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HIV exposure as a risk factor for death among neonates with healthcare-associated bloodstream infections in South Africa, 2016-2017

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DECLARATION

I, Thembekile Zwane, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science in Field Epidemiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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(Signature of candidate)

_____22____day of___February_____2021_____in__Germiston _____

DEDICATION

I dedicate this research report to my family who did not see me or enjoy my company at times because I needed to complete this. My son (Malachi) at the tender age of 3 years had come to understand that mommy comes back tired and often falls asleep on the couch and would then ask mommy to go sleep in the bed. My partner (Alain) who sacrificed a lot of his time and goals to help me study and stay with our son during the lockdown period so I could go to work. Most importantly, I appreciate his encouragement on those days when this seemed impossible. Lastly, I would like to give thanks to the Lord Almighty for carrying me up to this point in time.

ABSTRACT

Background: Hospitalised neonates (aged 0-28 days) are at high risk of developing bloodstream infections (BSI). Bacterial infections account for 26% of deaths during the neonatal period in low- and middle-income countries. HIV-exposed neonates may have lower specific antibody levels at birth compared to HIV-unexposed neonates, which makes them vulnerable to various infectious agents. We hypothesized that HIV exposure was associated with death among neonates with *Staphylococcus aureus*, carbapenem-resistant Enterobacterales (CRE) or *Candida* BSI.

Methods: A cross-sectional study was conducted at five hospitals participating in GERMS-SA surveillance for culture-confirmed BSIs, 2016-2017. A case was defined as a neonate with isolation of *S. aureus*, CRE or *Candida* from a blood culture specimen. Demographic, clinical and in-hospital outcome was collected by chart review/interview. We defined neonatal exposure to maternal HIV infection as any neonate with an HIV polymerase chain reaction (PCR) test result, assuming that this test would be ordered only if the mother was known to be HIV-seropositive. We used classical and multivariable logistic regression analyses to determine the association between HIV exposure and in-hospital mortality. We adjusted for age, sex, birthweight, gestational age, a central venous catheter (CVC) *in situ*, antimicrobial use and looked for effect modification of HIV exposure on mortality by birth weight in the final adjusted model.

Results: There were 380 cases and 86.8% (330/380) of the cases had a known outcome and HIV status. The percentage of missing information varied across cases and therefore the total for each variable was different. The median age was 11 days (IQR 7-18) and males accounted for 52.3% (195/373). Cases with CRE accounted for 5.2% (20/380), *Candida* 37.4% (142/380) and *S. aureus* cases 57.4% (218/380). The percentage of HIV-exposed neonates was 32.7% (108/330), of these 108 had a known outcome. The overall crude case-fatality ratio (CFR) was 25.4% (84/330). The CFR among HIV-exposed neonates was 26.8% (29/108). HIV-exposed neonates had 14% higher crude odds of death compared to HIV-unexposed neonates (odds ratio [OR] 1.14, 95% CI: 0.68-1.93). After adjustment, the OR for death was 1.19 (95% CI: 0.60-2.37, p=0.62). Among babies with birthweight ≥2.5 kg, the adjusted OR for death was 4.01 (95% CI: 0.14-113.86, p=0.42) among HIV-exposed versus HIV-unexposed neonates. Among neonates with a low birth weight, the stratum-specific OR for the effect of HIV exposure on mortality was 1.12 (95% CI: 0.55-2.28, p=0.74).

Conclusion: HIV-exposed neonates with a BSI had 19% higher adjusted odds of death than HIV-unexposed neonates, though the 95% CI spanned one. Birth weight modified the effect of HIV exposure on mortality and this was stronger among neonates with normal birthweight (≥2.5 kg). Our total sample size may not have been sufficiently large. Missing data probably resulted in selection bias. However, we recommend using a prospective cohort study design for the analysis to accurately measure 30-day in hospital mortality from admission date.

Keywords: HIV-exposed, neonate, bloodstream infections, mortality, pathogens, hospital-based surveillance, South Africa

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ABBREVIATIONS AND SYMBOLS

AMR: Antimicrobial resistance

ART: Antiretroviral therapy

AST: Antimicrobial susceptibility testing

BSI: Bloodstream infection

CI: Confidence interval

CRE: Carbapenem-resistant Enterobacterales

CHARM: Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses, National Institute for Communicable Diseases

