive study was conducted at King Edward VIII Hospital in Durban between January 2014 and December 2019. Laboratory and clinical data of neonates admitted to the neonatal unit with possible bacterial infection were collected. The organisms and their susceptibility profiles from blood cultures, cerebrospinal fluid and endotracheal aspirates were reviewed.

Results:

A total of 276 neonates were included in the final analysis, 50.7% of which were HIV-exposed. Group B Streptococcus was the predominant organism isolated in the HIV-exposed neonates in early-onset sepsis while Group B *Streptococcus* and *Klebsiella pneumoniae* in the HIV-unexposed. Gram-negative bacilli accounted for 65.8% of the bloodstream organisms causing late-onset sepsis of which *Klebsiella pneumoniae* was the most common Gram-negative pathogen with 61% being extended-spectrum β-lactamase (ESBL) producing and 23% carbapenemase-producing. Antimicrobial resistance was common in endotracheal aspirates which included ESBL producing and carbapenemase resistant *Klebsiella pneumoniae*, ESBL producing *E. coli* and multidrug-resistant (MDR). *Acinetobacter* spp. as well as Gram-positive bacteria that were resistant to Cloxacillin. HIV-exposure was found to be associated low birth weight ($p < 0.001$).

Conclusion:

Group B *Streptococcus* remains the most common pathogen causing early-onset sepsis in both HIV-exposed and unexposed neonates and is covered by the current empiric antibiotics

prescribed in the unit. Resistant gram-negative bacteria caused the majority of the episodes of late-onset sepsis in both groups. A review of antibiotic treatment for late-onset sepsis and infection prevention and control policies is warranted.

CHAPTER ONE: INTRODUCTION

1.1 Background:

KwaZulu-Natal is one of the provinces with the highest seroprevalence of HIV (human immunodeficiency virus) among pregnant women (Naidoo, Sartorius & Tshimanga-Tshikala, 2016). Administration of combined antiretroviral agents in HIV-infected pregnant women has markedly reduced the risk of mother-to-child transmission (MTCT) of the virus, leading to an increasing number of HIV-exposed uninfected (HEU) infants (Evans, Jones & Prendergast, 2016). These infants are known to have more severe disease, more hospitalisations and higher mortality than HIV-unexposed (HU) children (Slogrove, Goetghebuer, Cotton, Singer & Bettinger, 2016).

The Zimbabwe Vitamin A for Mothers and Babies Project (ZVITAMBO) trial was the first study to demonstrate higher mortality and morbidity in HEU infants definitively (Slogrove et al., 2016). This study had the largest cohort of HIV-exposed uninfected and HIV-unexposed infants. The most common causes of death in HEU infants were acute respiratory infections (57.7%), diarrheal illness or dysentery (16.1%), malnutrition (13.3%), sepsis (6.0%), and meningitis (4.8%). The highest absolute mortality for HEU infants was in the neonatal period.

Neonatal infections cause nearly a quarter (23%) of all neonatal deaths, with neonatal sepsis accounting for 15% of these deaths (Folgori et al., 2017b). There is a paucity of data from low-income and middle-income countries concerning pathogen distribution, epidemiology and resistance patterns unlike the well-established surveillance networks in high-income countries (Okomo et al., 2019). Although the risk of mortality from neonatal infections is high in sub-Saharan Africa, there is a substantial gap in aetiology-specific data (Okomo et al., 2019).

Neonatal sepsis is defined as a systemic infection occurring in infants during the first 28 days of life (Simonsen, Anderson-Berry, Delair & Davies, 2014). Sepsis occurring in the first three days of life is defined as early-onset sepsis (EOS) and beyond three days as late-onset sepsis (LOS). Epidemiological studies have observed a reduction in EOS, while LOS incidence has been increasing (Dong & Speer, 2015). Group B *Streptococcus* (GBS) has historically been the predominant organism causing early-onset sepsis in neonates. With the introduction of prenatal screening and intrapartum prophylaxis, current trends show a decrease in GBS frequency (Dong & Speer, 2015).

Implementing interventions to improve the survival of premature infants has led to the incidence of LOS increasing (Dong & Speer, 2015). Prolonged hospitalisation of these premature and low birth weight infants has led to a change in the microbial characteristics of LOS globally. Common pathogens for late-onset sepsis include coagulase-negative *staphylococcus* (CoNS), *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, gram-negative bacilli, and *Candida*.

The incidence of severe infections caused by multidrug-resistant (MDR) pathogens is currently rising worldwide. An increasing number of neonates and children with serious bloodstream infections due to resistant bacteria are being reported (Folgori & Bielicki, 2019). Multi-drug resistant is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (Magiorakos et al., 2012).

Antimicrobial resistance (AMR) is responsible for approximately 30% of neonatal deaths due to sepsis, globally (Laxminarayan et al., 2016). These multidrug-resistant pathogens have become a challenge for both high and low-middle income countries and affect predominantly premature infants (Folgori, Bielicki, Heath & Sharland, 2017a).

The epidemiology of neonatal sepsis is changing, and increases in antimicrobial resistance is a global concern. The Delhi Neonatal Infection Study (DeNIS) followed up a cohort of 88 636 newborn infants in three large hospitals in Delhi (Agarwal, Sankar, Health & Centre, 2016). It represents one of the largest studies to date of neonatal sepsis and resistance in the Indian subcontinent. The study showed high rates of *Acinetobacter* spp and coagulase-negative *staphylococcal* infections among the pathogens.

Nearly half of the pathogens that cause severe neonatal bacterial infections are resistant to the first-line (ampicillin or penicillin, and gentamicin) and second-line (third-generation cephalosporins) WHO-recommended treatments (Folgori et al., 2017b). Due to resistant pathogens, infections necessitate early use of broad-spectrum antibiotics due to the ineffectiveness of recommended first-line antibiotics (Dramowski et al., 2020).

A few studies have reported on AMR from different neonatal units around the country. (Ballot et al. (2019) described an increase in multi-drug resistant organisms causing bloodstream infections (BSI) in a neonatal unit in Johannesburg, South Africa (Ballot et al., 2019). An observational study in the NICU (neonatal intensive care unit) of a regional hospital in the northern part KwaZulu-Natal found that of the144 neonates that developed a nosocomial infection, *Klebsiella pneumoniae* was the most prevalent organism followed by *Staphylococcus aureus* and *Acinetobacter baumanni* (Rameshwarnath & Naidoo, 2018). However, the real burden of antimicrobial resistance in South Africa is unknown due to the lack of national programmes for neonatal infection surveillance (Dramowski et al., 2020). Regular monitoring and bench-marking causes of neonatal sepsis and their susceptibility patterns are vital to update policies and improve clinical practices (Giannoni et al., 2018).

1.2 Aim:

The study aimed to:

- identify risk factors that are associated with neonatal infections in HIV-exposed and unexposed newborns at the King Edward VIII Hospital Neonatal Unit;
- establish the aetiology and susceptibility patterns of organisms responsible for neonatal infections; and
- document the outcome of neonates with proven infection.

1.3 Objective:

- To assess the risk factors associated with neonatal sepsis;
- To investigate the organisms isolated from blood cultures, respiratory and cerebrospinal fluid samples responsible for neonatal sepsis in the unit;
- To establish the antibiotic susceptibility profile of these organisms to antimicrobial agents used; and
- To determine the outcome of these infections – the primary outcome being mortality and secondary being morbidity.

CHAPTER 2: LITERATURE REVIEW

2.1 Neonatal sepsis

Sepsis remains the leading cause of neonatal mortality and morbidity (Giannoni et al., 2018). Neonates are prone to infections as their immune systems are still developing and not as robust (Basha, Surendran & Pichichero, 2014). Neutrophils, which are a major component of the innate immune system, are immature and have reduced phagocytic and chemotaxis ability. Neonatal antigen-presenting cells, mainly monocytes and dendritic cells, have impaired activity and are found in low numbers (Basha et al., 2014). The adaptive immune system is also underdeveloped due to reduced numbers of neonatal lymphocytes, B-cell immaturity and cytokine production being downregulated. HIV-exposed neonates are shown to have low concentrations of maternally derived antibodies at birth and numerous T-cell abnormalities, including low CD4 counts (Evans et al., 2016).

The incidence of neonatal sepsis varies in different geographic regions depending on the availability of resources, risk factors for both mother and infant, and prevention strategies (Giannoni et al., 2018). EOS presents within 72 hours after birth, affecting 0.5-1 out of 1000 infants in high-income countries, with a case fatality rate of 10%-15% (Giannoni et al., 2018). LOS is characterized by onset beyond 72 hours after birth with reported rates of hospital-acquired LOS as high as 40% in extremely preterm newborns (Giannoni et al., 2018).

Neonatal sepsis can range from subclinical infection to severe systemic disease. These infections include common childhood infections like pneumonia, meningitis and diarrheal disease, but morbidity and mortality are reported to be higher in HIV-exposed infants (Slogrove et al., 2016). Pathogens are acquired intrauterine, during the delivery process or in the post-natal period, and the clinical manifestations depend on pathogens and routes of infection.

2.2 Early Onset Sepsis vs Late-Onset Sepsis

Early-onset sepsis is caused by maternally transmitted pathogens and occurs during the intrapartum period or just before delivery and reflects transplacental or ascending infections from the maternal genital tract (Simonsen et al., 2014). The bacteria involved are part of the maternal gastrointestinal and genitourinary tract's normal bacterial flora, resulting in subsequent colonisation and the fetus's infection (Cortese et al., 2016). Newborns exposed to

these bacteria can become ill due to aspiration of infected amniotic fluid in utero or during the delivery process. Risk factors for infection include chorioamnionitis, prolonged rupture of membranes and inadequate intrapartum antibiotic prophylaxis (Zea-Vera & Ochoa, 2015).

LOS is caused by microorganisms acquired from caregivers and the environment. Premature infants that require prolonged hospitalisation are at risk, especially in those with very low birth weight (VLBW) (Dong & Speer, 2015). Risk factors include underlying respiratory and cardiovascular diseases and strategies implemented to improve the premature neonate's survival like the use of invasive devices, endotracheal intubation, vascular catheterization and prolonged antibiotics (Dong & Speer, 2015). The causative pathogens characteristically represent colonisation with the microflora of the nosocomial environment. Hospital-acquired LOS, therefore, represents a preventable disease. It may change over time within the same hospital due to patients' demographic characteristics, microflora colonisation of the nosocomial environment, and antibiotic use (Dong & Speer, 2015).

Neonatal meningitis is a life-threatening disease that develops following primary bacteraemia with secondary spread to the central nervous system (Bedetti et al., 2019). Mortality occurs in approximately 10% of affected neonates while 20–50% of the survivors develop cognitive deficiencies, motor abnormalities and sensory impairments (Gordon, Srinivasan & Harris, 2017). The causes of neonatal meningitis in the high-income countries are GBS, *Escherichia coli*, *Listeria monocytogenes*, Gram-negative enteric bacteria and *Streptococcus pneumoniae* (Furyk, Swann & Molyneux 2011). In low- and middle-income countries, Gram-negative bacilli such as *Klebsiella pneumoniae* and *E. coli* appear to be more important pathogens, especially in LOS (Khalessi & Afsharkhas, 2014).

Neonatal pneumonia is defined as a pulmonary infection occurring within the first 28 days of life and a common problem in sub-Saharan Africa (Green & Kolberg, 2016). Risk factors for neonatal pneumonia include prolonged rupture of membranes, prematurity, meconium aspiration syndrome and mechanical ventilation. With preterm birth complications and intrapartum complications, pneumonia remains one of the leading causes of neonatal mortality (Green & Kolberg, 2016). Aiken reported significantly higher rates of congenital pneumonia based on lung pathology in neonates of HIV-infected mothers (Aiken, 1992).

The causes of pneumonia in the neonatal period include bacteria and viruses. Perinatally, pneumonia is caused by vertically transmitted organisms such as GBS and *E. coli* (Green & Kolberg, 2016). *Ureaplasma urealyticum* and *Mycoplasma hominis* are being increasingly recognised as organisms that cause congenital pneumonia (Green & Kolberg, 2016). Common viral causes include herpes simplex virus (HSV) and respiratory syncytial virus (RSV). Viral infections are more often acquired via nosocomial transmission from staff and infected patients in the neonatal unit, but HSV is acquired during delivery through an infected maternal genital tract (Green & Kolberg, 2016).

Mechanical ventilation is a major risk factor for the development of LOS. Pneumonia in mechanically ventilated patients is due to the introduction of organisms into the trachea via the microaspiration of gastric and oropharyngeal secretions. The oropharyngeal flora may be colonised with the neonate's endogenous flora or exogenously from the intensive care environment, from the hands of healthcare workers or contaminated ventilator circuits and humidifiers (Safdar, Crnich & Maki, 2005). This provides a potential route of entry for hospital-acquired bacteria.

Ventilation-associated pneumonia (VAP) is a complication of mechanical ventilation and one of the most common hospital-acquired infections in the neonatal ICU (Cernada, Brugada, Golombek & Vento, 2014). The incidence of VAP in neonates is influenced by the gestational age and income level of the country, with an incidence of 2.7 to 10.9 episodes per 1000 ventilator days reported from high-income countries and higher rates of 37.2 cases per 1000 ventilator days in low- and middle-income countries (Cernada et al., 2014). The pathogens implicated in VAP also vary according to the geographic region. Gram-negative bacilli were found to be exceptionally high in Asia while in Europe and North America, *Staphylococcus aureus* was predominant (Aelami, Lotfi & Zingg, 2014). Therefore, knowledge about the commonest pathogens colonising trachea in mechanically ventilated patients is important at the institute level.

2.3 Organisms causing Early Onset Sepsis

Group B *Streptococcus* has historically been the predominant organism causing early-onset sepsis in neonates (Cortese et al., 2016). Group B *Streptococcus* is a Gram-positive bacterium that primarily colonises both the gastrointestinal and genital tracts of healthy women (Mukesi

et al., 2019). Twenty to forty percent of healthy women are asymptomatically colonised with GBS. Newborns are at risk of acquiring GBS during labour and delivery as they swallow or aspirate the bacterium while passing through the birth canal. Disease manifestations include meningitis, pneumonia, sepsis, and can lead to long-term disabilities and death (Gizachew et al., 2019). Cutland et al. (2015) evaluated invasive GBS disease in young infants in a high maternal HIV prevalence setting and found the incidence of GBS disease greater in HIV-exposed than unexposed infants (Cutland et al., 2015). Serotype distribution was similar in both groups with serogroup III, causing more meningitis and serotype V bacteraemia (Cutland et al., 2015).

Maternal antibiotic prophylaxis for GBS has led to an increase in Gram-negative organisms, causing sepsis. *Escherichia coli* is the second leading cause of EOS in neonates, accounting for about 24% of all EOS episodes (Simonsen et al., 2014). Like other coliforms, *E. Coli* colonises the maternal recto-vaginal area, and the newborn can acquire it during delivery. Very low birth weight (VLBW) infants showed higher E. coli infection rates in prospective surveillance of EOS by Stoll et al. (2011). Rates of infection with *E. coli* was higher than GBS in neonates weighing <2500g while in infants with a birth weight of >2500g, GBS rates were higher (0.35 vs 0.07 per 1000 Live births) (Stoll et al., 2011).

The remaining causes of EOS include *Staphylococcus aureus*, *Enterococcus faecalis, Enterococcus faecium*, *Listeria monocytogenes* and other gram-negative bacilli (Simonsen et al., 2014). *Streptococcus pyogenes* and *Streptococcus viridans*, *Streptococcus pneumoniae, H. Influenzae* and *Pseudomonas aeruginosa* are uncommon sources for EOS, but several reports have documented neonatal infections by these agents (Coetzee, Mbowane & de Witt, 2017).

A recent study by Velaphi et al. (2019) on the aetiology and incidence of EOS in neonates from a large academic hospital in Soweto, South Africa found that the most common organisms isolated were Gram-positive bacteria. Unlike reports from other sub-Saharan African countries, the most common organism in that study was GBS instead of *Staphylococcus aureus*. Gram-negative bacteria accounted for only 25% of EOS overall, with 8% being *E. coli* and *Acinetobacter species* isolated in 4% (Velaphi et al., 2019).

2.4 Organisms causing Late-Onset Sepsis

The development of LOS is inversely related to gestational age and birth weight. Epidemiological data on very low birth weight (VLBW) infants shows that the predominant pathogens are Coagulase-negative *staphylococci*, followed by Gram-negative bacilli and fungi (Dong & Speer, 2015). Coagulase-negative *staphylococci* have emerged as the predominant pathogen of LOS. It accounts for 53.2%–77.9% of LOS in industrialised countries and 35.5%–47.4% in some low- and middle-income regions (Dong & Speer, 2015). The main gram-negative bacilli responsible for neonatal LOS include *Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp. and *Pseudomonas* spp. In some regions, *Candida* spp. is reported to be one of the major pathogens for LOS (Dong & Speer, 2015). Due to the difficulties in prompt diagnosis of LOS and the high risk of mortality and long-term neurodevelopmental sequelae, empirical antibiotic treatment is initiated on suspicion of LOS.

2.5 Diagnosis of Neonatal Sepsis

The clinical diagnosis of neonatal sepsis is difficult as signs and symptoms are nonspecific and unreliable in a newborn. The use of maternal intrapartum antimicrobial prophylaxis can confirm sepsis in the neonate challenging (Iroh Tam & Bendel, 2017). Laboratory diagnosis is limited due to the low sensitivity of most specimens. The gold standard for microbiological diagnosis for neonatal sepsis has historically relied on culture from a sterile site, including blood, cerebrospinal fluid or urine (Coetzee et al., 2017). Blood culture positivity is affected by several factors. The current recommended minimal blood volume inoculated is 1ml, and the sensitivity is reported to decrease by 10% to 40% when 0.5 ml is inoculated (Reyes, 2018). Culture negative results may also result from prior maternal antimicrobial treatment and intermittent or low-density bacteraemia found in neonates (Zea-Vera & Ochoa, 2015).

Endotracheal aspirates are readily obtained from ventilated patients, but sensitivity and specificity are low (Coetzee et al., 2017). Clinicians often use semi-quantitative neutrophil counts and quantitative bacterial culture results from endotracheal aspirates in conjunction with clinical and radiographic signs to diagnose VAP (Ergenekon & Çataltepe, 2020). Although a negative tracheal aspirate culture has a high negative predictive value for excluding VAP, positive cultures may identify the organisms that are colonising the airway (Coetzee et al., 2017). Patil (2014) looked at tracheal colonization in 100 mechanically ventilated patients for seven days doing tracheal aspirates on Day 1, 4 and 7. They noted that the isolation rate

increased with increased ventilation duration from 18.6% to 44.6% (Patil, 2014). Among the positive cultures, *Pseudomonas aeruginosa* was the most commonly isolated organism (37.4%). This was followed by *Klebsiella pneumonia*e (28.5%), *Staphylococcus epidermidis* (14.7%) and *Staphylococcus aureus* (4.36%). The isolation rate of *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e increased with the duration of ventilation, but a significant increase was seen with Coagulase negative *staphylococcus* (Journal & Science, 2014).

Bacterial meningitis causes high mortality and morbidity, and positive CSF culture is the gold standard for its diagnosis. Bedetti et al. reported that nearly one-quarter had concurrent meningitis in septic neonates with positive blood cultures (Bedetti et al., 2019). However, CSF culture is of poor sensitivity; therefore, clinicians rely on other CSF parameters like CSF glucose, white blood cell (WBC) count and protein (Bedetti et al., 2019).

The use of molecular diagnostics has been evaluated for the diagnosis of neonatal sepsis. These predominantly amplification methods, such as polymerase chain reaction (PCR) are more rapid and sensitive than culture-based methods (Iroh Tam & Bendel, 2017). They carry promise in neonatal infections where intrapartum exposure to antimicrobials, low-density bacteremia and culture-negative sepsis is common. Velaphi et al. (2019) evaluated the combination of blood culture and a polymerase chain reaction (PCR)-based test to determine the incidence and aetiology of EOS. The molecular assay used identified more organisms from blood culture, including non-culturable organisms like *Ureaplasma* species. However, a combination of blood culture and this assay improved the detection of organisms in neonates with sepsis (Velaphi et al., 2019).

2.6 Neonatal HIV Infection

Maternal HIV infection can have severe adverse effects on pregnancy. It leads to preterm delivery, low birth weight infants at risk for postnatal morbidity and mortality (Xiao et al., 2015). A significant finding by Naidoo et al. (2016) was that HIV-positive mothers were four times more likely to have preterm delivery than their HIV-negative counterparts (OR 4.09, 95% CI: 1.37–12.17) ($p = 0.010$) (Naidoo et al., 2016). HEU infants experience numerous exposures during fetal and early life that could increase their vulnerability to infectious diseases during infancy compared to HIV-unexposed infants (Slogrove et al., 2016). Diagnosing and treating paediatric HIV infection early, improves morbidity and mortality. Infant mortality is reduced

by 76% with early HIV diagnosis and antiretroviral therapy, while the progression of HIV is slowed by 75% (Templer et al., 2016).

Early HIV testing in infants is crucial for immediate access to treatment and care programmes. Serologic tests are unreliable (Templer et al., 2016). Passively transferred maternal antibodies may be detected and may not indicate infant infection. The universal birth HIV PCR testing programme was introduced in 2015 by the WHO to diagnose HIV-infected neonates as early as possible and initiate ART. Current guidelines recommend antiretroviral drugs to all newborns with perinatal exposure to HIV (South African National Department of Health, 2019).

2.7 Antimicrobial Resistance

The epidemiology of neonatal sepsis is changing, with resistant infections often occurring in the first days of life (Dramowski et al., 2020). A systemic review of studies from sub-Saharan Africa and the Indian subcontinent found the incidence of antibiotic resistance among neonates ranged from 2.9 (95% CI 1.9–4.2) to 24 (95% CI 21.8–25.7) for 1000 live births (Huynh et al., 2015). Mortality from drug-resistant bacteria is higher in low- and middle-income countries than in high-income countries (Crellen et al., 2019). These infection-attributable deaths affect more preterm and low-birthweight neonates (Dramowski et al., 2020). Multi-drug resistant gram-positive pathogens include Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant Enterococcus and penicillin-non-susceptible *Streptococcus pneumoniae*. These infections are being contained by improved infection control measures, development of new antibiotics and vaccines (Folgori & Bielicki, 2019). Gram-negative pathogens have been reported to have high resistance levels with a large proportion being resistant to the first and second-line treatment for neonatal sepsis recommended by the WHO (Folgori & Bielicki, 2019). These include ESBL- and carbapenemase-producing *K. pneumoniae*, *Acinetobacter baumanii*, *P aeruginosa*, and *Enterobacter* species have been referred to as "ESKAPE" pathogens. Greater than 80% of the *Acinetobacter* spp. in the Delhi Neonatal Infection Study were untreatable and pan-resistant with high mortality (Mathur, Li, Folgori, Sharland & Heath, 2016; Agarwal et al., 2016)

Gram-negative bacteria display antimicrobial resistance by expressing antibiotic-inactivating enzymes and non-enzymatic mechanisms such as efflux pumps and alterations by loss of outer

membrane porins (Ruppé, Woerther & Barbier, 2015). Resistance to beta-lactam antibiotics (β-lactams) in Enterobacteriaceae is due primarily to β-lactamase-mediated antibiotic hydrolysis, while efflux pumps and porins play a minor role (Gazin et al., 2012). Production of beta-lactamase may be chromosomally mediated or plasmid-borne where the genes are carried on plasmids and transposons (Ruppé et al., 2015). These horizontally transferable genes can also carry genes that encode resistance to aminoglycosides and fluoroquinolones (Ruppé et al., 2015).

Beta-lactamases are divided into four classes based upon their amino acid sequences according to the Ambler classification. This is the most widely used classification, and they are divided into four classes (A, B, C, and D) (Hall & Barlow, 2005). Extended-spectrum β-lactamases (ESBL) belonging to Ambler class A are most abundant and include the sulfhydryl reagent variable β-lactamase (SHV), Temoniera β-lactamase (TEM) and cefotaxime-M β-lactamase (CTX-M) types. These clinically significant extended-spectrum β-lactamases have spread rapidly between *Klebsiella pneumoniae*, *Enterobacter* spp. and *E. coli* and have become endemic in many hospitals globally (Ruppé et al., 2015).

Carbapenems remained the last-resort treatment for ESBL related infections. However, its increasing use has led to resistance. Mechanisms of carbapenem resistance are in the form of porin loss, active expulsion from the periplasmic space and beta-lactamases (Meletis, 2016). These carbapenemases are divided into metallo-carbapenemases (zinc-dependent Class B) and non-metallo-carbapenemases (zinc independent Class A, C and D) (Lee et al., 2016). The most common Enterobacteriaceae exhibiting carbapenem resistance is *Klebsiella pneumoniae* and the class A enzyme *K. pneumoniae* carbapenemase (KPC), is the most frequent class identified worldwide (Lee et al., 2016). Other carbapenemases in this class include IMI (imipenem-hydrolyzing β-lactamase), NMC-A (not metalloenzyme carbapenemase), SME (Serratia marcescens enzyme) and GES (Guiana extended-spectrum) enzymes (Meletis, 2016).

Class B carbapenemases are the metallo-β-lactamases (MBLs) that require zinc for the β-lactam hydrolysis and include IMP (active on imipenem), VIM (Verona integron-encoded MBL), and New Delhi metallo-β-lactamases (Meletis, 2016). Class C includes AmpC β-lactamase enzymes with weak hydrolytic activity against carbapenems, but their resistance is due to the added mechanisms of decreased outer membrane permeability and efflux pump (Meletis, 2016). Ambler class D carbapenemases are oxacillinases OXA (oxacillin-hydrolyzing) which

can hydrolyze oxacillin and are commonly found in *Pseudomonas aeruginosa* and *Acinetobacter* spp. (Meletis, 2016).

The geographic distribution of carbapenemases is similar in adult and pediatric populations (Chiotos, Han, & Tamma, 2016). The KPC-producing strains account for up to 80% of carbapenem resistance in the United States and dominate in Italy, Israel, Greece and Portugal. NDM-1 metallo-β-lactamases is endemic in the Indian region and South Asia, while OXA-type carbapenemases have been reported in the Middle East, North Africa and is endemic inTurkey (Folgori & Bielicki, 2019).

Case reports and case series currently guide treatment for Carbapenemase-producing Enterobacteriaceae (CPE) infections in neonates as optimal treatment regimes, and therapeutic agents are unavailable (Folgori & Bielicki, 2019). The majority of CPE isolates are resistant to the most antibiotic classes. Therefore, Polymyxins have re-emerged as a treatment option for serious infections due to carbapenem-resistant organisms (Folgori & Bielicki, 2019). Tigecycline has shown to have in vitro activity and has been used when other options are exhausted in paediatric patients, however, neonatal data is lacking.

CHAPTER THREE: MATERIALS AND METHODS

This was a retrospective record review conducted in the King Edward VIII Hospital (KEH) Neonatal Unit in Durban between July 2014 and December 2019. KEH has 852 beds with approximately 22 000 outpatients monthly and provides tertiary services to the whole of the KZN province and part of the Eastern Cape.

3.1. Ethics Approval

The Biomedical Research Ethics Committee of the University of KwaZulu-Natal approved this study (BE342/13) (**Appendix 5**). Gatekeeper Permission was obtained from King Edward VIII Hospital (**Appendix 6**). Permission was obtained from the Department of Health to conduct research at King Edward VIII Hospital (**Appendix 7**).

3.2. Study site

The study was conducted in the Neonatal Unit at King Edward VIII Hospital, Durban, KwaZulu-Natal, South Africa. KEH is a regional hospital that delivers approximately 670 babies per month. Of these, around 300 are admitted to the nursery for care.

3.3 Study Population

The study population included neonates admitted to the KEH Neonatal Unit between July 2014 and December 2019. Neonates with suspected sepsis who had blood culture, CSF, sputum or endotracheal aspirates sent to the National Health Laboratory Service (NHLS) microbiology laboratory, were included in the study. The clinical data of these patients were reviewed.

3.4. Specimen Collection

A septic workup was done on any neonate showing signs and symptoms of infection, including poor feeding, irritability, lethargy and apnoea. Specimens sent to microbiology laboratory included blood cultures, sputum specimens, cerebrospinal fluid if indicated, and endotracheal aspirates from ventilated patients.

Blood cultures were obtained from neonates that were admitted to the KEH Neonatal Unit with suspected sepsis. One to three millilitres of blood were taken using aseptic technique and

inoculated into Paediatric blood culture bottles. These samples were processed at the NHLS microbiology laboratory at KEH. The fully automated continuous monitoring blood culture system BacT/Alert Microbial Detection System (BioMerieux, France) was used.

Cerebrospinal fluid: All lumbar punctures were performed using aseptic technique and at least 0.5ml of CSF in a sterile additive-free tube was sent to the laboratory.

Sputum samples or endotracheal aspirates (ETA) from ventilated patients and gastric aspirates were sent to the microbiology laboratory. Gastric aspirates were sent for *Mycobacterium tuberculosis* (TB) work-up.

HIV samples were sent for early infant diagnostic testing. Dried Blood Spot for HIV was done at the Department of Virology, Inkosi Albert Luthuli Central Hospital. The analysis was done in the following categories: HIV-exposed vs HIV-unexposed neonates.

3.5. Laboratory Investigations

Positive blood cultures were removed from the instrument and microscopy and culture performed. Blood cultures were considered negative if there was no growth after continuous incubation for up to 7 days.

Gram stain was performed on positive bottles, and Gram-negative bacilli were reported immediately to the KEH Neonatal Unit. Identification of bacterial subcultures was performed using the automated Vitek 2 AES (BioMerieux, France). Susceptibility results were interpreted using the Clinical and Laboratory Standards Institute criteria.

ETAs and sputum specimens were subjected to Gram staining, and the rest of the samples were immediately subcultured onto a blood agar plate, a MacConkey plate, and a chocolate agar plate. Gastric aspirates were cultured for TB as per laboratory standard operating procedures.

CSF: Once received, the macroscopic appearance was recorded, and the sample was centrifuged at 1000 × g for 10-15 min. Microscopy included a cell count and a Gram stain. The sediment was then subcultured onto blood, chocolate and MacConkey agar plates and incubated at 37°C with 5 per cent CO_2 for 18-24 hours.

3.5.1 Bacterial Isolation

Plates were examined for pure cultures or the dominant pathogens. The selected colonies were plated out onto blood agar plates to ensure purity. These plates were then incubated at 37 °C for 24 hours. A sufficient number of morphologically similar colonies from the purity plate was used to prepare a homogenous organism suspension with a density equivalent to the appropriate McFarland standard using the VITEK 2 DensiCHEK Plus. The prepared suspension was then transferred into the appropriate cards. All fungal cultures were sent to the Mycology laboratory at Inkosi Albert Luthuli Central Hospital for further identification and susceptibility testing.

3.5.2 Identification

The VITEK 2 system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing. The Advanced Expert System (AES) is a database that comprises information on the specific organism and its specific minimum inhibitory concentration results based on previous findings.

Yeasts were identified using the Vitek 2 or API® 20C AUX (BioMerieux). Antifungal susceptibility testing was performed using ETESTs® or Vitek 2 (BioMerieux). The susceptibility values were interpreted using Clinical and Laboratory Standards Institute guidelines taking into account the species-specific clinical breakpoints.

3.6 Data Collection

A data collection sheet was used to record the data retrospectively. (**Appendix 2**).

Data included paper-based hospital case notes including patient's demographics, gestational age, birth weight, mode of delivery, admission diagnosis, underlying and associated conditions, use of central venous catheters and peripherally inserted central catheters (PICC), use of total parenteral nutrition, feeding, mechanical ventilation, maternal HIV status, antibiotic administration and surgical or invasive procedures.

The maternal HIV status included antiretroviral treatment and CD4 counts where available and Neonatal HIV-PCR test results.

The universal birth HIV PCR testing programme was introduced in 2015 by the WHO in order to diagnose HIV-infected neonates as early as possible and initiate ART.

3.7 Definitions:

Outcome:

The outcome was documented as discharged or as a poor outcome if the neonate demised during the current admission.

Empirical treatment

Neonates received penicillin G or ampicillin and gentamicin as first-line therapy or cefotaxime and ampicillin if meningitis was suspected as per unit policy. Empirical treatment for healthcare-associated infections was piperacillin-tazobactam and amikacin, with escalation to meropenem in suspected meningitis cases.

Prematurity was defined according to WHO definition:

Normal weight at term delivery is 2500–4200 g

Low birth weight (LBW) is defined as a birth weight of an infant less than 2500 grams

Very low birth weight (VLBW) is defined as a birth weight of an infant less than 1500 g

Extremely low birth weight (ELBW), is defined as a birth weight of an infant which is less than 1000 g

The WHO classification was used for gestational age:

Full Term is 38–41 weeks,

Moderately preterm is 32–37 weeks

Very Preterm 28-32 weeks

Extremely preterm is less than 28 weeks

3.8 Inclusion and Exclusion critera

Inclusion criteria:

- All neonates (days 1 - 28) admitted to KEH nursery for > 24 hours that were suspected of having infection/sepsis by the clinician were eligible for enrolment in the study. Sepsis was defined as nonspecific symptoms and signs of infection accompanied by bacteraemia or positive culture from a sterile site in the first 28 days of life. Neonates

with suspected sepsis who had blood culture, CSF, sputum or endotracheal aspirates sent to the National Health Laboratory Service (NHLS) microbiology laboratory, were included in the study.

Exclusion criteria:

- No consent obtained
- Neonates with severe congenital or chromosomal abnormalities will be excluded from the study.
- < 24 hours stay in the nursery

The following organisms were considered to be contaminants:

1. *Micrococcus* species
2. *Bacillus* species
3. *Corynebacterium* species
4. *Streptococcus viridans*

We chose to exclude Coagulase-negative *staphylococcus* in our analysis despite current literature implicating it as a cause of LOS. Determining clinical significance is problematic. Coagulase negative *staphylococcus* (CoNS) is a known blood culture contaminant, and it requires repeat positive cultures to establish significance.

3.9 Statistical Analysis

Data was captured in Ms Excel spreadsheet and loaded into R Statistical computing software for analysis. Simple descriptive statistics and inferential statistics were used for data analysis. The data consisted of categorical variables. The descriptive statistics included counts and percentage frequencies only. The descriptive statistics were further visualised using simple and multiple bar charts. A component bar chart was used to visualize the susceptibility of the *Klebsiella pneumoniae*. Pareto charts were also used to present the prevalence of organisms in blood cultures. The bivariate association between the categorical variables was assessed using either the Chi-square test and Fisher's exact test depending on the cross-tabulations' cell distribution. Fisher's exact test was specifically applied where the expected frequency of at least one of the cross-tabulation cells was less than 5%.