CFR: Case fatality ratio

CLSI: Clinical and Laboratory Standards Institute

DNA: Deoxyribonucleic acid

ECV: Epidemiologic cut-off

ESBL: Extended spectrum beta-lactamase

EUCAST: European Committee for Antimicrobial Susceptibility Testing

HABSI: Healthcare-associated bloodstream infection

HAI: Healthcare-associated infections

HIV: Human immunodeficiency virus

HREC: Human Research Ethics Committee

IPC: Infection prevention and control

IQR: Interquartile range

IV: Intravenous line

MIC: Minimum inhibitory concentration

MDR: multidrug-resistant

MRSA: Methicillin-resistant *Staphylococcus aureus*

MSSA: Methicillin-susceptible *Staphylococcus aureus*

NHLS: National Health Laboratory Service

NICD: National Institute for Communicable Diseases

NICU: Neonatal Intensive Care Unit

OR: Odds ratio

PCR: Polymerase chain reaction

PICU: Paediatric intensive care unit

PMTCT: Prevention of mother to child transmission

RR: Risk ratio

SA: South Africa

SAFETP: South African Field Epidemiology Training Programme

sd: standard deviation

µg/ml: micrograms per millilitre

VLBW: very low birthweight

WHO: World Health Organization

DEFINITION OF KEY TERMS

Term	Definition for the purpose of the report
HIV-exposed neonate	An infant either HIV PCR-positive or HIV PCR-negative born to a woman who is HIV-seropositive. The infant is at risk of acquiring HIV infection from the mother. The infant/child may test HIV-positive or negative on HIV PCR at birth or later.
Multidrug-resistant pathogens	Multidrug-resistant bacterial infections are those from any pathogen which are resistant to three or more classes of antimicrobials Multidrug-resistant *Candida* infections are those that are resistant to at least two classes of antifungals (azoles, polynes, echinocandin).
Single episode – CRE and *S. aureus*	Bacterial episode was defined using a 21-day period after the day the first positive blood culture was collected for CRE and or S. *aureus*
Single episode – *Candida* spp.	Fungal episode was defined using a 30-day period after the day the first positive blood culture was collected for any *Candida* spp.
Viable isolates	Viable isolates were those tested for antimicrobial susceptibility at a reference laboratory
Antibiogram	The overall profile of antimicrobial susceptibility testing results of a bacterial or fungal species to a panel of antibiotic agents
MIC	The lowest concentration of an antimicrobial agent that prevents growth of a microorganism.
ECV	Defined as the highest MIC value that categorise microbial populations into wild type (with acquired mutation or non-wild-type (without mutational resistance).
Susceptible	A susceptible microorganism to a particular antimicrobial agent is when the microorganism is inhibited by the concentration of that antimicrobial
Intermediate	Is a category that includes isolates with MICs for which response rates may be lower than for susceptible isolates. The microorganism might or might not be inhibited by the concentration of that antimicrobial.
Resistant	This is a category where the usual concentration of the antimicrobial agent does not inhibit growth of the microorganism.
Length of hospital stay	Defined as duration of stay (number of days) in the hospital from admission to outcome (discharge/death/ referral)
Empiric antimicrobial therapy	Exposure to antimicrobials before or on the same day of blood collection or 2 days after blood collection.
Birth weight category	Very low birth weight was defined as weight < 1.5 kg Low birth weight was defined as weight of a neonate <2.5 kg Normal birthweight was defined as weight of a neonate ≥2.5kg

Prematurity / preterm defined by gestational age at birth	Gestational age at birth - is the gestational age in weeks recorded at birth, this was assessed by date of last menstrual period and date of physical exam recorded in medical charts.

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CHAPTER 1: Introduction

Hospitalised infants are at high risk of developing bloodstream infections (BSI). These infections are part of the broad problem of healthcare-associated infections (HAI) also known as nosocomial infections as a result of health care delivery.[1] For newborns, the source of healthcare-associated bloodstream infections (HABSI) is usually the hospital environment, after admission (≥48 hours) to nursery care.[2] Reported outbreaks in the neonatal units are caused by various pathogens which lead to morbidity and mortality.[3] Early infant diagnosis (EID) of HIV and management has improved over the years in South Africa. However, prematurity and low birth weight are common birth outcomes among HIV-infected pregnant women. [4–6] These and other factors such as invasive procedures meant for the survival of these infants become the source of infections and adverse outcomes.[2] This study investigates the influence of concomitant neonatal HIV exposure (from mother) on mortality among neonates with HABSI by *Staphylococcus aureus* and carbapenem-resistant Enterobacterales (CRE) and *Candida* species.

Chapter one provides a brief background on HABSI among neonates including causes and mortality. The research was then directed at specific pathogens that were the focus of the study, while still giving a brief overview of risk factors associated with both infections and mortality. It then introduces the main exposure (HIV) and how that connects to HABSI in neonates. The problem statement and justification of the study are stated, which leads to formulation of the research question and hypothesis. Finally, the aim of the study and the objectives are outlined.

1.1. Background

1.1.1. Healthcare-associated Bloodstream infections (HABSI) among neonates

HAIs are understood to indicate a complication of patients receiving healthcare and result in increased morbidity, in-hospital mortality and healthcare cost.[2,7] Hospitalised neonates (babies in the first 28 days of life) are at high risk of acquiring HAIs and the most common type of HAI among neonates is BSI.[8–10] The HABSIs are caused by bacterial, viral, parasitic and fungal pathogens.[9] Bacterial pathogens from the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), *Escherichia coli* and fungal pathogens particularly *Candida* spp. have been reported as causative agents in HAI worldwide.[11,12] HAIs are not only an issue in terms of patient safety but are recognised as major drivers of antimicrobial resistance (AMR).[13]

Some of the antimicrobial resistant pathogens causing HABSIs include methicillin-resistant *S. aureus* (MRSA), penicillin-resistant pneumococci, extended-spectrum beta-lactamase (ESBL)-producing organisms and CRE.[13,14] Colistin-resistant *Pseudomonas*, *Acinetobacter* and Enterobacterales are considered multidrug-drug resistant (MDR) pathogens that threaten effective control and treatment of HAIs and other infections worldwide.[13] MDR is defined as resistance to one or more agents in three or more classes of antimicrobials.[15] However, MRSA is also considered MDR because resistance to oxacillin or cefoxitin predicts resistance to the class of beta-lactam antimicrobials except anti-MRSA cephalosporin.[15] The ESKAPE pathogens also include agents that are highly resistant to multiple antimicrobials i.e. *Enterobacter* spp. and *K. pneumoniae*.[16]

In sub-Saharan Africa, the majority of BSIs in neonates are caused by Gram-negative organisms including ESBL-producing *Klebsiella* spp. and *E. coli.*[17] This highlights the importance of identifying common pathogens and their susceptibility patterns among neonates with HABSI in different areas, to select appropriate empirical antimicrobials.[18] Additionally, it is important to understand the risk factors associated with mortality from pathogen-specific HABSIs to inform specific target groups (e.g. HIV exposed neonates) for preventative and control measures.[19]

This study explores factors associated with mortality in neonates with HABSIs from *S. aureus*, CRE and *Candida* spp. using surveillance data. We also explore HIV exposure as the main risk factor for death among neonates with HABSI from these pathogens. To understand pathogen-specific HABSI from CRE, *S. aureus* and *Candida*, it is important to understand their

pathogenicity, how they cause invasive infection, their antimicrobial susceptibility profile and factors associated with death in adults and in neonates.