Univariate and multiple logistic regression was applied to assess the risk factors associated with neonatal sepsis and outcome

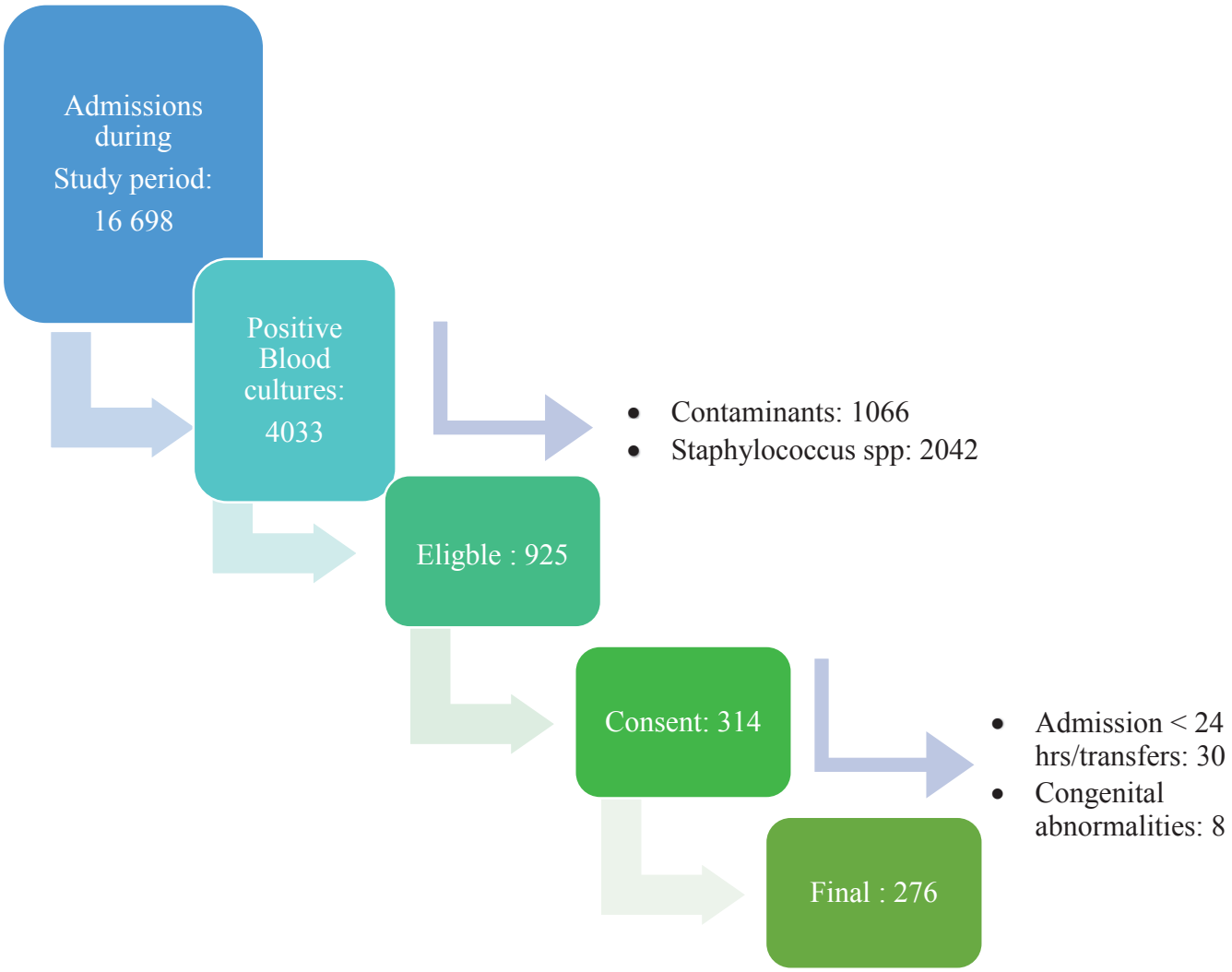
CHAPTER FOUR: RESULTS

4.1 Characteristics of Study Population, Clinical attributes and Interventions

4.1.1 Characteristics of the Study population

The study population comprised of 276 neonates. Neonates with suspected sepsis who had blood culture, CSF, sputum or endotracheal aspirates sent to the National Health Laboratory Service (NHLS) microbiology laboratory, were eligible. Consent was obtained from 314 mothers, but 38 neonates were excluded. The flow diagram shows how the sample size was reached. (Figure 1)

Figure 1. Flow diagram of positive blood cultures



There was an almost equal proportion of patients, 49.3% (n = 136) that were HIV-unexposed and 50.7% (n = 140) that were HIV-exposed (**Table 1**). There were more male than female neonates in both groups – 58.1% vs 41.9% for HIV-unexposed and 55% vs 45% in the HIV-exposed group. (**Table 1**) Eighty-eight percent of the neonates were born inside the healthcare facility, and eleven percent were out-born either at home or another facility. More neonates were delivered via caesarean section in both the HIV-exposed (47.9% vs 40.7%) and unexposed (47.1% vs 41.2%) in-borns. The proportions of gender (p = 0.605) and delivery modes (p = 0.962) were not significantly different between the groups.

Antenatal care was documented for the majority of the mothers. Prematurity was the most common admission diagnosis, and the major clinical presentation was respiratory distress. Twenty-six neonates were seropositive for other concurrent congenital infections including Syphilis, Cytomegalovirus, Rubella and Parvovirus.

CD4 counts were documented for 81 of HIV-positive mothers. Median CD4 cell count was 534 cell/mm^3 with 59% having a CD4 cell count >350/mm^3. The universal birth HIV PCR testing programme was introduced in 2015 by the WHO to diagnose HIV-infected neonates as early as possible and initiate ART. Of the 140 exposed neonates, 28 had no HIV PCR performed at birth as it was only introduced into the standard of care in late 2015 at KEH. One hundred and ten neonates had negative birth PCR results, while two neonates had positive results.

Table 1. Study population characteristics by HIV exposure and outcome

HIV exposure	Unexposed (N=136)	Exposed (N=140)	p-value	Overall (N=276)
Sex			0.605	
Female	57 (41.9%)	63 (45.0%)		120 (43.5%)
Male	79 (58.1%)	77 (55.0%)		156 (56.5%)
Delivery			0.962	
Normal Vaginal Delivery	56 (41.2%)	57 (40.7%)		113 (40.9%)
Caesarian section	64 (47.1%)	67 (47.9%)		131 (47.5%)
Out-born	16 (11.8%)	15 (10.7%)		31 (11.2%)
Missing	0 (0%)	1 (0.7%)		1 (0.4%)
Gestation			**<0.001**	
Term (>37 weeks)	54 (39.7%)	28 (20.0%)		82 (29.7%)
Moderately pre-term (32-37weeks)	21 (15.4%)	46 (32.9%)		67 (24.3%)
Very pre-term (28-32 weeks)	45 (33.1%)	48 (34.3%)		93 (33.7%)
Extremely pre-term (<28 weeks)	16 (11.8%)	18 (12.9%)		34 (12.3%)
Weight group			**<0.001**	
AGA >2500g	54 (39.7%)	26 (18.6%)		80 (29.0%)
LBW <2500g	19 (14.0%)	18 (12.9%)		37 (13.4%)
ELBW <1000g	41 (30.1%)	44 (31.4%)		85 (30.8%)
VLBW <1500g	22 (16.2%)	52 (37.1%)		74 (26.8%)

The p-values are based on non-missing cases only(tableStack).

4.1.2 The association between HIV exposure and clinical attributes

Majority of the neonates 194/276 (70%) in this study, were born preterm. HIV-exposure was found to be associated with prematurity (p <0.001) and low birth weight (p <0.001). The most notable differences for gestation was a higher proportion of Term neonates in the HIV-unexposed 39.7% (54/136) as compared to the HIV-exposed 20.0% (28/140).

Mortality was higher in the HIV-exposed neonates (10% vs 6.6%) (p=0.309) although it was not statistically significant. (**Table 2**)

Table 2. Clinical presentation and outcome by HIV exposure

HIV exposure	Unexposed (N=136)	Exposed (N=140)	p-value	Overall (N=276)
MAS	20 (14.7%)	9 (6.4%)	**0.026**	29 (10.5%)
Respiratory symptoms	100 (73.5%)	106 (75.7%)	0.677	206 (74.6%)
Meningitis	6 (4.4%)	4 (2.9%)	0.538	10 (3.6%)
Hepatosplenomegaly	3 (2.2%)	3 (2.1%)	1.000	6 (2.2%)
NEC	4 (2.9%)	3 (2.1%)	0.720	7 (2.5%)
Outcome: Demised	9 (6.6%)	14 (10.0%)	0.309	23 (8.3%)

The p-values are based on non-missing cases only(tableStack).

4.1.3 The association between HIV exposure and Interventions

Respiratory interventions included intermittent positive pressure ventilation (IPPV) and nasal continuous positive airway pressure (CPAP). More HIV-exposed babies required ventilation by IPPV (51.8% vs 40.9%) and CPAP (50.4% vs 41.6%). Interventions, including IPPV and umbilical artery catheterisation were associated with poor neonatal outcome p=0.006 and p=0.022, respectively. (**Table 3**)

Table 3. Clinical attributes and Interventions by the outcome

Outcome	Survived (N=253)	Demised (N=23)	p-value	Overall (N=276)
Meningitis symptoms	7 (2.8%)	3 (13.0%)	**0.042**	10 (3.6%)
NEC	4 (1.6%)	3 (13.0%)	**0.014**	7 (2.5%)
IPPV	111 (43.9%)	17 (73.9%)	**0.006**	128 (46.4%)
UAC	56(22.1%)	10(43.5%)	**0.022**	66(23.9%)

Risk factors associated with poor outcome were analysed and adjusted for HIV, sex and weight group. (**Table 4**)

Table 4. Risk factors associated with Outcome

	OR (univariable)	OR (multivariable)
Extremely Pre-term (<28 weeks)	3.28 (1.01-11.05, p=0.047)	0.64 (0.04-14.25, p=0.768)
NEC	9.34 (1.74-45.27, p=0.005)	12.10 (1.29-122.51, p=0.030)
Meningitis	5.25 (1.07-20.54, p=0.023)	0.58 (0.20-1.64, p=0.299)
IPPV	3.62 (1.45-10.32, p=0.009)	2.83 (0.81-11.87, p=0.120)
UAC	2.71 (1.10-6.48, p=0.026)	1.60 (0.49-5.11, p=0.426)

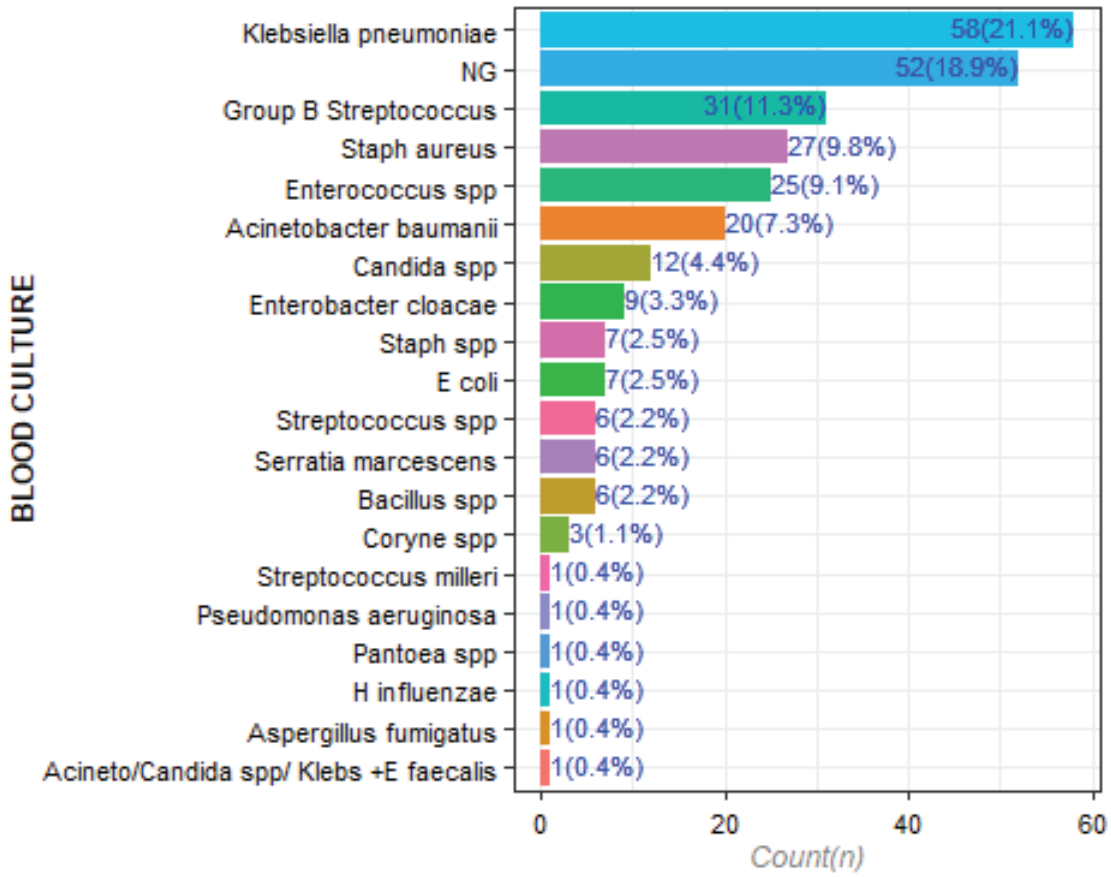
Neonates that were ventilated with IPPV were 3.62 times more likely to have a poor outcome. After controlling for the other risk factors like HIV exposure, sex and weight, the likelihood of death decreased to 2.8X.

4.2 Microbiology Results

There were 133 cerebrospinal fluid samples, 276 blood cultures and 121 endotracheal aspirates received for microbiological workup. Poly-microbial infections were found in blood cultures and the endotracheal aspirates.

4.2.1 Blood cultures

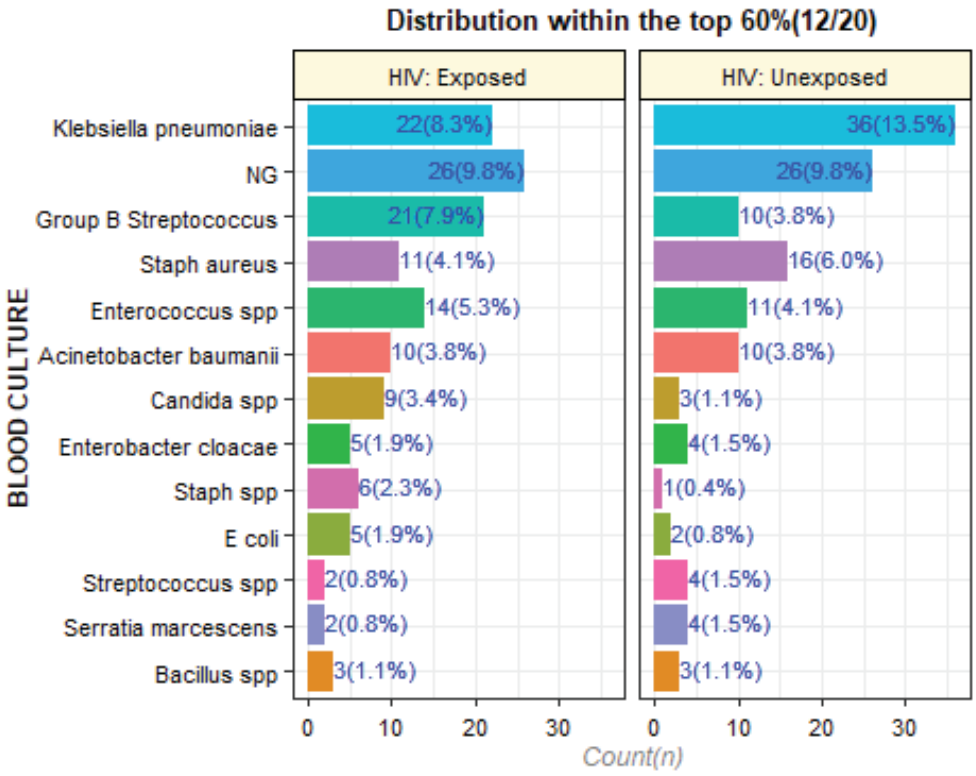
Of the 276 blood cultures received, bacterial growth occurred in 222 of the samples. The most common isolate causing bloodstream infections in the unit was *Klebsiella pneumoniae* (**Figure 2**). This was followed by gram-positive bacteria including Group B *Streptococcus*, *Staphylococcus aureus* and *Enterococcus* spp. Coagulase-negative *staphylococci* (CoNS) and *Corynebacterium spp* were excluded from the analysis.



Key Note: NG – no growth

Figure 2. Profile of organisms from blood cultures

In the HIV-unexposed neonates, *Klebsiella pneumoniae* and *Staphylococcus aureus* were found to be the predominant organisms isolated while in the HIV-exposed neonates *Klebsiella pneumoniae* and Group B *Streptococcus* were found. (**Figure 3**) Other Gram-negative bacteria included *Acinetobacter* spp. and *Enterobacter cloacae*, which were similar in both groups. *E. coli* (1.9% vs 0.8%) and *Candida spp* (3.4% vs 1.1%) were more predominant in the HIV-exposed neonates.



Key Note: NG – no growth

Figure 3. Distribution of the top 60% of blood culture isolates

When analysed by birth weight, *Klebsiella pneumoniae* was the main cause of sepsis in HIV-unexposed infants for all weight categories. (**Figure 4**) However, the spectrum of the pathogens was different among the HIV-exposed infants. For the HIV-exposed neonates with normal birth weight *Group B Streptococcus* was the most common bacteria isolated while in the low birth weight neonates *Klebsiella pneumoniae* was predominant. *Enterococcus* spp. followed by *Candida* spp. were the main causes of sepsis in the HIV-exposed VLBW.

Klebsiella pneumoniae and *Staphylococcus aureus*, were predominant in the HIV-unexposed ELBW neonates, while *Klebsiella pneumoniae* and *Enterococcus* spp. were found in the HIV-exposed ELBW neonates.