It is important to describe mortality in HIV-exposed neonates with and without HABSI to understand if there is a relationship. This chapter gives a brief background on each pathogen as it relates to infection in neonates. We further expand on the different factors associated with infection in the following chapter on the literature review.

1.1.2. Pathogen-specific HABSI: CRE, S. *aureus* and *Candida* spp.

The Centres for Disease Control and Prevention (CDC) defines CRE as organisms resistant to carbapenems (imipenem, meropenem, doripenem, or ertapenem) or confirmation that isolates are carbapenemase-producing (e.g. *E. coli*, *Klebsiella* spp.).[20] Carbapenems are beta-lactam antibiotics that have broad-spectrum activity and have been termed "last resort" antibiotics. Until recently carbapenems were mostly used in treating serious bacterial infections (e.g. bacteraemia, complicated urinary tract infections, meningitis and others).[21,22] Because of their broad-spectrum activity, they act against Gram-negative and Gram-positive bacteria including anaerobes. However, they are mostly used against Gram-negative pathogens.[22]

The most commonly reported CRE among neonates is *K. pneumoniae*.[23] Patient-level risk factors for CRE infection have been described among children including neonates. These are comprised of prior antibiotic exposure, frequent or prolonged hospitalisations, invasive medical devices and age. [21] Antibiotic exposure was reported as a risk factor for death associated with CRE infection and increased with prolonged use (more than five days after birth) compared to those with no exposure to antibiotics.[24,25]

Candidaemia (BSI caused by *Candida* spp.) is associated with morbidity and mortality specifically in very low birth weight neonates (VLBW).[26,27] A study done in an Italian hospital reported candidaemia as the third most common nosocomial BSI among neonates with late-onset sepsis.[28] In sub-Saharan Africa, there is paucity of studies on neonatal candidaemia. Different studies have reported that it is responsible for 25% to 30% of morbidity in neonatal intensive care units (NICUs).[27,29] *C. albicans* followed by *C. parapsilosis* have been reported as the most frequent causes of candidaemia in neonates.[27,30] The treatment of candidaemia using appropriate antifungals

i.e. fluconazole, amphotericin B deoxycholate and micafungin should be initiated promptly after diagnosis.[26] Some of the risk factors for neonatal candidaemia include prolonged NICU stay, use of a catheter or endotracheal tube, broad-spectrum antibiotic use and total parenteral nutrition among others.[28]

S. aureus is another significant pathogen in neonatology and an important cause of morbidity.[31] Humans are carriers of *S. aureus*, through the nasopharynx, skin and axillae.[32,33] Neonates can be exposed and colonised shortly after birth through direct contact from caregivers or through the environment (i.e., hospital equipment). [32] Colonisation may be a precursor to invasive infection.[32] The prevalence of methicillin-resistant *S. aureus* (MRSA) varies among different populations and age groups, some studies have reported 39% prevalence among children and an observed increased colonisation with MRSA.[34,35] MRSA in neonatal wards has been reported to spread horizontally by contact with healthcare workers or the hospital environment.[32,36] In addition, MRSA remains the frequent source of infection among premature and critically ill infants in NICU compared to methicillin-susceptible *S. aureus* (MSSA) due to resistance to multiple types of antibiotics.[36] MRSA is resistant to cloxacillin and all beta-lactam antibiotics (e.g. penicillins, cephalosporins).[32] In one study, MRSA colonisation or infection was associated with significant morbidity and length of hospital stay. However, it was not significantly associated with an increased risk of mortality in their NICU.[37]

HABSI-associated mortality in neonates due to CRE, S. *aureus* and *Candida* spp.

Bacterial infections in the neonatal period causes high mortality and, in developing countries, are responsible for 26% of deaths.[38] Fungal HABSI has been associated with high mortality rates in neonates, where mortality following *Candida* BSI is as high as 40%.[27,39] It has been reported that among newborns with birth weight less than 1000g, 4-8% develop candidaemia, which has a 30% mortality in this group of patients.[28] In South Africa (SA), overall mortality due to neonatal sepsis was reported to be between 21% and 23% and was mostly due to Gram-negative bacterial pathogens (69%-80%).[18]

1.1.3. HIV exposure and mortality in neonates

In addition to HAI, exposure and infection by HIV may be a significant cause of neonatal mortality in communities with a high prevalence of HIV infection. According to the 2017 South African

national antenatal survey, HIV prevalence among women attending antenatal care remains high (30.7%).[40] As a result of successful HIV prevention of mother to child transmission (PMTCT) programmes, there is a growing population of HIV-exposed but uninfected infants.[41] HIV seems to impact the developmental changes in infant immunity in both HIV-infected and HIV-exposed uninfected children, however, the mechanism is poorly understood.[41]

1.1.4. The gap – Does HIV exposure increase the risk of death among neonates with HABSI?

Due to the HIV PMTCT intervention, fewer infants are becoming infected with HIV. Therefore, more attention is given to the survival of infants who are HIV-exposed, but uninfected.[42] A study done among infants in South Africa reported that "antenatal HIV exposure was associated with lower specific antibody responses in HIV-exposed uninfected infants compared with HIV-unexposed infants at birth".[43] Few South African studies have looked at HABSI in HIV-exposed infants. HIV exposure and infection have been identified as novel risk factors for HAI among children.[8,44] It has been reported that maternal HIV infection impacts on pregnancy outcomes i.e., low birth weight and preterm delivery, which are important risk factors for post-neonatal mortality and morbidity.[6] A study done in an urban district hospital in KwaZulu-Natal indicated that of the 92 (37%) HIV-positive mothers, 13 (14%) delivered preterm neonates, compared to 10(6%) of the 158 HIV-negative mothers.[45] The study further highlighted that conclusion cannot be drawn on the increased risk of mortality in preterm HIV-exposed infants compared to the unexposed group owing to the small sample size. However, the results of the study confirmed that maternal HIV infection is a significant risk factor for preterm delivery.[45]

At the National Institute for Communicable Diseases (NICD), the GERMS-SA surveillance programme has collected data on CRE, *S. aureus* and *Candida* spp. BSI. We specifically selected these three pathogens because they were monitored simultaneously at five hospitals from 2016-2017. Therefore, this was a convenient population to study. In summary, these are cases of patients with laboratory-confirmed isolates from blood cultures where demographic, clinical information and HIV status in addition to other risk factors were collected.

1.2. Statement of the Problem

There is a paucity of data regarding the effect of HIV exposure on mortality among neonates presenting with HABSI. A retrospective study done in Cape Town showed that the HABSI crude mortality rate was 7.1%, with neonates having a higher case fatality ratio (6/40; 15%) than children including infants less than 12 months (5/116; 4.3%).[46] Investigators further reported a higher crude mortality rate in HIV-infected children without specifying age.[46] A retrospective paediatric study reported HIV infection (risk ratio (RR)=2.44, 95%CI: 1.59-3.74) as one of the significant risk factors for death among children with ESBL-producing *K. pneumoniae* HABSI admitted to a paediatric intensive care unit (PICU) in Cape Town; however, the report was on all children less than five years as opposed to neonates .[44]

We have identified a gap in information concerning HIV exposure as a risk factor for mortality among neonates with HABSI. Various factors such as low birth weight, exposure to antibiotics, poor feeding and AMR pathogens could lead to mortality in neonates with HIV exposure and HABSI. Taking into account the complexity of different factors that lead to neonatal death, our research question is; does HIV exposure increase the risk of death among neonates with culture-confirmed HABSI from CREs, *S. aureus* and *Candida* spp.? In addition, is this effect modified by low birth weight? We hypothesize that the effect of HIV-exposure on death is stronger in premature low birth weight infants because a randomized clinical trial in Uganda showed that low birth weight and gestational age were associated with increased odds of death in a cohort of HIV-exposed infants.[47]

1.3. Justification

Importance of the study

South Africa has an established surveillance programme for bacterial, viral and fungal infections of public health importance through GERMS-SA.[48] Data generated are important for understanding the pathogens associated with healthcare-associated morbidity and mortality. EID of HIV is crucial for timely initiation of antiretroviral therapy (ART) for HIV infected neonates.[49] Identifying high-risk neonates and initiating ART when needed is critical to reduce morbidity and mortality.[5] Although prevention of HIV transmission is possible, different studies have reported

death occurring among HIV-exposed infants, calling for new comprehensive interventions to reduce mortality in this growing population.[47] There is an important need for data linkage of HIV-exposed neonates in the first days of life and the risk of acquiring HABSI to prevent adverse outcomes.

The overall case fatality ratio (CFR) among neonates was reported to be 15% at a tertiary hospital in Cape Town. Preliminary unpublished data by the same authors suggests that it has increased to 30% (reasons and time period were unknown) with no differences between HIV-infected and HIV-uninfected infants.[46] We hypothesize that HIV exposure does increase the risk of death among neonates with HABSI because HIV-exposed neonates experience immunological alterations *in utero* due to HIV exposure.[50] Therefore, exposure to infectious agents could lead to an increased risk of infection-associated morbidities and mortality compared to HIV-unexposed neonates.[51,52]

1.4. Aim

Our study aimed to determine whether HIV exposure was associated with in-hospital mortality among neonates with HABSI from CRE, *S. aureus* and *Candida* spp. The information obtained from this study could be used to contribute to programmes on antimicrobial stewardship and infection prevention and control (IPC) in different participating hospitals.

1.5. Objectives

1.5.1. To describe the demographic and clinical characteristics of neonates with HABSI caused by CRE, *S. aureus* and *Candida* spp. at five hospitals in South Africa in 2016 and 2017.

1.5.2. To describe pathogens and their antimicrobial susceptibility profiles from neonates with HABSI.

1.5.3.

i. To calculate the CFR among neonates with HABSI due to CRE, *S. aureus* and *Candida* spp. in five hospitals.

ii. To determine whether HIV exposure increases the risk of mortality among neonates with HABSI from these specific pathogens, considering other confounders.

iii. To do a post hoc analysis to determine if HIV exposure effect on death is modified by birth weight of a neonate. This analysis was done after completing the analysis in (ii) and we had not planned to look at this initially.

CHAPTER 2: A literature review

This chapter covers in-depth the concepts introduced in the first chapter by explaining pathogen-specific HABSI and risk factors associated with mortality including HIV exposure. It also explains how HIV exposure and other risk factors may contribute to mortality using a body of work that has been published highlighting important variables to be considered for the study.