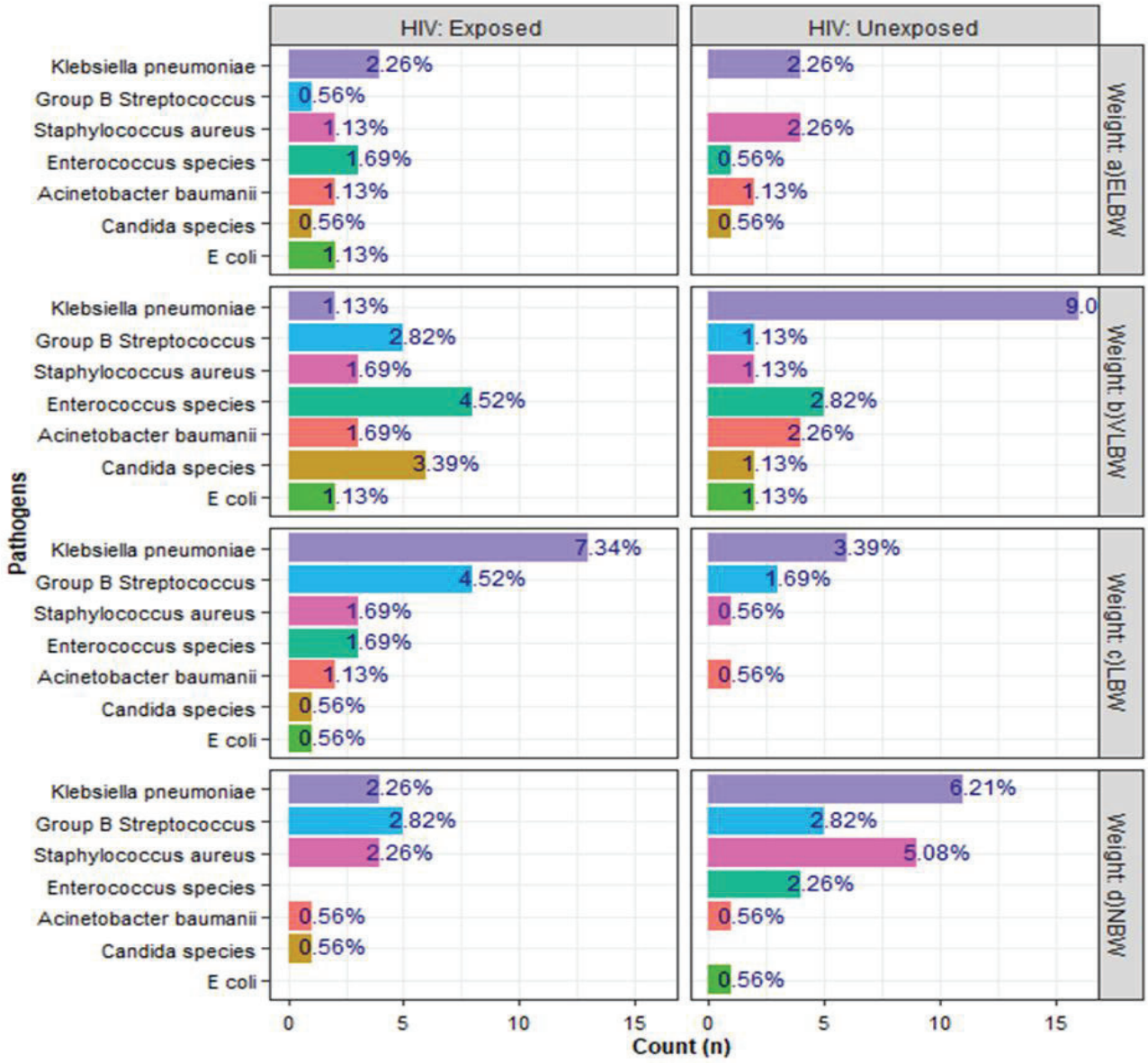


Figure 4. Blood culture results stratified by birth weight

4.2.2 Profile of Pathogens in Early Onset Sepsis vs Late-Onset Sepsis

Early Onset Sepsis

From the bloodstream infections (BSI), there were 66 cases of culture-confirmed EOS, of which 25/197(13%) organisms were in the HIV-unexposed, and 41/197(21%) in the HIV-exposed. (**Table 5**) For HIV-exposed infants with early-onset sepsis, GBS was the most common organism. In the unexposed neonates, GBS and *Klebsiella pneumoniae* were both found to be predominant. Overall for EOS, Gram-positive bacteria were found to predominate in both HIV-exposed and unexposed neonates 15/25(60%) for unexposed and 29/41(71%) in exposed) and

included GBS, *Staphylococcus aureus* and *Enterococcus* spp. *Candida* spp. was isolated in two exposed neonates with EOS.

Table 5. Organisms isolated from EOS vs LOS

Organism isolated	Total no. of organisms	EOS		LOS	
		Unexposed	Exposed	Unexposed	Exposed
	197(%)	25	41	71	60
Escherichia coli	7(3.6)	0(0)	0(0)	2(2.8)	5(8.3)
Klebsiella pneumoniae	59(30)	8(32)	4(9.8)	28(39.4)	19(31.7)
Enterobacter species	7(3.6)	1(4)	3(7.3)	1(1.4)	2(3.3)
Acinetobacter species	20(10)	1(4)	2(4.9)	9(12.7)	8(13.3)
Serratia marcescens	6(3)	0(0)	1(2.4)	4(5.6)	1(1.7)
Psuedomonas aeruginosa	1(0.5)	0(0)	0(0)	0(0)	1(1.7)
Group B Streptococcus	31(15,7)	8(32)	21(51)	2(2.8)	0(0)
Staphylococcus aureus	27(13.7)	5(20)	4(9.8)	11(15.5)	7(11.7)
Enterococcus species	25(12.7)	2(8)	4(9.8)	9(12.7)	10(16.7)
Candida Species	13(6.6)	0(0)	2(4.9)	4(5.6)	7(11.7)
Aspergillus	1(0.5)	0(0)	0(0)	1(1.4)	0(0)

Late-Onset Sepsis

In infants with late-onset sepsis, the Gram-negative bacilli accounted for 80/131(61 %) of the organisms. *Klebsiella pneumoniae* was the most common in both HIV-exposed and unexposed. *Staphylococcus aureus* and *Enterococcus* spp. were the predominant gram-positive organisms with only two cases of GBS recorded. (**Table 5**)

In the HIV-unexposed neonates with LOS, *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most common bacteria isolated in BSI followed by *Acinetobacter* spp and *Enterococcus spp*. *Serratia marcescens* was predominantly found in the HIV-unexposed neonates with LOS.

In HIV-exposed neonates with LOS, *Klebsiella pneumoniae* and *Enterococcus* spp were the most common bacteria isolated, followed by *Acinetobacter* spp and *Staphylococcus aureus*. Among the 13 isolates of *Candida* spp. found, 4/13(31%) occurred in HIV-unexposed neonates, and 7/13(54%) in HIV-exposed neonates.

4.2.3 Antibiotic susceptibility

Gram-positive organisms

Gram-positive susceptibilities were performed using the Vitek 2 system. The tested antibiotics included Ampicillin, Clindamycin, Cloxacillin, Erythromycin, Gentamicin, Linezolid and Vancomycin (**Table 6**).

Table 6. Antimicrobial susceptibility profile of Gram-Positive Bacteria

	% Susceptible		
Antibiotic	***Streptococcus agalactiae* (=n=31(%)**	***Staphylococcus aureus* n=27(%)**	***Enterococcus* spp. n=25(%)**
Ampicillin	31(100)	NA	10(40)
Clindamycin	28(90)	10(37)	0(0)
Cloxacillin	NA	7(26)	NA
Erythromycin	28(90)	9(33)	0(0)
Gentamicin	NA	12(44)	NA
Linezolid	31(100)	27(100)	25(100)
Vancomycin	31(100)	27(100)	24(96)

Key Note: NA – not applicable

All the GBS isolates were susceptible to penicillin, ampicillin, linezolid and vancomycin. Resistance to erythromycin and clindamycin was found in 3/31(10%) of the isolates.

Among the Enterococcus isolates, 9/25(36%)were *E faecalis* that were all susceptible to ampicillin. Fifteen of the twenty-five (60%) were *E faecium* that were resistant to ampicillin. All Enterococcal isolates were susceptible to linezolid and vancomycin apart from one isolate of *E gallinarum*, which was expected.

All 27(100%) isolates of *S. aureus* were susceptible to Vancomycin and Linezolid. Susceptibility to Cloxacillin was found in 7/27(26%) of the isolates, and they were also susceptible to Clindamycin, Erythromycin and Gentamicin. The remaining 20/27(74%) of the *Staphylococcus aureus* were methicillin-resistant with 17/27(63%) and 18/27(67%) of these isolates resistant to Clindamycin and Erythromycin respectively. Gentamicin susceptibility was found in 12/27(44%) of the isolates.

The susceptibility patterns of Gram-negative bacteria are presented in **Table 7**.

Table 7. Antimicrobial susceptibility profile Gram-negative bacteria

Gram-negative bacteria n=99(%)					
	Klebsiella pnuemoniae **(n=59)**	***Acinetobacter baumanii*** **(n=20)**	***E. coli*** **(n=7)**	***Serratia marcescens*** **(n=6)**	***Enterobacter*** **Spp. (n=7)**
Ampicillin	0(0)	0(0)	0(0)	0(0)	0(0)
Amoxicillin-clavulanate	15(25)	0(0)	7(100)	0(0)	0(0)
Piperacillin- tazobactam	25(42)	3(15)	7(100)	0(0)	5(71)
Cefotaxime	17(29)	2(10)	3(43)	0(0)	0(0)
Imipenem	47(80)	3(15)	7(100)	6(100)	7(100)
Meropenem	46(78)	3(15)	7(100)	6(100)	7(100)
Gentamicin	13(22)	5(25)	3(43)	6(100)	5(71)
Amikacin	46(78)	13(65)	7(100)	6(100)	7(100)
Ciprofloxacin	34(58)	5(25)	5(71)	6(100)	7(100)
Tigecycline	57(97)	17(85)	7(100)	NA	NA

Key Note: NA – not applicable

All isolates were resistant to ampicillin. Resistance to third-generation cephalosporins occurred in more than half of the isolates.

All isolates of *E. coli* were fully susceptible to piperacillin-tazobactam, amikacin, imipenem and meropenem. Three of the seven isolates (43%) were susceptible to cefotaxime and

gentamicin while five isolates (71%) showed ciprofloxacin susceptibility. Four (57%) isolates were ESBL producing.

All isolates of *Serratia marcescens* and *Enterobacter* spp. were susceptible to meropenem, ciprofloxacin, gentamicin and amikacin.

Acinetobacter spp. showed limited susceptibility to gentamycin (25%), amikacin (25%), ciprofloxacin (25%), piperacillin-tazobactam (15%), Cefotaxime (10%), Imipenem (15%) and Meropenem (15%). Eighty-five percent of the isolates were susceptible to tigecycline.

Susceptibility of *Klebsiella pneumoniae*

There were 59 isolates of *Klebsiella pneumoniae*. Of these, 12(20%) were fully susceptible to cefotaxime (3rd generation cephalosporins), amikacin, ciprofloxacin, and the carbapenems-imipenem, and meropenem. Susceptibility to other antibiotics included 83% to gentamicin, 75% to piptazobactam), and 75% to amoxicillin clavulanate.

Thirty-four (57%) of the 59 isolates were ESBL producers, and 13(22%) were carbapenem-resistant. They were all resistant to Ampicillin, and 78% were resistant to Gentamicin. The overall prevalence of ESBL producing *Klebsiella pneumoniae* was 56%. High resistance rates were observed for the beta-lactam agents amoxicillin clavulanate (82%) and piperacillin-tazobactam(52%). Gentamicin resistance was found in 91% and ciprofloxacin resistance in 41%.

Multi-drug resistance was documented for 13/59 (22.3%) of the *Klebsiella pneumoniae* isolates. Among the Carbapenem-resistant *Klebsiella pneumoniae*, only 23% showed sensitivity to amikacin, 15% to ciprofloxacin and 92% to tigecycline. (**Figure 5**)

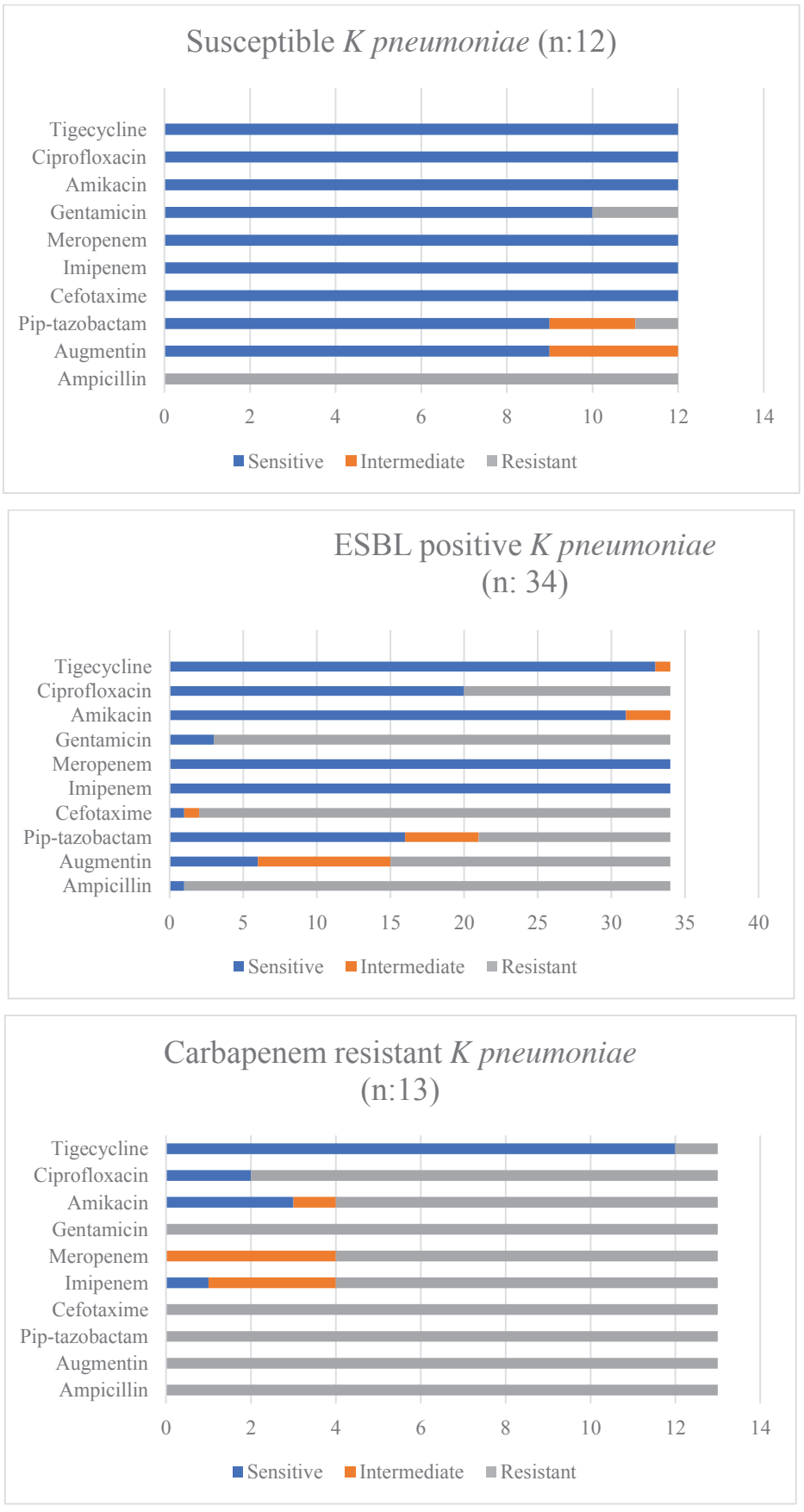


Figure 5. Antibiotic resistance patterns of susceptible, ESBL producing and Carbapenem-resistant *Klebsiella* spp.

Fungal isolates

*Candida parapsilosi*s accounted for 11/13(84%) of the cases of neonatal candidemia. *C albicans* and *C glabrata* were the other species found (8% each). Fluconazole resistance was found in 4/11(36%) of the *C parapsilosis* isolates. Voriconazole susceptibility testing was introduced in 2015, and resistance was detected in one isolate.

4.2.4 Cerebrospinal fluid

CSF cultures were conducted on 133 neonates (48%). Of these, four yielded positive results (4/133 = 3%). One CSF was from an HIV-unexposed neonate which cultured a *Serratia marcescens*. The other three that were positive were from HIV-exposed neonates, two with ESBL producing *Klebsiella pneumoniae* and one neonate was positive for *Mycobacterium tuberculosis*. From the 31 neonates that had GBS on blood culture, CSF cultures were sent from 20 neonates. All 20 specimens had no growth on culture.

4.2.5 Endotracheal aspirates

A total of 121 respiratory samples were sent, 37 (30.5%) had no bacterial growth. Sixteen were from HIV- exposed and 21 from HIV-unexposed neonates. Eighty-four were culture-positive, of which eight had more than one organism and all organisms were included in the analysis. (**Table 5**) Thirty-seven of the culture-positive samples were from HIV-unexposed neonates, and 47 from HIV-exposed neonates.

Table 8. Organisms isolated in endotracheal aspirates

	Exposed n=48(%)	Unexposed n=42(%)	Total n=90(%)
Gram-negative organisms			
Klebsiella pneumoniae	17(35)	12(29)	29(32)
Acinetobacter spp.	9(19)	10(24)	19(21)
E. coli	5(10)	5(12)	10(11)
Enterobacter cloacae	0(0)	3(7)	3(3)
Serratia marcescens	0(0)	2(4)	2(2)
Pseudomanas aeuruginosa	1(2)	0(0)	1(1)
Stenotrophomonas maltophilia	0(0)	1(2)	1(1)
Gram-positive organisms			
Staphylococcus aureus	4(8)	3(7)	7(8)
Staphylococcus spp.	5(10)	2(4)	7(8)
GBS	1(2)	0(0)	1(1)
Enterococcus spp.	1(2)	1(2)	2(2)
Bacillus spp.	1(2)	0(0)	1(1)
Fungal			
Candida spp.	3(6)	2(4)	5(6)
Mycobacterium tuberculosis	1(2)	1(2)	2(2)

The pathogen distribution was similar for HIV-exposed and unexposed neonates. Gram-negative bacteria (65/90)(72%) were the most commonly isolated organisms in the endotracheal aspirates. The commonest Gram-negative organism was *Klebsiella pneumoniae* (29/90)(32%), followed by *Acinetobacter* spp. (19/90)(21%), and *E. coli* (10/90)(11%). **(Table 8)** Gram-positive organisms constituted 20% (18/90) of the organisms isolated. *Staphylococcus aureus* and *Staphylococcus* spp. predominated amongst the Gram-positive organisms. Yeasts were cultured from five neonates and *Mycobacterium tuberculosis* from two neonates.

Resistance among the endotracheal isolates was high for both Gram-positive and Gram-negative bacteria. The majority of the *Klebsiella pneumoniae* (93%) isolated displayed resistance to first and second-line antibiotics used in the unit. ESBL-producing strains were found in 16/29 (55%) of the *Klebsiella pneumoniae*, and 11/29 (37%) were resistant to

Carbapenems. Of the 17 HIV-exposed neonates with *Klebsiella pneumoniae*, 10/17 (58%) were ESBL-producing, and 6/17 (35%) were Carbapenem-resistant. Findings were similar in the HIV-unexposed group with 6/12 (50%) being ESBL-producing and 5/12 (42%) CRE. (**Figure 6**)

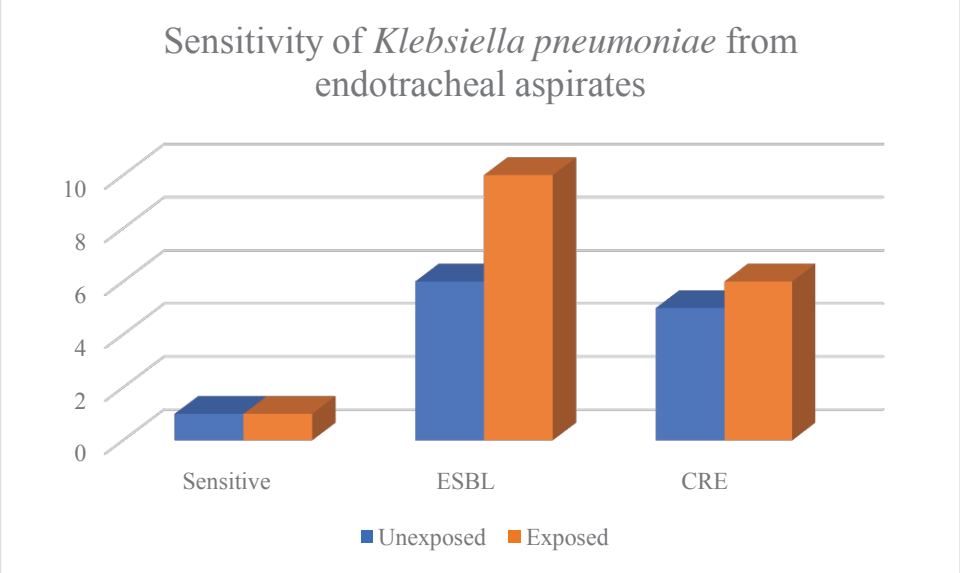


Figure 6. Sensitivity of *Klebsiella pneumoniae* from Endotracheal aspirates

All *Acinetobacter* spp were XDR, and 40% of *E. coli* were ESBL producers.

Amongst the Gram-positive bacteria, all *Staphylococcus aureus* were methicillin-resistant *Staphylococcus aureus* (MRSA) strains, and all Coagulase-negative *staphylococcus* were cloxacillin resistant from both HIV-exposed and HIV-unexposed neonates. All *Candida* isolates from ETAs were Fluconazole resistant. Three of the *Candida* spp were from HIV-exposed neonates and two from unexposed. *Mycobacterium tuberculosis* isolated from the HIV-exposed and unexposed neonates were sensitive to standard anti-Tb treatment regimes. One exposed neonate from a mother with an antenatal diagnosis of MTB had both, an endotracheal aspirate and a positive CSF. The second neonate required IPPV from birth. Both neonates were term with weight >2500g.

CHAPTER FIVE: DISCUSSION

In the present study, we describe the organisms responsible for neonatal sepsis in HIV-exposed and unexposed infants in a tertiary neonatal unit in Durban. GBS was the predominant cause of early-onset neonatal sepsis in HIV-exposed neonates while GBS and *Klebsiella pneumoniae* was found in the HIV-unexposed neonates. *Klebsiella pneumoniae* was the most common pathogen in both groups with a high proportion being ESBL producing and some resistant to carbapenem antibiotics for late-onset neonatal sepsis.

Evidence suggests that HIV-exposed infants experience greater mortality and hospitalisation than unexposed due to infectious morbidity (Slogrove et al., 2016). We found that HIV exposure was associated with a poor outcome, especially in the VLBW and ELBW neonates. Mortality risks were increased if they presented with meningitis or necrotising enterocolitis and had interventions like umbilical artery catheterization and mechanical ventilation.

In early-onset sepsis, we found GBS to be the predominant organism in HIV-exposed neonates. Similar findings were noted by Slogrove et al. where the risk Group B *Streptococcus* and *Streptococcus pneumoniae* was greater in HIV-exposed infants and a Belgium study that reported an increased incidence of neonatal GBS in HIV-exposed neonates (Slogrove et al., 2016; Dauby, Chamekh, Melin, Slogrove & Goetghebuer, 2016). In the HIV-unexposed group, GBS, as well as *Klebsiella pneumoniae*, were present. Studies have reported a predominance of Gram-positive organisms in EOS. Velaphi et al. (2019) found Group B *streptococcus*, *Viridans streptococcus* and *Enterococcus* species as the most common bacteria in EOS and *Escherichia coli* in 10% of cases in a study done in Soweto. In a study in Khayelitsha, South Africa, the most frequent pathogens in neonates <28 days of age (n=40) were Group B *Streptococci* (20/40, 50.0%), *S. aureus* (7/40, 17.5%) and *E. coli* (6/40, 15.0%) (Crichton et al., 2018). There were no *E. coli* isolated as a cause of EOS in this study.

The epidemiology and microbiology of LOS are very diverse and depends on geographic areas and socio-economic factors (Giannoni et al., 2018). High-income countries have reported a prevalence of Gram-positive organisms, including coagulase-negative *staphylococci* and *Staphylococcus aureus*. A 10-year review from Queensland Australia showed that 73% of LOS was due to Gram-positive organisms of which 39.8% was coagulase-negative *staphylococci*. In comparison, the United Kingdom's surveillance data found an equal proportion of Gram-

positive and Gram-negative bacteria, with 10% being fungal (Dong, Speer & Glaser, 2018). The predominant organisms were *S aureus*, *E. coli* and *Candida albicans* (Cailes et al., 2018). We chose to exclude CONS in this study due to difficulty in determining its significance. Repeat blood cultures are needed to establish the significance of CONS in neonatal sepsis (Coetzee et al., 2017).

In late-onset sepsis, *Klebsiella pneumoniae* was the predominant pathogen in both HIV-unexposed and exposed neonates followed by *Staphylococcus aureus* in HIV-unexposed and *Enterococcus* spp. in HIV-exposed neonates. A retrospective review at a tertiary care Neonatal Unit in Johannesburg, South Africa, also found Gram-positive and Gram-negative infections prevalent in LOS. However, the Gram-negative bacteria, especially *E. coli* and *K. pneumoniae*, accounted for more deaths (Ballot et al., 2019). In this study, Gram-negative bacteria accounted for 60% of the LOS and included *Klebsiella pneumoniae*, *Acinetobacter* spp., *E. coli*, and *Serratia marcescens*. This increase in Gram-negative pathogens is consistent with other reports from low- and middle-income countries, noting a significant change in pathogens, particularly the emergence of *A. baumannii* LOS (Ballot, Nana, Sriruttan & Cooper, 2012). Li et al. (2018) found that greater than 50 % of LOS in preterm infants with poor outcome was due to Gram-negative bacteria (Li et al., 2018).

Eighty-five percent of the *Klebsiella pneumoniae* in our study displayed resistance to first-line antibiotics with 61% being ESBL producing and 23 % carbapenemase-producing. Thirteen neonates cultured carbapenem-resistant *Klebsiella pneumoniae* from the blood culture with four neonates having the organism on ETA. Sixty-two percent of these were in HIV-unexposed neonates and most occurred when there was a carbapenem-resistant *Klebsiella pneumoniae* outbreak in the unit. All carbapenem-resistant *Klebsiella pneumoniae* infected neonates had a good outcome in this study. The case fatality rate in neonates with carbapenem-resistant *Klebsiella pneumoniae* vary with 33.3% reported in the Johannesburg Neonatal Unit while a study in Egypt reported 44.3% (Ballot et al., 2019, Nour et al., 2017). Mortality was significantly associated with lower birth weight, necrotising enterocolitis and mechanical ventilation (Ballot et al., 2019). In this study, carbapenem-resistant *Klebsiella pneumoniae* infected neonates had a good outcome despite 69% being ventilated. Sixty-two percent of these infections occurred in HIV-unexposed neonates.

The other Gram-negative bacteria also showed resistance to the first-line antibiotics in the study. *Acinetobacter* spp. isolated from 17 neonates with LOS were all MDR. Fifty-seven percent of the *E. coli* were ESBL positive, and five neonates had sepsis due to *Serratia marcescens*, a known AmpC producer. Viswanathan et al. (2014) reported that almost one-third (32.1%) of positive blood cultures in the NICU were caused by glucose-non-fermenting Gram-negative bacilli, of which *Acinetobacter species* was the most common organism and 50% of those isolates were MDR (Viswanathan et al., 2014). A study in Nepal reported that 77% of their isolates being Gram-negative with *Klebsiella species* and *Enterobacter species* being most common (Pokhrel, Koirala, Shah, Joshi & Baral, 2018). Greater than 50% of the bloodstream infections in a Neonatal Unit in Johannesburg were due to MDR organisms with *Klebsiella pneumoniae* being the most common isolate (Ballot et al., 2019).

Resistance was also high amongst the Gram-positive organisms. *Staphylococcus aureus* and *Enterococcus* spp. were causes of LOS in both HIV-unexposed and exposed neonates. Of the 18 *Staphylococcus aureus* isolates, 89% were MRSA. Nineteen neonates had *Enterococcus* species of which 63% were the more resistant *Enterococcus faecium*. MRSA is a significant pathogen, particularly in preterm low birth weight neonates (Dong et al., 2018). Colonisation is associated with a 24.2 times increased risk of infection (Washam, Woltmann, Haberman, Haslam & Staat, 2017). Risk factors for colonisation include very preterm infants (born <32 weeks' gestation) and VLBW infants (born <1,500 g) (Washam et al., 2017). In our study, 50% of neonates with MRSA bacteraemia were below 1500g, but all had a good outcome.

Enterococcus spp. was the second most common pathogen in LOS found in HIV-unexposed and exposed neonates. The majority were *Enterococcus faecium* isolated in neonates were low birth weight. Six with VLBW and three neonates with ELBW. *Enterococci* species have evolved from harmless commensals to becoming important pathogens associated with nosocomial infection. In recent years, the incidence of Vancomycin-Resistant Enterococci in neonatal ICUs has become a growing concern (Shantala, Nagarathnamma, Pooja, Harsha & Karthik, 2014). Fortunately, we had only one case of VRE in the study with a favourable outcome.

Candida bacteraemia was found in 11 neonates with LOS and two with EOS. The majority were VLBW with three ELBW. Nine of these neonates were exposed of which two demised. Low birth weight infants are at risk of candidaemia due to their immature immune system being

unable to eliminate the pathogen (Fu et al., 2018). Other risk factors, especially in VLBW, include mechanical ventilation, medical devices like catheters, broad-spectrum antibiotic use and total parenteral nutrition (Fu et al., 2018). In this group, most of the candidaemia occurred in HIV-exposed neonates, of which 23% were on TPN, and 69% had umbilical catheters. Mortality occurred in two neonates.

Candida parapsilosis was the dominant species in this study, found in 85% of the newborns. This is consistent with findings from other centres. *C. parapsilosis* caused 54.2% neonatal BSIs in a Johannesburg hospital (Ballot, Bosman, Nana, Ramdin & Cooper, 2013). An Italian study reported 58.5% Candida rates *parapsilosis* compared to 34.1% *C albicans* in their neonatal intensive care unit (Caggiano et al., 2017).

Due to high invasive candidiasis rates, fluconazole prophylaxis is currently recommended in neonates with birth weights <1000g (Pappas et al., 2016). Laboratory-based surveillance of neonatal and adult candidaemia in public and private hospitals in South Africa between 2009–2010 found a dominance of *C. parapsilosis* with only 37% susceptible to fluconazole and voriconazole (Govender et al., 2016). The rising resistance to fluconazole is a concern, and 31% of the isolates were fluconazole-resistant in this study.

Mycobacterium tuberculosis was isolated in two neonates. Prematurity and low birth weight have been described in neonates with congenital MTB (Bekker, Schaaf, Draper, Kriel & Hesseling, 2016). A recent prospective study conducted in Cape Town on HIV-infected and uninfected pregnant and postpartum women with TB found 3% of the newborns infected while in the pre-antiretroviral era, Adhikari et al. (2011) reported the incidence of 15% (Bekker et al. 2016; Adhikari et al., 2011). The improvement of MTB diagnosis with the introduction of the molecular platforms like the Xpert MTB/RIF will assist with the early detection in pregnant women.

The HIV-exposed neonates were more likely to have lower birth weight and were of lower gestation than the unexposed neonates in this study. This is in keeping with other studies that show that HIV-positive pregnant women have significantly more preterm deliveries than their HIV-negative counterparts (Naidoo et al., 2016). Parekh et al. (2011) found an increased risk of very-small-for-gestational-age among HIV-exposed infants in Botswana (Parekh et al., 2011). A meta-analysis by Peng-Lei Xiao et al. (2015) found similar findings. HIV-infected

women are at higher risk of having a low birth weight infant or a preterm delivery infant compared with HIV-uninfected women (Xiao et al., 2015).

IPPV was an independent risk factor for mortality. A significant proportion of the neonates admitted to NICU required ventilation. From the neonates that demised in this study, 73.9% (17/23) were on IPPV. Survival rates of 64% and 67.9% have been described from other similar studies in low-middle income countries (Trotman, 2006; Karthikeyan & Hossain, 2002). Weight <2500g and gestation <34 weeks are some of the predictors of mortality in ventilated neonates (Iqbal et al., 2015).

The significance of Coagulase negative *staphylococci* from endotracheal aspirates is not well established, but several studies have described its presence. Madan, Meyer & Amortequi (1988) studied autopsy specimens of lung tissue from neonates that had demised from congenital pneumonia. The most frequently isolated organism was *Staphylococcus epidermidis* (18%), *Group B Streptococcus* (13%), *E. coli* (9%) and *Ureaplasma urealyticum* (9%) (Madan et al., 1988)

Resistance amongst ventilated patients was high in both the HIV-unexposed and exposed. The majority of the neonates on IPPV had Gram-negative bacteria on endotracheal aspirates. Gram-positive bacteria were *Staphylococcus aureus* and coagulase-negative *staphylococcus*. Both were resistant to cloxacillin which meant use of vancomycin or linezolid. Gram-negative bacteria included ESBL producing and carbapenemase resistant *Klebsiella pneumoniae*, ESBL producing *E. coli* and MDR *Acinetobacter*. Despite the presence of highly resistant organisms, the main challenge was the diagnosis of VAP. Currently, the Centers for Disease Control and Prevention (CDC) guidelines do not offer algorithms for neonates. There is a lack of a gold standard case definition and diagnostic tests for intubated newborns. ETA cultures are a problem due to low sensitivity (Claassen & Keenan, 2019). The aetiology of ventilator-associated pneumonia is often polymicrobial, and differentiating colonisation from infection is difficult (Claassen & Keenan, 2019). Due to the strong association between IPPV and mortality, mechanisms to reduce VAP in the unit is vital. This warrants looking at introducing Quality Control program to reduce the rate of VAP in the unit by interventions like VAP preventive bundle.

The study highlights the high rate of resistant organisms among HIV-exposed and unexposed neonates in the unit. Increase in resistance was seen in both Gram-positive and Gram-negative bacteria. For EOS, the organisms were susceptible, and the unit's ampicillin and gentamicin policy provided good empirical coverage. However, for LOS, most pathogens were resistant to the second-line antibiotics, and Carbapenems and Vancomycin provided the greatest in-vitro cover for bloodstream infections in the unit.

Strategies to reduce the MDR rates include infection-prevention and control (IPC) programmes and antimicrobial stewardship programmes. Basic infection-prevention measures are currently practised, but specialised unit-specific protocols may be needed given the procedures undertaken like umbilical catheter placement, surfactant administration, incubator and radiant warmer care, preparation, and storage maternal and donor breast milk that is unique to neonatal environments. Regular training of all staff and the use of care bundles can improve the quality of NICU practices and ensure adhering to evidence-based guidelines and prevent HAIs.

Antimicrobial stewardship programmes are essential to help conserve antibiotics and delay the progression of AMR. Regular audits are required to track antimicrobial usage and monitor resistance, and this will help with the development of unit-specific antimicrobial treatment protocols based on local susceptibility data. Due to the increasing incidence of hospital-acquired infections and antimicrobial resistance seen in neonatal units, The SA Neonatal Sepsis Task Force was launched (Dramowski et al., 2020). The focus will be on preventing and managing AMR infections and/or neonatal HAIs in public and private sector facilities.