2.1. Hospital care among neonates or new-borns

Many interventions have been developed for the care of newborn infants with a special focus on high-risk group i.e., those born prematurely or with low birth weight.[53] These interventions are aimed at improving newborn health outcomes and reducing neonatal mortality which accounts for a third of all deaths in children under five years of age.[54] An example of these interventions for newborns at the hospital level includes the provision of PMTCT of HIV, resuscitation of newborns, care for small/ill newborns in NICU and Kangaroo Mother Care for stable low birth weight infants. In addition to these interventions, early diagnosis, treatment of infections and good infection control (especially handwashing) is essential. Therefore, quality assurance programmes in hospitals are crucial for monitoring the quality of care that is provided to newborns.[55]

Prematurity or preterm birth results from an infant being born before 37 weeks completed gestation and it is usually marked by low birth weight (weight less than 2500g) at birth.[56] Preterm delivery has been reported as a significant risk factor for neonatal death.[45] In addition, HIV-infected preterm infants are at a higher risk of death, compared to HIV-uninfected infants.[57] Several studies carried out in different regions globally have confirmed that maternal HIV infection is a risk factor for preterm delivery.[58–61] HIV-exposed preterm infants generally require better healthcare, which might lead to prolonged stay in hospital and increased cost due to the treatment of associated infections.[45] Preterm infants also develop an abnormal pattern of microbial colonisation, which may contribute to infection.[62] Because their intestines along with immunity are immature, there is an altered intestinal mucus and decreased gut barrier function. This decreased barrier function causes an increase in contact between the gut of the preterm and colonizing bacteria.[48] The NICU environment also influences the microbiome of preterm infants.[63] This includes clinical interventions i.e., formula feeding, instrumentation and exposure to antibiotics.[64] The microbiome

greatly influences the health of the infant and continues to develop as the infant matures, playing an important role in acquiring and preventing disease.[63]

Care for preterm infants or low birth weight infants often requires a longer stay in the hospital. With a prolonged stay in the hospital, there is exposure to risks associated with the environment including HAI which might impact neonatal mortality.[65] One study reported that length of stay is primarily influenced by gestational age at birth and other medical conditions such as persistent apnoeic spells, or the need for tube feeding.[66] This was further supported by another study which reported that prolonged postoperative stay in the ICU is associated with high hospital and post-discharge mortality among children in the first year of life.[67]

2.2. Specialised care for HIV-exposed neonates

HIV exposure may not be a direct cause of death among neonates. However, maternal HIV status is known to increase the risk of stillbirth and neonatal or infant death even among HIV-exposed uninfected babies.[68] This may be due to interaction with other factors such as maternal illness and infection among others.[69] It has been established that HIV-exposed newborns have a higher mortality rate compared to HIV-unexposed newborns.[70] A prospective cohort study in Botswana reported no significant mortality difference by HIV exposure status; 27% (35/130) of HIV-exposed neonates died compared with 20% (55/271) of HIV-unexposed neonates (p=0.19).[52]

HIV PMTCT in South Africa has resulted in a reduction in HIV transmission from mother to child and therefore an increased number of HIV-exposed uninfected infants. In South Africa, it is estimated that 270 000 HIV exposed infants are born annually.[71] HIV-exposed infants have been shown to have higher rates of lower respiratory tract infection, meningitis and mortality is higher (up to 4-fold) in the first year of life.[72] Infants born to HIV-infected mothers are generally born prematurely or with low birth weight.[73] Additionally, exposure to HIV has been associated with immunological alterations among exposed infants that may increase their risk of infections, especially in the absence of or with shorter breastfeeding.[59]

2.3. Other factors associated with HABSI and adverse outcomes among neonates:

Neonates are generally the population at higher risk of HABSI among hospitalised patients due to inherent (weak immune system) and external factors including low birth weight, respiratory distress syndrome due to ventilation and presence of invasive medical devices.[9] Unnecessary antibiotic exposure in the neonatal period may lead to colonisation with MDR microorganisms among neonates due to a disturbance in the microbial flora.[25] Antibiotic therapy in this period also leads to an increased risk of early adverse outcomes such as necrotizing enterocolitis (NEC) and fungal infections.[74]

2.3.1. Antimicrobial exposure

HABSIs require treatment; exposing neonates to various invasive medical devices such as intravenous (IV) lines to administer treatment. If the infection does not clear immediately or is a result of MDR (resistance to at least one agent in 3 or more antimicrobial classes) pathogens, it can lead to prolonged exposure to antibiotics which has been associated with an increased risk of NEC and death among preterm neonates.[15,25]

Due to the challenges relating to the diagnosis of neonatal infections, clinicians often have to commence empiric treatment based on clinical signs while awaiting blood culture results.[75] For this reason, neonates are started on antimicrobial therapy early until the presence or absence of the offending pathogen is identified.[76] Appropriate choice of treatment and administration should be guided by antimicrobial stewardship to limit the inappropriate use of antimicrobials and prevent the development of AMR.[77] Therefore local surveillance of antimicrobial susceptibility patterns or antibiograms are in important for the selection of empiric therapy, especially because pathogens vary considerably by region and neonatal unit.[78]

Antimicrobial surveillance is also important to monitor resistance patterns of microorganism and the emergence of new resistant strains.[79] For this reason, there are organizations that provide reference methods and breakpoints for classifying microorganisms as susceptible or non-susceptible (resistant) to antimicrobial agents. These organisations are the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST). They provide standardized criteria to guide interpretation of antimicrobial susceptibility testing (AST) results based on data routinely generated from surveillance systems.[79] Certain antimicrobials are effective in treating specific infections. Infections due to *S. aureus* are

empirically treated with parenterally administered vancomycin or cloxacillin plus gentamicin.[32] For MRSA strains, which are often resistant to multiple types of antibiotics, drugs of choice include vancomycin and gentamicin.[80] Fluconazole and amphotericin B are the most frequently used antifungal agents in neonates and fluconazole is regarded as an excellent choice for prophylaxis.[12] The sensitivity of the most common *Candida* spp. (*C. albicans* and *C. parapsilosis*) and safety of fluconazole in neonates has been established, although a large proportion of *C. parapsilosis* isolates are fluconazole resistant .[81]

2.3.2. Invasive medical devices: Central lines

Invasive devices are essential in the support of the lives of neonates. An example includes IV lines and central venous catheters (CVC), which are important in the administration of fluids and treatment. Extra caution in the insertion and removal of these devices in compliance with aseptic standards is necessary in the NICU.[82] The use of these devices is one of the main extrinsic factors associated with HABSIs. Previous studies have shown that medical catheter retention increased the risk of mortality due to candidaemia (OR 2.50, 95% CI:1.06–5.91), and early removal of catheters significantly reduced candidaemia-associated mortality (OR 20.5, 95% CI:3.9–106.5).[27] Also, infants with bacteraemia have fewer infection-related complications when the CVC is removed promptly.[83] This highlights the importance of implementing appropriate medical practices i.e. early removal of medical devices in improving the prognosis among infants.

2.4. Effect of maternal and neonatal HIV on the outcome

In one study involving children in South Africa, HIV was identified as a risk factor for HAI. The study authors mentioned that the risk of HIV infection was probably linked to the underlying immunodeficiency, as 50% of the HIV-infected children (less than five years of age) were not on antiretroviral therapy (ART) at the time of acquiring *K. pneumoniae* HABSI.[44] As previously mentioned, few studies report neonatal mortality in neonates with HABSI exposed to HIV infection in Southern Africa.[8,44,46,52] Results in these studies were not comparable as some report on HIV-infected vs HIV-uninfected infants with varying age ranges (more than 28 days).

The South African consolidated national guidelines for HIV state that the HIV DNA PCR test should be performed at birth and again at 10 weeks for infants who are exposed to maternal HIV.[84] Some studies have hypothesised that the provision of ART to HIV-infected pregnant women might

help reduce neonatal mortality. However, data are limited as studies include few women receiving ART or are limited to retrospective analyses of clinical data.[47] This highlights the important information that was used to identify HIV-exposed neonates for our study.

2.5. Mortality attributable to HIV exposure and other factors among neonates

Neonatal mortality is the outcome of a complex relationship between neonatal, maternal and healthcare-related factors.[85] Survival rates among neonates with low birth weight and preterm birth have improved over the years. However, HABSI remains a significant challenge in this group because of infection and high mortality rates.[9,86] All the factors mentioned above play a role in the acquisition of HABSI and neonatal mortality. Very few factors influence both HIV exposure and mortality. These include birth weight, prematurity or preterm birth and sex according to literature. The rest are mostly factors associated with HABSI and mortality in the neonatal period caused by specific pathogens. Due to exposure to HIV, neonates tend to be born with low birth weight requiring longer hospitalization and often treatment and these directly influence mortality. Therefore, in addition to HIV exposure, it is also worth exploring low birth weight as an effect modifier and the association of other factors to neonatal mortality.

CHAPTER 3: Methods

This chapter describes the study design, population and the settings of the study. Case definitions that were used are also described with their applications. Cases that were excluded in the analysis of each objective were discussed. We also discussed the methods used to analyse data and the sources that were used. We then outlined the data management steps and the analysis of each objective.

3.1. Study design

This was a retrospective secondary data analysis of GERMS-SA surveillance data at the NICD. We used GERMS-SA data that were collected from 2016 to 2017 at five enhanced surveillance hospitals in South Africa.

3.2. Setting

The five selected hospitals included Charlotte Maxeke Johannesburg Academic, Groote Schuur, Tygerberg, Steve Biko and Rahima Moosa Mother and Child hospitals. These were selected because they are GERMS-SA sentinel sites for enhanced surveillance. These sites collect additional patient information. We chose the five hospitals because S. *aureus* data were collected in 2016 and 2017 while *Candida* spp. and CRE data were collected nationally for the same period. These are public referral hospitals with specialized neonatal care services.

3.3. Population and period

The study population consists of neonates with HABSI from the specific pathogens admitted at five different hospitals from 2016-2017. A neonate was defined as an infant from birth to 28 days of life.

3.4. Data sources

3.4.1. GERMS-SA data

GERMS-SA has enhanced surveillance hospitals where demographic and clinical information including patient outcome is collected by the surveillance officers at each hospital. The surveillance officers either use a medical chart or interview the parents or guardian to complete

the case report form for those meeting the surveillance case definitions. Follow-up of cases is required until an outcome is noted which could be one of these; still admitted, deceased, referred, absconded or refused treatment. The case report form used by the surveillance officers also requires that HIV status be indicated for the mother and child; however, this is usually incomplete. In addition to the completion of the case report form, isolates where the pathogen was identified are sent to NICD reference laboratory for further identification and characterization (viable isolates). Sometimes isolates are not sent to NICD. However, these missing cases are detected by the audit of the laboratory information system, hence are referred to as audit isolates.