CHAPTER SIX: CONCLUSION

GBS remains the primary cause of EOS in HIV-exposed and unexposed neonates and is sensitive to the current first-line antibiotics, ampicillin and gentamicin used in the unit. For LOS, 60% were caused by Gram-negative bacteria found in both HIV-exposed and unexposed neonates. These included ESBL producing *Klebsiella pneumoniae*, Carbapenem-resistant *Klebsiella pneumoniae* and *MDR Acinetobacter* spp. The antibiotic policy for treatment of LOS in the unit needs to review as well as infection prevention and control and antimicrobial stewardship programmes need to be intensified to curb the rising problem of resistance in the unit.

Limitations

Limitation of study is the absence of non-culture based methods for the detection of sepsis. The microbiological workup of CSF, ETA and blood culture only detected culturable organisms. The use of PCR-based assays to detect the 16S ribosomal RNA in clinical specimens may help detect non-culturable organisms.

Twenty-eight neonates had no HIV PCR performed at birth and we were unable to characterise these neonates as “HIV-exposed uninfected” or as “HIV-exposed infected

REFERENCES

Adhikari, M., Jeena, P., Bobat, R., Archary, M., Naidoo, K., Coutsoudis, A., … Nair, N. (2011). HIV-Associated Tuberculosis in the Newborn and Young Infant. *International Journal of Pediatrics, 2011*, 1–10. https://doi.org/10.1155/2011/354208

Aelami, M. H., Lotfi, M. & Zingg, W. (2014). Ventilator-associated pneumonia in neonates, infants and children. *Antimicrob Resist Infect Control 3*, 30 (2014). https://doi.org/10.1186/2047-2994-3-30

Agarwal, R., Sankar, J., Health, N., & Centre, K. (2016). Characterization and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. *The Lancet Global Health, 4*(10, E752-E760. https://doi.org/10.1016/S2214-109X(16)30148-6

Ballot, D. E., Bandini, R., Nana, T., Bosman, N., Thomas, T., Davies, V. A., … Lipman, J. (2019). A review of -multidrug-resistant Enterobacteriaceae in a neonatal unit in Johannesburg, South Africa. *BMC Pediatrics, 19*(1), 320. https://doi.org/10.1186/s12887-019-1709-y

Ballot, D. E., Bosman, N., Nana, T., Ramdin, T., & Cooper, P. A. (2013). Background changing patterns of neonatal fungal sepsis in a developing country. *Journal of Tropical Pediatrics, 59*(6), 460–464. https://doi.org/10.1093/tropej/fmt053

Ballot, D. E., Nana, T., Sriruttan, C., & Cooper, P. A. (2012). *Bacterial Bloodstream Infections in Neonates in a Developing Country*. ISRN Pediatrics, 2012, 1–6. https://doi.org/10.5402/2012/508512

Basha, S., Surendran, N., & Pichichero, M. (2014). Immune responses in neonates. *Expert Review of Clinical Immunology, 10*(9), 1171–1184. https://doi.org/10.1586/1744666X.2014.942288

Bedetti, L., Marrozzini, L., Baraldi, A., Spezia, E., Iughetti, L., Lucaccioni, L., & Berardi, A. (2019). Pitfalls in the diagnosis of meningitis in neonates and young infants: the role of lumbar puncture. *Journal of Maternal-Fetal and Neonatal Medicine, 32*(23), 4029–4035. https://doi.org/10.1080/14767058.2018.1481031

Bekker, A., Schaaf, H. S., Draper, H. R., Kriel, M., & Hesseling, A. C. (2016). Tuberculosis disease during pregnancy and treatment outcomes in HIV-infected and uninfected women at a referral Hospital in Cape Town. *PLoS ONE, 11*(11).

https://doi.org/10.1371/journal.pone.0164249

Caggiano, G., Lovero, G., De Giglio, O., Barbuti, G., Montagna, O., Laforgia, N., & Montagna, M. T. (2017). Candidemia in the Neonatal Intensive Care Unit: A Retrospective, Observational Survey and Analysis of Literature Data. *BioMed Research International, 2017*, 7901763. https://doi.org/10.1155/2017/7901763

Cailes, B., Kortsalioudaki, C., Buttery, J., Pattnayak, S., Greenough, A., Matthes, J., … Heath, P. T. (2018). Epidemiology of UK neonatal infections: the neonIN infection surveillance network. *Archives of Disease in Childhood - Fetal and Neonatal Edition, 103*(6), F547 LP-F553. https://doi.org/10.1136/archdischild-2017-313203

Cernada, M., Brugada, M., Golombek, S., & Vento, M. (2014). Ventilator-associated pneumonia in neonatal patients: An update. *Neonatology, 105*(2), 98–107. https://doi.org/10.1159/000355539

Chiotos, K., Han, J. H., & Tamma, P. D. (2016). Carbapenem-Resistant Enterobacteriaceae Infections in Children. *Current Infectious Disease Reports, 18*(1), 2. https://doi.org/10.1007/s11908-015-0510-9

Claassen, C. C., & Keenan, W. J. (2019). Challenging the "Culture" of the Tracheal Aspirate. *NeoReviews, 20* (3) e145-e151. doi: https://doi.org/10.1542/neo.20-3-e145

Coetzee, M., Mbowane, N. T., & de Witt, T. W. (2017). Neonatal sepsis: Highlighting the principles of diagnosis and management. *SAJCH South African Journal of Child Health, 11*(2), 99–103. https://doi.org/10.7196/SAJCH.2017.v11i2.1244

Cortese, F., Scicchitano, P., Gesualdo, M., Filaninno, A., De Giorgi, E., Schettini, F., … Ciccone, M. M. (2016). Early and Late Infections in Newborns: Where Do We Stand? A Review. *Pediatrics and Neonatology, 57*(4), 265–273. https://doi.org/10.1016/j.pedneo.2015.09.007

Crellen, T., Turner, P., Pol, S., Baker, S., Nguyen, T. N. T., Stoesser, N., Day, N. P. J., Turner, C., & Cooper, B. S. (2019). Transmission dynamics and control of multidrug-resistant klebsiella pneumoniae in neonates in a developing country. *ELife, 8*, 1–24. https://doi.org/10.7554/eLife.50468

Crichton, H., O'Connell, N., Rabie, H., Whitelaw, A. C., & Dramowski, A. (2018). Neonatal and paediatric bloodstream infections: Pathogens, antimicrobial resistance patterns and prescribing practice at Khayelitsha District Hospital, Cape Town, South Africa. *South*

African Medical Journal, *108*(2), 99–104. https://doi.org/10.7196/SAMJ.2018.v108i2.12601

Cutland, C. L., Schrag, S. J., Thigpen, M. C., Velaphi, S. C., Wadula, J., Adrian, P. V, … Madhi, S. A. (2015). Increased risk for group B Streptococcus sepsis in young infants exposed to HIV, Soweto, South Africa, 2004-2008(1). *Emerging Infectious Diseases*, *21*(4), 638–645. https://doi.org/10.3201/eid2104.141562

Dauby, N., Chamekh, M., Melin, P., Slogrove, A. L., & Goetghebuer, T. (2016). Increased Risk of Group B Streptococcus Invasive Infection in HIV-Exposed but Uninfected Infants: A Review of the Evidence and Possible Mechanisms. *Frontiers in Immunology*, *7*, 505. https://doi.org/10.3389/fimmu.2016.00505

Dong, Y., & Speer, C. P. (2015). Late-onset neonatal sepsis: recent developments. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *100*(3), F257 LP-F263. https://doi.org/10.1136/archdischild-2014-306213

Dong, Y., Speer, C. P., & Glaser, K. (2018). Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity. *Virulence*, *9*(1), 621–633. https://doi.org/10.1080/21505594.2017.1419117

Dramowski, A., Velaphi, S., Reubenson, G., Bekker, A., Perovic, O., Finlayson, H., … Govender, N. P. (2020). National Neonatal Sepsis Task Force launch: Supporting infection prevention and surveillance, outbreak investigation and antimicrobial stewardship in neonatal units in South Africa. *South African Medical Journal*, *110*(5), 360. https://doi.org/10.7196/SAMJ.2020.v110i5.14564

Ergenekon, E., & Çataltepe, S. (2020). Ventilator-associated pneumonia in the NICU: time to boost diagnostics? *Pediatric Research*, *87*(7), 1143–1144. https://doi.org/10.1038/s41390-019-0672-5

Evans, C., Jones, C. E., & Prendergast, A. J. (2016). HIV-exposed, uninfected infants: new global challenges in the era of paediatric HIV elimination. *The Lancet. Infectious Diseases*, *16*(6), e92–e107. https://doi.org/10.1016/S1473-3099(16)00055-4

Folgori, L., & Bielicki, J. (2019). Future Challenges in Pediatric and Neonatal Sepsis: Emerging Pathogens and Antimicrobial Resistance. *Journal of Pediatric Intensive Care*, *08*(01), 017–024. https://doi.org/10.1055/s-0038-1677535

Folgori, L., Bielicki, J., Heath, P. T., & Sharland, M. (2017a). Antimicrobial-resistant Gram-

negative infections in neonates: burden of disease and challenges in treatment. *Current Opinion in Infectious Diseases, 30*(3). https://journals.lww.com/co-infectiousdiseases/Fulltext/2017/06000/Antimicrobial_resistant_Gram_negative_infections.5.aspx

Folgori, L., Ellis, S. J., Bielicki, J. A., Heath, P. T., Sharland, M., & Balasegaram, M. (2017). Tackling antimicrobial resistance in neonatal sepsis. *The Lancet. Global health, 5*(11), e1066–e1068. https://doi.org/10.1016/S2214-109X(17)30362-5

Fu, J., Ding, Y., Jiang, Y., Mo, S., Xu, S., & Qin, P. (2018). Persistent candidemia in very low birth weight neonates: risk factors and clinical significance. *BMC Infectious Diseases, 18*(1), 558. https://doi.org/10.1186/s12879-018-3487-9

Furyk, J. S., Swann, O., & Molyneux, E. (2011). Systematic review: Neonatal meningitis in the developing world. *Tropical Medicine and International Health, 16*(6), 672–679. https://doi.org/10.1111/j.1365-3156.2011.02750.x

Gazin, M., Paasch, F., Goossens, H., Malhotra-Kumar, S., & MOSAR WP2 and SATURN WP1 Study Teams (2012). Current trends in culture-based and molecular detection of extended-spectrum-β-lactamase-harboring and carbapenem-resistant Enterobacteriaceae. *Journal of clinical microbiology, 50*(4), 1140–1146. https://doi.org/10.1128/JCM.06852-11

Giannoni, E., Agyeman, P. K. A., Stocker, M., Posfay-Barbe, K. M., Heininger, U., Spycher, B. D., … Schlapbach, L. J. (2018). Neonatal Sepsis of Early Onset, and Hospital-Acquired and Community-Acquired Late Onset: A Prospective Population-Based Cohort Study. *Journal of Pediatrics, 201*, 106-114.e4. https://doi.org/10.1016/j.jpeds.2018.05.048

Gizachew, M., Tiruneh, M., Moges, F., Adefris, M., Tigabu, Z., & Tessema, B. (2019). Streptococcus agalactiae from Ethiopian pregnant women; prevalence, associated factors and antimicrobial resistance: alarming for prophylaxis. *Annals of clinical microbiology and antimicrobials, 18*(1), 3. https://doi.org/10.1186/s12941-019-0303-3

Gordon, S. M., Srinivasan, L., & Harris, M. C. (2017). Neonatal meningitis: Overcoming challenges in diagnosis, prognosis, and treatment with omics. *Frontiers in Pediatrics, 5*, 1–10. https://doi.org/10.3389/fped.2017.00139

Govender, N. P., Patel, J., Magobo, R. E., Naicker, S., Wadula, J., Whitelaw, A., …Zietsman,

I. L., & TRAC-South Africa group (2016). Emergence of azole-resistant Candida parapsilosis causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa. *The Journal of antimicrobial chemotherapy, 71*(7), 1994–2004. https://doi.org/10.1093/jac/dkw091

Green, R. J., & Kolberg, J. M. (2016). Neonatal pneumonia in sub-Saharan Africa. *Pneumonia, 8*(1), 0–1. https://doi.org/10.1186/s41479-016-0003-0

Hall, B. G., & Barlow, M. (2005). Revised Ambler classification of β-lactamases. *Journal of Antimicrobial Chemotherapy, 55*(6), 1050–1051. https://doi.org/10.1093/jac/dki130

Huynh, D. T., Estorninos, E., Capeding, R. Z., Oliver, J. S., Low, Y. L. & Rosales, F. J. (2015). Longitudinal growth and health outcomes in nutritionally at-risk children who received long-term nutritional intervention. *Journal of human nutrition and dietetics: the official journal of the British Dietetic Association, 28*(6), 623–635. https://doi.org/10.1111/jhn.12306

Iqbal, Q., Younus, M. M., Ahmed, A., Ahmad, I., Iqbal, J., Charoo, B. A., & Ali, S. W. (2015). Neonatal mechanical ventilation: Indications and outcome. *Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine, 19*(9), 523–527. https://doi.org/10.4103/0972-5229.164800

Iroh Tam, P. Y., & Bendel, C. M. (2017). Diagnostics for neonatal sepsis: Current approaches and future directions. *Pediatric Research, 82*(4), 574–583. https://doi.org/10.1038/pr.2017.134

Karthikeyan, G., & Hossain, M. M. (2002). Conventional ventilation in neonates: experience from Saudi Arabia. *Indian journal of pediatrics, 69*(1), 15–18. https://doi.org/10.1007/BF02723768

Khalessi, N., & Afsharkhas, L. (2014). Neonatal meningitis: Risk factors, causes, and neurologic complications. *Iranian Journal of Child Neurology, 8*(4), 46–50. https://doi.org/10.22037/ijcn.v8i4.5309

Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J. A., Klugman, K., & Davies, S. (2016). Access to effective antimicrobials: A worldwide challenge. *The Lancet, 387*(10014), 168–175. https://doi.org/10.1016/S0140-6736(15)00474-2

Lee, C.-R., Lee, J. H., Park, K. S., Kim, Y. B., Jeong, B. C., & Lee, S. H. (2016). Global Dissemination of Carbapenemase-Producing Klebsiella pneumoniae: Epidemiology,

Genetic Context, Treatment Options, and Detection Methods. *Frontiers in Microbiology, 7*, 895. https://doi.org/10.3389/fmicb.2016.00895

Li, J.-Y., Chen, S.-Q., Yan, Y.-Y., Hu, Y.-Y., Wei, J., Wu, Q.-P., … Lin, J. (2018). Identification and antimicrobial resistance of pathogens in neonatal septicemia in China-A meta-analysis. *International Journal of Infectious Diseases, 71*, 89–93. https://doi.org/10.1016/j.ijid.2018.04.794

Madan, E., Meyer, M. P., & Amortequi, A. (1988). Chorioamnionitis: a study of organisms isolated in perinatal autopsies. *Annals of clinical and laboratory science, 18*(1), 39–45. Retrieved from http://www.annclinlabsci.org/content/18/1/39.full.pdf+html (02 October 2020)

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., … Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Mathur, S., Li, G., Folgori, L., Sharland, M., & Heath, P. T. (2016). DeNIS collaboration: setting the future research agenda. *The Lancet Global Health, 5*(1), e36. https://doi.org/10.1016/S2214-109X(16)30286-8

Meletis, G. (2016). Carbapenem resistance: overview of the problem and future perspectives. *Therapeutic Advances in Infectious Disease, 3*(1), 15–21. https://doi.org/10.1177/2049936115621709

Mukesi, M., Iweriebor, B. C., Obi, L. C., Nwodo, U. U., Moyo, S. R., & Okoh, A. I. (2019). Prevalence and capsular type distribution of Streptococcus agalactiae isolated from pregnant women in Namibia and South Africa. *BMC Infectious Diseases, 19*(1). https://doi.org/10.1186/s12879-019-3809-6

Naidoo, M., Sartorius, B. & Tshimanga-Tshikala, G. (2016). Maternal HIV infection and preterm delivery outcomes at an urban district hospital in KwaZulu-Natal 2011. *Southern African Journal of Infectious Diseases, 31*(1), 25–28. https://doi.org/10.1080/23120053.2016.1118838

Nour, I., Eldegla, H. E., Nasef, N., Shouman, B., Abdel-Hady, H., & Shabaan, A. E. (2017). Risk factors and clinical outcomes for carbapenem-resistant Gram-negative late-onset

sepsis in a neonatal intensive care unit. *Journal of Hospital Infection*, *97*(1), 52–58. https://doi.org/10.1016/j.jhin.2017.05.025

Okomo, U., Akpalu, E. N. K., Le Doare, K., Roca, A., Cousens, S., Jarde, A., … Lawn, J. E. (2019). Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. *The Lancet Infectious Diseases*, *19*(11), 1219–1234. https://doi.org/10.1016/S1473-3099(19)30414-1

Pappas, P. G., Kauffman, C. A., Andes, D. R., Clancy, C. J., Marr, K. A., Ostrosky-Zeichner, L., … Sobel, J. D. (2016). Clinical Practice Guideline for the Management of Candidiasis: 2016 *Update by the Infectious Diseases Society of America*. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *62*(4), e1–e50. https://doi.org/10.1093/cid/civ933

Parekh, N., Ribaudo, H., Souda, S., Chen, J., Mmalane, M., Powis, K., … Shapiro, R. L. (2011). Risk factors for very preterm delivery and delivery of very-small-for-gestational-age infants among HIV-exposed and HIV-unexposed infants in Botswana. *International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics*, *115*(1), 20–25. https://doi.org/10.1016/j.ijgo.2011.04.008

Patil, T. (2014). The study of the organisms colonizing trachea in mechanically. *International Journal of Medical Science and Education*, *1*(1), 39–48. Retrieved from http://www.ijmse.com/uploads/1/4/0/3/14032141/ijmse_2014_vol_1_issue_1_p39-48.pdf (02 October 2020)

Pokhrel, B., Koirala, T., Shah, G., Joshi, S., & Baral, P. (2018). Bacteriological profile and antibiotic susceptibility of neonatal sepsis in neonatal intensive care unit of a tertiary hospital in Nepal. *BMC Pediatrics*, *18*(1), 208. https://doi.org/10.1186/s12887-018-1176-x

Rameshwarnath, S., & Naidoo, S. (2018). Risk factors associated with nosocomial infections in the Neonatal Intensive Care Unit at Mahatma Gandhi Memorial hospital between 2014 and 2015. *Southern African Journal of Infectious Diseases*, *33*(4), 93–100. https://doi.org/10.1080/23120053.2018.1453641

Reyes, A. (2018). Ending the culture of culture-negative sepsis in the neonatal ICU. *Revista Chilena de Infectologia*, *35*(2), 216–217. https://doi.org/10.4067/s0716-

10182018000200216

Ruppé, É., Woerther, P.-L., & Barbier, F. (2015). Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Annals of Intensive Care*, *5*(1), 61. https://doi.org/10.1186/s13613-015-0061-0

Safdar, N., Crnich, C. J., & Maki, D. G. (2005). The Pathogenesis of Ventilator-Associated Pneumonia: Its Relevance to Developing Effective Strategies for Prevention. *Respiratory Care*, *50*(6), 725–741. http://rc.rcjournal.com/content/50/6/725

Shantala, G. B., Nagarathnamma, T., Pooja, D. R., Harsha, T. R., Karthik, R. (2014). Neonatal septicaemia caused by vancomycin resistant enterococcus faecium-a case report. *Journal of clinical and diagnostic research*. *JCDR*, *8*(11), DD03–DD4. https://doi.org/10.7860/JCDR/2014/10284.5220

Simonsen, K. A., Anderson-Berry, A. L., Delair, S. F., & Davies, H. D. (2014). Early-Onset Neonatal Sepsis. *Clinical Microbiology Reviews*, *27*(1), 21–47. https://doi.org/10.1128/CMR.00031-13

Slogrove, A. L., Goetghebuer, T., Cotton, M. F., Singer, J., & Bettinger, J. A. (2016). Pattern of Infectious Morbidity in HIV-Exposed Uninfected Infants and Children. *Frontiers in Immunology*, *7*, 164. https://doi.org/10.3389/fimmu.2016.00164

South African National Department of Health. (2019). *Guideline for the Prevention of Mother to Child Transmission of Communicable Infections*, October 2019. Available from: https://www.nicd.ac.za/wp-content/uploads/2019/11/Guidelines-for-the-Prevention-of-Transmission-of-Communicable-Diseases-from-mother-to-child_28-October.pdf (Accessed on 29 July 2020).

Stoll, B. J., Hansen, N. I., Sánchez, P. J., Faix, R. G., Poindexter, B. B., Van Meurs, K. P., … Network, for the E. K. S. N. I. of C. H. and H. D. N. R. (2011). Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. *Pediatrics*, *127*(5), 817–826. https://doi.org/10.1542/peds.2010-2217

Templer, S. P., Seiverth, B., Baum, P., Stevens, W., Seguin-Devaux, C., & Carmona, S. (2016). Improved Sensitivity of a Dual-Target HIV-1 Qualitative Test for Plasma and Dried Blood Spots. *Journal of clinical microbiology*, *54*(7), 1877–1882. https://doi.org/10.1128/JCM.00128-16.

Trotman, H. (2006). The neonatal intensive care unit at the University Hospital of the West

Indies: The first few years' experience. *The West Indian medical journal, 55*(2), 75–79. https://doi.org/10.1590/s0043-31442006000200002

Velaphi, S. C., Westercamp, M., Moleleki, M., Pondo, T., Dangor, Z., Wolter, N., … Madhi, S. A. (2019). Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. *PLOS ONE, 14*(4), e0214077. https://doi.org/10.1371/journal.pone.0214077

Viswanathan, R., Singh, A. K., Basu, S., Chatterjee, S., Roy, S., & Isaacs, D. (2014). Multi-Drug-Resistant, non-Fermenting, gramnegative bacilli in neonatal sepsis in Kolkata, India: A 4-Year study. *Paediatrics and International Child Health, 34*(1), 56–59. https://doi.org/10.1179/2046905513Y.0000000072

Washam, M., Woltmann, J., Haberman, B., Haslam, D., & Staat, M. A. (2017). Risk factors for methicillin-resistant Staphylococcus aureus colonization in the neonatal intensive care unit: A systematic review and meta-analysis. *American Journal of Infection Control, 45*(12), 1388–1393. https://doi.org/10.1016/j.ajic.2017.06.021

Xiao, P.-L., Zhou, Y.-B., Chen, Y., Yang, M.-X., Song, X.-X., Shi, Y., & Jiang, Q.-W. (2015). Association between maternal HIV infection and low birth weight and prematurity: a meta-analysis of cohort studies. *BMC Pregnancy and Childbirth, 15*(1), 246. https://doi.org/10.1186/s12884-015-0684-z

Zea-Vera, A., & Ochoa, T. J. (2015). Challenges in the diagnosis and management of neonatal sepsis Clinical Review. *Journal of Tropical Pediatrics, 61*, 1–13. https://doi.org/10.1093/tropej/fmu079

APPENDICES

APPENDIX 1: STUDY PROTOCOL

Spectrum of organisms and outcome of neonatal infections in HIV exposed and unexposed newborns at a tertiary care hospital in KZN

P Mahabeer

Supervisor: Prof K Mlisana
Co-supervisor: Prof M Adhikari
Collaborators: Dr R Singh

Aim:

The aim of the study is to:
- establish the aetiology and susceptibility patterns of organisms responsible for neonatal infections in HIV exposed and unexposed newborns with suspected sepsis at King Edward VIII Hospital neonatal unit
- identify risk factors that are associated with these neonatal infections.
-document the outcome of neonates with proven infection

Objective:

- To investigate the organisms isolated from blood cultures, respiratory and cerebrospinal fluid samples responsible for neonatal sepsis in that unit.
- To establish the antibiotic susceptibility profile of these organisms to antimicrobial agents used.
- To assess the risk factors associated with neonatal sepsis
- To determine outcome of these infections – primary outcome being mortality and secondary being morbidity.

Background:

Neonatal sepsis is a major cause of infant morbidity and mortality. The main causes of death in the neonatal period are prematurity, perinatal asphyxia, birth trauma, congenital abnormalities and infections respectively. Common infections include septicaemia, meningitis, respiratory infections, diarrhoea and neonatal tetanus.[1]

The immature immune system in the neonate exposes them to higher risk of infections. Whilst babies are born without endogenous microbial flora they rapidly become colonised with microbes encountered in the maternal genital tract and their immediate postnatal environment.[2] As the immature immune system is unable to provide a robust defence against microbes, the risk of developing invasive infections increases.

Maternal risk factors for neonatal sepsis include chorioamnionitis, prolonged rupture of membranes and meconium-stained amniotic fluid. Tuberculosis (TB) and HIV are also well-recognised risk factors for maternal and infant mortality and morbidity. KwaZulu-Natal has

been described as having one of the highest rates of HIV and Tuberculosis in South Africa.[3] Congenital TB carries a high risk of infant death, with reported mortality rates of up to 38%. Postnatal acquisition of TB infection by young infants is also associated with a high risk of disseminated and rapidly fatal TB disease.[4]

Highly active antiretroviral therapy (HAART) has markedly improved the health and the long-term prognosis of HIV-infected patients and reduced the risk for mother-to-child transmission (MTCT) of the virus. This has led to an increase in the number of HIV-exposed uninfected (HEU) infants. [3] These infants may be more susceptible to infections than infants born to HIV-uninfected mothers but data on the outcomes of these newborns is lacking. King Edward VIII (KEH) hospital is a tertiary hospital with over 7000 births /annum. The prevalence of HIV at the KEH antenatal clinic is around 40% and 35-40% of the admissions to nursery are HIV exposed neonates (personal communication: Dr R Singh, KEH Neonatal Unit). For effective management of these patients, establishing the spectrum of commonly infecting organisms and risk factors for disease is vital.

Neonatal practices vary in different countries and these geographical differences are reflected in the diverse patterns of neonatal sepsis. The pathogen distribution, epidemiology and resistance patterns are different for developing and developed countries. A review by Vergnano et al. found that in the developing world, Gram-negative bacteria such as *Klebsiella pneumoniae, E coli, Serratia marscesens, Pseudomonas aeruginosa*, and *Salmonella* spp and the Gram positive organisms, *Staphylococcus aureus* and *coagulase-negative Staphylococcus* (CoNS) are the predominant pathogens. [5] In the developed countries *Group B Streptococcus* (GBS) remains the most frequent pathogen in term infants, and *E. coli* the most significant pathogen in preterm infants with early onset sepsis.[6]

The data on aetiology of neonatal sepsis in South Africa is diverse, but remains limited. In a large study with a cohort of over 8000 mother-baby pairs delivered at a public hospital in Soweto, maternal and neonatal factors associated with neonatal sepsis and perinatal death were assessed.[7] There were 289 cases of early-onset sepsis (EOS) and of the ten percent (29/289) that were culture confirmed, GBS was the most common pathogen. *Escherichia coli* was the leading cause of late-onset sepsis (LOS) followed by GBS.

Blood culture audits done at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) neonatal unit found varying aetiology over time. An audit done in 2002-2003 found gram negative bacilli to be the leading organisms in EOS and *coagulase-negative Staphylococcus* in LOS [8], while a review of blood culture isolates in 2009-2010 found *S. agalactiae* as the major cause of EOS. [9] Current data on the aetiology of neonatal sepsis in KZN is lacking.

Isolation of microorganisms from blood culture or cerebrospinal fluid has remained the most definitive way of making a diagnosis of infection in a neonate. Urine samples are often unreliable in a newborn as they are usually bag specimens that are frequently contaminated. Adjunctive tests like the haematological indices and acute phase reactants have been used but are not sufficiently sensitive and specific to exclude or confirm the diagnosis of neonatal sepsis.[10]

Blood cultures remain the mainstay of investigation of potential sepsis in infants. The sensitivity of the culture methods is frequently low, due to the concomitant antibiotic therapy, or to the combination of small blood sample volume and low colony counts.[10] Despite improvements in automated blood culture systems, results of blood culture can be delayed by up to 48 hours.

Pathogens can be directly identified from patient samples or after initial growth in blood cultures. Although the major benefit of a molecular test is speed, none have shown to have a sensitivity and specificity sufficient to replace standard blood culture techniques, which carry the additional important advantage of antibiotic susceptibility. [10] The cost-effectiveness and clinical impact of molecular assays still need evaluation but has the potential of being an adjunctive to blood cultures.

Bacterial organisms causing neonatal sepsis have developed increased drug resistance to commonly used antibiotics, making management a challenge for both the public and private health sectors. [8] These multidrug resistant organisms such as *Klebsiella spp*, *Pseudomonas spp*, and *Acinetobacter* spp ,decrease therapeutic options resulting in serious challenges in poorly resourced countries where access to alternative antibiotics is severely limited. This resistance is fuelled by poor infection-control practices and gross inappropriate use of antibiotics. The powerful selective pressure of inappropriate and prolonged antimicrobial use favours the emergence and amplification of resistance in hospital nurseries.[2]

Neonatal sepsis is associated with significant morbidity and mortality justifying prompt initiation of empirical antibiotic therapy. Knowledge of the common pathogens causing septicemia in neonates and their local antimicrobial susceptibility is essential to select appropriate antimicrobial treatment. [11] We therefore aim to prospectively observe the risk factors, aetiology and susceptibility profile of the organisms causing neonatal infections and document the outcome of these infections in HIV exposed and unexposed infants at KEH This will help evaluate the empiric guidelines currently used in the unit and guide the development of future studies exploring the use of rapid microbiological and molecular techniques to enable early recognition and management of neonatal sepsis.

References:

1. Costello A, Francis V, Byrne A, et al. The state of the world's newborns. Washington: Save the Children Fund, 2001.

2. Anita K M Zaidi, W Charles Huskins, DurraneThaver, Zulfiqar A Bhutta, Zohair Abbas, Donald A Goldmann. Hospital-acquired neonatal infections in developing countries. Lancet 2005; 365: 1175–88.

3. Goga AE, Dinh TH, Jackson DJ for the SAPMTCTE study group. Evaluation of the Effectiveness of the National Prevention of Mother-to-Child Transmission (PMTCT) Programme Measured at Six Weeks Postpartum in South Africa, 2010. South African Medical Research Council, National Department of Health of South Africa and PEPFAR/US Centers for Disease Control and Prevention. 2012.

4. Lynne M. Mofenson and Barbara E. Laughon. Human Immunodeficiency Virus, *Mycobacterium Tuberculosis*, and Pregnancy: A Deadly Combination. Clinical Infectious Diseases 2007; 45:250–3.

5. S Vergnano, M Sharland, P Kazembe, C Mwansambo, P T Heath. Neonatal sepsis: an international perspective. Arch Dis Child Fetal Neonatal Ed 2005;90:F220–F224.

6. Edmond K, Zaidi A . New Approaches to Preventing, Diagnosing, and Treating Neonatal Sepsis. PLoS Med 7(3): e1000213. doi:10.1371/journal.pmed.1000213.

7. Stephanie J. Schrag, DPhil, Clare L. Cutland, Elizabeth R. Zell, Locadiah Kuwanda, Eckhart J. Buchmann, Sithembiso C. Velaphi, Michelle J. Groome, Shabir A. Madhi, and the PoPS Trial Team. Risk Factors for Neonatal Sepsis and Perinatal Death Among Infants Enrolled in the Prevention of Perinatal Sepsis Trial, Soweto, South Africa
The Pediatric Infectious Disease Journal. Volume 31, Number 8, August 2012.

8. Motara F, Ballot DE, Perovic O: Epidemiology of Neonatal Sepsis at Johannesburg Hospital. Southern Afr J Epidemiol Infect 2005, 20:90-93.

9. Daynia E. Ballot, Trusha Nana, Charlotte Sriruttan, and Peter A, Cooper. Bacterial Bloodstream Infections in Neonates in a Developing Country International Scholarly Research Network, Pediatrics Volume 2012.

10. J Gaetano Chirico, Cristina Loda. Laboratory aid to the diagnosis and therapy of infection in the neonate. Pediatric Reports 2011; volume 3.

11. Sindhu Sivanandan, Amuchou S. Soraisham, and Kamala Swarnam. Choice and Duration of Antimicrobial Therapy for Neonatal Sepsis and Meningitis. International Journal of Pediatrics Volume 2011.

Study design:

A prospective descriptive study: Routine samples sent to the microbiology laboratory from infants with suspected sepsis will be analysed. Their clinical and demographic details, risk factors, treatment and outcome of neonatal sepsis will be documented.

Study population:

The study population will comprise of HIV exposed and unexposed infants with suspected sepsis that are admitted to KEH nursery for > 24hours. Written informed consent will be obtained from parents or guardian.

Sampling strategy:

As per the unit policy, the following samples will be taken during a septic workup:
-Blood culture, CSF (if clinically indicated) and ETA for ventilated patients.
-FBC and CRP
A separate whole blood sample will be collected in a yellow top vacutainer for storage of serum.

Sample size:

Statistical projection for sample size is 370 based on delivery rate ,suspected sepsis rate and the 40% maternal HIV prevalence.

Inclusion criteria:

- All neonates (days 1 - 28) admitted to KEH nursery for > 24 hours that are suspected of having infection/sepsis by the clinician will be eligible for enrolment in the study

Exclusion criteria:

- No consent obtained

- Neonates with severe congenital or chromosomal abnormalities will be excluded from the study.
- < 24 hours stay in nursery

Data collection methods:

All blood cultures, cerebrospinal fluids and respiratory samples received from neonates admitted to the unit during the study period will be included in the study.

Sample Collection, Handling and Transport:
-Blood Cultures: Using aseptic technique about 1 – 3ml of blood will be inoculated directly into BACT/ALERT PF Paediatric culture vials and transported to the KEH Microbiology Laboratory for subsequent processing.

Bottles will be loaded into the BACT/ALERT 3D Microbial Detection System which is a fully automated blood culture system.

Positive blood cultures will be removed from instrument and microscopy and culture performed.

Blood cultures will be considered negative if there is no growth after continuous incubation for up to 7 days

-Cerebrospinal fluid: All lumbar punctures will be performed using aseptic technique and at least 0.5ml of CSF in a sterile additive free tube will be sent to laboratory. Once received, the macroscopic appearance will be recorded. Microscopy will include a cell count and gram stain. The sediment will be cultured onto blood, chocolate and MacConkey agar plates and incubated.

-Respiratory Samples: will include sputum specimens, endotracheal aspirates, bronchoalveolar lavage fluid and gastric aspirates for TB. The most purulent portion of the specimen will be used for gram stain and innoculated onto blood, chocolate and MacConkey agar plates. Gastric aspirates will be cultured for TB as per laboratory standard operating procedures.

-Whole Blood Sample: the sample will be left to stand for 15-30 min for blood to clot. The clot will be removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge. The supernatant – which is the serum will be transferred into a clean polypropylene tube using a Pasteur pipette. The serum will be apportioned into 0.5ml aliquots and stored at – 20°C for future workup.