All this information is stored under different pathogen name databases in the GERMS-SA surveillance programme. Under the GERMS-SA database, healthcare-associated infections are divided according to the type of pathogen under surveillance using Microsoft Access. There is a candidaemia database and the antimicrobial resistance reference lab (AMRRL) database. The candidaemia database stores data on *Candida* species (from the mycology reference lab) from 2012-2018. The AMRRL database stores data for the following pathogens: CRE, *S. aureus*. *S. aureus* surveillance took place from 2015-2017, while CRE surveillance was from 2015 to date.

3.4.2. NHLS CDW data

The National Health Laboratory Service (NHLS) offers HIV testing for EID among other services for public hospitals in South Africa. This data is then deposited in a central repository called the Corporate Data Warehouse (CDW). Permission to access this data for the identified cases was obtained after a formal application was made to request the data. The criteria used to request data from CDW included neonates with a documented HIV PCR test and test results for neonates admitted at the five hospitals in 2016 and 2017. Maternal data including mother's HIV status are limited in our surveillance data; therefore, we focused on HIV exposure as best defined by the availability of the neonates' HIV test results at birth for those born to HIV-positive mothers.

3.5. Case definitions

A case was defined as a laboratory-confirmed positive blood culture for any of the three pathogens (CRE, *S. aureus* and *Candida* spp.) from a neonate admitted at any of the five hospitals during 2016-2017.

- ***Fungal incident episode*** was defined using a 30-day period from the day the first positive blood culture was collected for any *Candida* spp. If a subsequent positive blood culture for *Candida* spp. was drawn from the same neonate more than 30 days after the first isolation, it was regarded as a recurrent episode.
- ***Bacterial episode*** was defined using a 21-day period from the day the first positive blood culture was collected for any bacterial pathogen (CRE and or *S. aureus*). A recurrent episode was defined as isolation of CRE or S. *aureus* more than 21 days after the first episode.
- ***HIV-exposed neonate*** was defined as a neonate who had either maternal HIV status recorded as positive (GERMS-SA) **OR** a HIV PCR result (either negative or positive from NHLS CDW) **OR** both.

  NOTE: Some cases did not have the mother's HIV status documented, which was the reason for merging data. Maternal HIV status was recorded (Negative or Positive) for some cases (GERMS database) and HIV- PCR results were available (NHLS CDW) for neonates who were exposed (born to HIV-exposed mothers) while neonates with uninfected mothers had no HIV PCR done. We used the national guidelines and literature to make the assumption that all neonates with HIV PCR are HIV-exposed as testing is compulsory at birth (2015) and neonates with no HIV PCR recorded are not HIV-exposed.[69] This correlated with absent HIV PCR test results for neonates whose mother's HIV status was available and negative.

- ***Patient outcome*** (still admitted, deceased, referred, absconded or refused treatment) was categorised into alive or died for the study. In-hospital mortality or death regardless of the cause was measured as death occurring in the hospital during the period from admission to the final outcome.

3.6. Inclusion and Exclusion

- ***Inclusion***: Cases with positive blood cultures, antimicrobial susceptibility (AST) results, hospital outcome of patient and HIV status. All cases meeting the case definition were included for objective one and three. Only cases with viable isolates and ASTs were included in the analysis of objective two.
- ***Exclusion***: Positive blood cultures of patients >28 days, from any other hospital apart from the five selected and from any period outside 1 January 2016 to 31 December 2017. Cases with incomplete CRF and unknown or missing hospital outcome and infant HIV status were excluded specifically for objective three.

3.7. Data management

GERMS data were accessed on Microsoft Access and exported to Microsoft Excel. Microsoft Excel was used to append the cases from different datasets into one dataset. The appended dataset was imported onto Stata version 15.1 for data cleaning, merging using hospital number with HIV results from CDW and data analysis.

For data retrieved from GERMS-SA, the data collection instrument is not the same across all the three datasets. Therefore, variables were standardized and renamed (for similarity). Case records were then appended on the same Excel file using standardized variables. Duplicate cases were removed according to the case definition for bacterial and fungal episodes. If there were two episodes of the same pathogens (according to definitions described above) and or different organisms isolated even though the date of collection was the same, this was regarded as one case.

HIV PCR data obtained from NHLS CDW was merged with de-duplicated cases from the appended GERMS-SA file. Dates and string variables were formatted, new variables were generated and existing variables re-labeled before analysis.

3.7.1. Variable definitions / data dictionary

Table 3.7.1: Data dictionary with variable definitions

Variable	Definition
HIV exposure status	• HIV exposed neonate was defined as a neonate that had either maternal HIV status recorded as positive or a HIV PCR result (either negative or positive) or both. • HIV-unexposed neonate was defined as a neonate with maternal HIV status recorded as negative or where HIV PCR results could not be obtained from the database (suggesting that HIV PCR testing was not done)
In-hospital outcome	Defined as patient outcome recorded as one of the following: deceased, still admitted, discharged or referred. Two categories for our outcome were defined as alive (grouped all those with outcome other than deceased) and died.
Age	Age of the neonate calculated from the date of birth to the specimen (blood) collection date
Length of stay (LOS)	This was calculated using the date of admission and date of the outcome. Most neonates were admitted on the date of birth except for those referred from another facility.
Gestational age at birth	Defined as the original gestational age in weeks recorded at birth, this was assessed by date of last menstrual period and date of physical exam recorded in medical charts.
Birth weight	The weight of the neonate in grams/kilograms recorded at birth
ARV use/ received	Referring to whether the neonate or mother (during pregnancy) received ART treatment, which was either current (started at birth) or perinatal or previous for the mother.
Antimicrobial use	Refers to antimicrobials (antifungal and antibiotic therapy) received through the IV line by the neonate during the current admission marked as “yes” or “no”.
Referred from another facility	Neonates transferred from another facility to the current facility
Facility/hospital id	Refers to the facilities that were chosen for the study, each had a unique numeric identifier. 1- Charlotte Maxeke Johannesburg Academic Hospital **(CMJAH)** 16- Steve Biko Academic Hospital (**SBAH**) 17-Rahima Moosa Mother and Child Hospital (**RMMCH**) 49 -Groote Schuur Hospital (**GSH**) 50 -Tygerberg Hospital (**TH**)

3.8. Statistical Analysis

Stata version 15.1 was used for data analysis.