Culture and Identification:

Innoculated plates will be incubated for 18 hours at 35ºC and then inspected for bacterial growth. Bacterial colonies will be definitively identified using the VITEK 2 system (bioMérieux SA, France). Antimicrobial susceptibility testing will be done following Clinical Laboratories Standard Institute (CLSI) recommendations.

Definitions:
A blood culture will be considered contaminated if 1 or more of the following organisms are identified:

-*Coagulase-negative Staphylococcus spp*

-Viridans streptococci
-Micrococcus spp
-Bacillus spp
-Corynebacterium spp

Primary outcome: Mortality
Secondary outcome: Morbidity

Clinical and demographic data :

Clinical and demographic data for each patient including date and place of birth, mode of delivery, gestational age, Apgar score, HIV status, TB exposure, feeding options, specific clinical diagnosis, clinical signs of sepsis, antibiotic treatment and outcome will be obtained by attending paediatrician and captured on the datasheet below.
Datasheet: see appendix

Data Analysis:
Data will be entered and analysed in The SPSS software version 21.
Simple descriptive statistics and inferential statistics will be used for data analysis. Frequence distribution tables and graphs (bar/pie) will be constructed for categorical variables. Measures of central tendency and dispersion will be calculated for continuous variables. Multivariate statistical methods will be used to assess the risk factors associated with neonatal sepsis.

Study location:
Neonatal unit at King Edward VIII Hospital

Ethical Considerations:

Written, signed, informed consent will be obtained from parent/guardian/caregiver.
A study number given to each participant will be used on the datasheet and laboratory forms.
Ethical approval will be obtained from University of KwaZulu-Natal Biomedical Research Ethics Committee

APPENDIX 2: DATA SHEET FOR NEONATAL MICRO STUDY

NEONATAL DATA:

Study no. :_____ Date of Birth:_____

Birth weight:_____Kg Gestational Age:_____ SEX:_____

Mode of Delivery: NVD / BBA / C/S Meconium Stained Liquor : N / Y : I /II / III

RH :_____ WR:_____

APGAR Score : 1min_____5 min_____

Respiratory Distress at Birth: _____ SRT :_____

Meningitis _____ Hepatosplenomeg_____NEC_____

IPPV: Y / N Duration:_____ CPAP: Y / N Duration:_____

UV line: Y / N Duration:_____ UA line: Y / N Duration:_____ PICC line: Y / N Duration:_____

TPN : Y / N Duration: ____

HIV : unexposed ____ exposed _____ infected_____ uninfected_____

TB : exposed Y/N infected Y/N

Lab investigations:

Sample	Date	AG no.	Results
FBC diff			
CD4			
U&E			
CRP			
Whole blood			
Blood culture			
CSF			
Resp sample ETA Gastric asp BAL Sputum			
Urine			
HIV PCR			

Management:

Feeding: Breastfed : Y / N Donor : Y /N Formula : Y /N

Bld T/f : Y/N Steroids : Y/N duration_____

Antibiotics: Y /N

1._____ Duration:_____

2._____ Duration:_____

3._____ Duration:_____

Antifungal : Y / N

1._____ Duration:_____

HAART : Y / N

1._____ Duration:_____

2._____ Duration:_____

Outcome: _____ Discharged : _____

Demise :_____Cause :_____ Age : Day _____

MATERNAL DATA:

Antenatal: PROM : Y / N Duration:_____ Pyrexia : Y / N Chorioamnionitis : Y / N

Antibiotics: Y / N Name : _____ Duration: _____

HIV_____ HAART: _____ Duration:_____ CD4 :_____

TB : Y / N Treatment: _____ Duration:_____

Investigations:_____

Clinical Outcome:_____

APPENDIX 3: INFORMATION SHEET AND CONSENT TO PARTICIPATE IN RESEARCH

Date:

Good day.
My name is Dr Prasha Mahabeer from Department of Microbiology, National Health Laboratory Service at King Edward Hospital.

You are invited to be part of a research study. The study involves looking at the germs or bacteria that cause disease in newborns - babies that are less than 28 days old. The point of this research is to find out what are the common bacteria/germs that cause different types of infections and to see if the antibiotics that are being used to treat them will help to cure the infection.

This study is expected to study 370 newborns that are admitted to the King Edward hospital Nursery with supposed infection. It will involve everyday procedures. Samples that will be used for the study are the tests that are done daily by the doctors looking after your baby. Extra tests will only be done if clinically needed. The length of time of your involvement if you choose to enroll and remain in the study will be the period of time your newborn stays in the nursery. You will not remain longer in the hospital for the study.

The study is funded by_____

We hope that the study will have the following benefits: better knowledge of germs currently causing disease in newborns and which antibiotics are effective to treat them.
This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (approval number_____).

In the event of any problems or concerns/questions you may contact the researcher at :
Department of Microbiology
King Edward Hospital
Tel: 031 360 3184/91
Email: mahabeerp2@ukzn.ac.za

or the UKZN Biomedical Research Ethics Committee, contact details as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Participation in this research is voluntary and you may withdraw participation at any point. In event of refusal/ withdrawal, you will not incur any loss of treatment or benefit to which your baby is normally entitled.

To ensure confidentiality, your baby will be allocated a study number. That study number will be used on all documentation and reports.

APPENDIX 4: INFORMED CONSENT FORM

I…… mother/legal guardian of ……., hereby give my permission to Dr Prasha Mahabeer to use my and my child's clinical information, laboratory specimens and radiological imaging in the study on "Spectrum of organisms and outcome of neonatal infections in HIV-exposed and unexposed newborns at a tertiary care hospital in KZN".

I understand that the study will be sent to a Journal for publication. It will not include any information that will allow me or my child to be identified.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that my child would be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher.

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

My consent is voluntary. The information has been explained to me in my own language and I fully understand and accept everything that has been explained to me.

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**

_____ _____

Signature of Translator **Date**

(Where applicable)

INFORMED CONSENT FORM FOR STORAGE OF HUMAN BIOLOGICAL MATERIAL FOR RESEARCH PURPOSES[1]

BIOMEDICAL RESEARCH ETHICS COMMITTEE, UNIVERSITY OF KWAZULU- NATAL

[Spectrum of organisms and outcome of neonatal infections in HIV-exposed and unexposed newborns at a tertiary care hospital in KZN.]

The Document consists of two parts:

1. Information Document
2. Certificate of Consent (Record of the agreement)

INFORMATION DOCUMENT

INTRODUCTION

Good Morning

My name is Dr Prasha Mahabeer from Department of Microbiology, National Health Laboratory Service at King Edward Hospital.

You are invited to be part of a research study. The study involves looking at the germs or bacteria that cause disease in newborns - babies that are less than 28 days old.

The purpose of this study is to find out what are the common bacteria/germs that cause different types of infections in babies and to see if the antibiotics that are being used to treat them at the moment are useful.

The study will look at specimens and blood taken from your baby. These may include sputum, fluid around the spine or blood, depending on where the infection is.

I, Dr Prasha Mahabeer from the Department of Microbiology at King Edward Hospital, NHLS/UKZN, would like your permission to store blood and any bacteria/germs cultured from those specimens for future testing.

USE AND STORAGE

The reason for storing the samples is to do further tests on it at a later stage. The samples will be stored in a freezer at -35^{0}C in the Department of Microbiology at King Edward Hospital for 5 years and thereafter discarded in a safe manner. My supervisor Prof K Mlisana and I, Dr Prasha Mahabeer will only have access to these samples.

BENEFITS

There are no direct benefits of the study to your baby but this study will improve the future treatment of babies with infections. The information obtained from the stored samples may be used for teaching, public health surveillance, research, to generate new knowledge, for publications, presentations and or academic qualifications. The sample will not be sold for profit.

RISKS

There are no risks to having your baby's sample stored.

CONFIDENTIALITY

To ensure confidentiality, your baby will be allocated a study number. That study number will be used on all documentation, reports and storage of samples. The results of future tests will not go into your baby's medical records.

PARTICIPANTS RIGHTS

You may not agree to have your baby's sample stored and used for future research. This will not affect your baby's participation in the study. Please feel free to tell me if there is something that you don't want your baby's sample used for or if you don't want their sample used at all.

You may withdraw permission at anytime – by contacting me: Dr Prasha Mahabeer at Department of Microbiology at King Edward Hospital, Tel: 031 360 3184.

You may ask any questions about any part of the information provided above.

Any research which uses the sample/s will have been approved by the University's Ethics Committee.

CERTIFICATE OF CONSENT

In the light of the information that I have received, and having had the opportunity to ask questions that have been answered, and if any of the biological material [specify, i.e. blood, isolates] I, parent/guardian of _____ have provided for this research project**: Spectrum of organisms and outcome of neonatal infections in HIV-exposed and unexposed newborns at a tertiary care hospital in KZN,** is unused or leftover or additional samples have been provided, I agree to participate in the research study and consent to the following:

	Yes	**No**
The samples [blood and isolates] to be disposed of lawfully, immediately	☐	☐
The sample/s [blood and isolates] to be disposed of lawfully after_5_ years.	☐	☐
.		
The sample/s [blood and isolates] to be stored for _5__years	☐	☐

AND if the sample is to be stored I consent to the following:

The sample/s collected during 2014-2019 may be stored at Microbiology Department at King Edward Hospital.	☐	☐
The sample/s [blood and isolates] to be stored and used in future research for the specific purposes of this study [Spectrum of organisms and outcome of neonatal infections in HIV-exposed and unexposed newborns at a tertiary care hospital in KZN] approved by BREC	☐	☐
The sample/s [blood and isolates] to be stored and used in future research of any type which has been approved by BREC.	☐	☐
The sample/s[blood and isolates] to be used for teaching, quality assurances, public health surveillance, clinical audit, publications and presentations approved by BREC	☐	☐
AND		
I want my child's identity to be removed from my sample/s [blood and isolates]	☐	☐
I want my child's identity to be kept with my samples [blood and CSF]	☐	☐

AND **Yes** **No**

I am willing to be re-contacted by the researcher/s about possible future use of my child's sample/s in the future ☐ ☐

I do not want to be re-contacted to provide more sample/s in the future or take part in future studies. ☐ ☐

I declare:

I have read the information, or it has been read to me. I have had the opportunity to ask questions about it and my questions have been answered to my satisfaction. I consent voluntarily to have my samples stored in the manner and for the purpose indicated above. I have been informed of my right to withdraw my consent to the storage and/or use of my samples at any time and without giving any reason and without prejudice to myself or my child's treatment. ☐ ☐

I have been informed that I will be given information from the research team concerning the progress and general results of the research studies upon my explicit request. I have also been informed that they will not communicate any individual results to me. ☐ ☐

*Name of Participant*_____

Signature of Participant _____

Date _____

Day/month/year

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

*Print name of witness*_____ ***Thumb print of participant***

Signature of witness _____

Date _____ *Time*_____

Day/month/year

STATEMENT BY THE RESEARCHER/PERSON TAKING CONSENT

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands.
I confirm that the participant was given an opportunity to ask questions about the nature and manner of storage of the samples, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed consent form has been provided to the participant.

*Name of Researcher/person taking the consent*_____

*Signature of Researcher /person taking the consent*_____

Date _____ *Time*_____

Day/month/year

APPENDIX 5: BIOMEDICAL RESEARCH ETHICS COMMITTEE OF UNIVERSITY OF KWAZULU-NATAL – LETTER OF APPROVAL

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 February 2014

Dr Prasha Mahabeer
201 Harbourlights
103 Cato Rd
Glenwood
Mahabeerp2@ukzn.ac.za

PROTOCOL: Spectrum of Organisms and Outcome of Neonatal Infections in HIV Exposed and Unexposed Newborns at a Tertiary Care Hospital in KZN: BE342/13

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 11 September 2013.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 15 January 2014 to queries raised on 31 October 2013 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 28 February 2014.

This approval is valid for one year from **28 February 2014.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **08 April 2014.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

[redacted]

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

Professor D Wassenaar (Chair)
Biomedical Research Ethics Committee
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban, 4000, South Africa
Telephone: +27 (0)31 260 2384 **Facsimile:** +27 (0)31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

APPENDIX 6: BIOMEDICAL RESEARCH ETHICS COMMITTEE OF UNIVERSITY OF KWAZULU-NATAL – RECERTIFICATION

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

18 March 2019

Dr Prasha Mahabeer
201 Harbourlights
103 Cato Rd
Glenwood
Mahabeerp2@ukzn.ac.za

PROTOCOL: Spectrum of Organisms and Outcome of Neonatal Infections in HIV Exposed and Unexposed Newborns at a Tertiary Care Hospital in KZN: BE342/13

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	28 February 2019
Expiration of Ethical Approval:	27 February 2020

I wish to advise you that your application for Recertification received on 05 March 2019 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 April 2019.

Yours sincerely

[redacted]

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

APPENDIX 7: GATEKEEPER APPROVAL – KING EDWARD VIII HOSPITAL

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

OFFICE OF THE HOSPITAL CEO
KING EDWARD VIII REGIONAL HOSPITAL
Private Bag X02, CONGELLA, 4013
Corner of Rick Turner & Sydney Road
Tel.031-3603853/3015; Fax.031-2061457;
Email.rejoice.khuzwayo@kznhealth.gov.za;
www.kznhealth.gov.za

Ref.: KE 2/7/1/ (55/2013)
Enq.: Mrs. R. Sibiya
Research Programming

6 December 2013

Dr. Prasha Mahabeer
201Harbourlights
103 Cato Road
GLENWOOD

Dear Dr. Mahabeer

Protocol: "Spectrum of Organisms and Outcome of Neonatal Infections in HIV exposed and unexposed newborns at a Tertiary Care Hospital in KZN" REF. BE342/13

Permission to conduct research at King Edward VIII Hospital is provisionally granted, pending approval by the Provincial Health Research Committee, KZN Department of Health.

Kindly note the following:-

- The research will only commence once confirmation from the Provincial Health Research Committee in the KZN Department of Health has been received.
- Signing of an indemnity form at Room 8, CEO Complex before commencement with your study.
- King Edward VIII Hospital received full acknowledgment in the study on all Publications and reports and also kindly present a copy of the publication or report on completion.

The Management of King Edward VIII Hospital reserves the right to terminate the permission for the study should circumstances so dictate.

Yours faithfully

[redacted]

SUPPORTED/~~NOT SUPPORTED~~

18/12/2013

DR. OSB BALOYI
ACTING CHIEF EXECUTIVE OFFICER

DATE

APPENDIX 8: KZN DEPARTMENT OF HEALTH APPROVAL

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM335 /13
Enquiries: Mrs G Khumalo
Telephone: 033 – 395 3189

23 December 2013

Dear Dr P Mahabeer

Subject: Approval of a Research Proposal

1. The research proposal titled **'Spectrum of organisms and outcome of neonatal infections in HIV exposed and unexposed newborns at a tertiary care hospital in KZN'**was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby **approved** for research to be undertaken at **King Edward VIII Hospital.**

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mrs G Khumalo on 033-395 3189.

Yours Sincerely

[redacted]

Dr. E Lutge
Chairperson, KwaZulu-Natal Health Research Committee
Date: 03/01/2014

APPENDIX 9: RESULTS

Interventions by HIV exposure

HIV exposure	Unexposed (N=136)	Exposed (N=140)	p-value	Overall (N=276)
IPPV			0.088	
No	80 (58.8%)	68 (48.6%)		148 (53.6%)
Yes	56 (41.2%)	72 (51.4%)		128 (46.4%)
CPAP			0.112	
No	80 (58.8%)	69 (49.3%)		149 (54.0%)
Yes	56 (41.2%)	71 (50.7%)		127 (46.0%)
Umbilical vein			0.620	
No	74 (54.4%)	72 (51.4%)		146 (52.9%)
Yes	62 (45.6%)	68 (48.6%)		130 (47.1%)
Umbilical artery			0.668	
No	105 (77.2%)	105 (75.0%)		210 (76.1%)
Yes	31 (22.8%)	35 (25.0%)		66 (23.9%)
PICC			0.097	
No	122 (89.7%)	133 (95.0%)		255 (92.4%)
Yes	14 (10.3%)	7 (5.0%)		21 (7.6%)
TPN			0.293	
No	108 (79.4%)	118 (84.3%)		226 (81.9%)
Yes	28 (20.6%)	22 (15.7%)		50 (18.1%)
Feeding			0.790	
Breast	125 (91.9%)	124 (88.6%)		249 (90.2%)
Formula	7 (5.1%)	8 (5.7%)		15 (5.4%)
Missing	4 (2.9%)	8 (5.7%)		12 (4.3%)
Transfusion			0.513	
No	97 (71.3%)	104 (74.3%)		201 (72.8%)
Yes	39 (28.7%)	35 (25.0%)		74 (26.8%)
Missing	0 (0%)	1 (0.7%)		1 (0.4%)

The p-values are based on non-missing cases only(tableStack).

APPENDIX 10: TURNITIN ORIGINALITY REPORT

Spectrum of organisms and outcome of neonatal infections in HIV-exposed and unexposed newborns at a tertiary care hospital in KwaZulu-Natal

ORIGINALITY REPORT

9%	5%	7%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of KwaZulu-Natal Student Paper	1%
2	"Neonatology", Springer Science and Business Media LLC, 2018 Publication	1%
3	fn.bmj.com Internet Source	1%
4	A Dramowski, S Velaphi, G Reubenson, A Bekker, O Perovic, H Finlayson, A Duse, N R Rhoda, N P Govender. "National Neonatal Sepsis Task Force launch: Supporting infection prevention and surveillance, outbreak investigation and antimicrobial stewardship in neonatal units in South Africa", South African Medical Journal, 2020 Publication	1%
5	researchspace.ukzn.ac.za Internet Source	1%

6	"Posters", Clinical Microbiology and Infection, 05/2009 Publication	1%
7	www.thieme-connect.com Internet Source	1%
8	"Posters", Clinical Microbiology and Infection, 2007 Publication	1%
9	hdl.handle.net Internet Source	1%

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