Table 3.8.1: Overview of the methodological approach used in the analysis of each objective (1, 2 & 3):

Objective 1: Description of study population by hospital and year	
Frequency and percentage of categorical variables	In-hospital outcome HIV exposure Sex Antimicrobial use Birthweight (<2.5kg vs ≥2.5kg) Cases at each hospital by year and pathogen ARV received
Median and Interquartile range for continuous variables	Age (days) LOS-length of stay (days) Gestational age at birth (weeks)
Objective 2: Antimicrobial susceptibility testing (AST) profile of viable isolates	
Count and percentages	AST profile for *Candida* spp.
Percentage in a stacked graph	Antibiogram for *S. aureus* Antibiogram for CRE
Objective 3: Risk factors for adverse outcome in neonates with HABSI (CRE, *S. aureus* and *Candida*) and potential confounders	
Main exposure	HIV exposure
Outcome	In-hospital mortality
Regression model	Logistic regression
Potential confounders (identified *a priori*)	Birth weight Gestational age at birth (indicating prematurity) ARV received by the mother
Biologically important variables	Sex Age
Other Covariates (effect modifiers or mediators)	Length of stay Antimicrobial use Central venous catheter inserted Previous surgery Referred from another facility
Excluded covariates (reasons)	Those that were not biologically important Those with strong correlation coefficients (r>0.5)
Post hoc analysis for effect modification by birth weight	Variables used in the final model including interaction term between HIV-exposed and birth weight

3.8.2. A detailed description of the methodological approach used for each objective

3.8.2.1. Objective one

Descriptive statistics were performed using frequency or proportions with percentages for categorical variables and median and IQR for continuous variables. For each variable, the total number of cases was presented irrespective of known outcome and HIV status.

3.8.2.2. Objective two

For this objective, only viable isolates were included with ASTs with no missing information (e.g., pathogen name). The antibiogram for each pathogen was presented separately as counts and percentages. The CLSI guidelines were used to identify susceptibility profiles specific to each pathogen. These are explained briefly below:

- **Antibiogram for *Candida spp*.**

For *Candida* spp., the 2017 CLSI guidelines (M60) which have undergone extensive revisions were used[87]. Minimum inhibitory concentrations (MIC) were used to interpret isolates as susceptible (S), indeterminate (I) and resistant (R) for each antifungal using defined breakpoints. Breakpoints are available for some *Candida* spp. vs anidulafungin, caspofungin, fluconazole, micafungin, and voriconazole. For *Candida* spp. without breakpoints, epidemiological cut-off values (ECVs) were used (e.g., for common *Candida* spp. and amphotericin B). ECVs are MIC values that separate populations of *Candida* into isolates with and without acquired or mutation-based resistance.

- **Antibiogram for bacterial pathogens: CRE and S. *aureus***

The CLSI guidelines (2017) were used to interpret MICs of isolates from S. *aureus* and CRE using pre-defined breakpoints for these pathogens[88]. The MIC interpretations I, S, and R were also used for each antibiotic tested for a specific pathogen. For this study, not all antibiotics tested were reported, only those known to be effective in the treatment of each pathogen.

3.8.2.3. Objective three

An analysis of included and excluded cases (due to missing data) for each variable was performed by calculating the percentage of missing information for the included cases. Characteristics of cases were tabulated for included and excluded cases (with missing data) to assess for selection bias due to missing data.

The overall CFR was calculated by taking the number of cases who died over the number of all cases from 2016-2017. The CFR was calculated for HIV-exposed and HIV-unexposed neonates as outlined in Table 3.8.2. The 95% confidence interval (CI) was presented for these proportions.

Table 3.8.2: Formula used to calculate the case fatality ratio

CFR	**Formula**
Overall	$\frac{Number\ of\ cases\ who\ died}{Total\ number\ of cases} x100$
HIV-exposed	$\frac{Number\ of\ \ HIV-exposed\ cases\ who\ died}{Total\ number\ of\ HIV-exposed\ cases} x100$
HIV-unexposed	$\frac{Number\ \ of\ HIV-unexposed\ cases\ who\ died}{Total\ number\ of\ \ HIV-unexposed\ cases} x100$

To test the association between HIV exposure and in-hospital outcome (mortality), the chi-square test was used for categorical variables. For continuous variables, the Student t-test and the Wilcoxon Rank-sum test was used. To test the normality of data, the normal probability plot was used. Bivariate logistic regression was also used to assess the associations between HIV exposure and mortality while adjusting for one confounder at a time.

Potential confounders and biologically important variables were chosen *a priori* based on literature and the variables that were collected in the surveillance data. Variables considered biologically important included age and sex; these were added to the final model irrespective of their statistical significance. Potential confounders identified *a priori* included; birth weight, gestational age at birth (prematurity) and whether the mother was receiving ARV.

Test of collinearity was performed for all independent variables to determine correlation to HIV exposure status. Variables that were highly correlated to HIV exposure ($r>0.5$) were not included in the final model. Confounders were determined by adding each variable to the model and assessing whether the odds ratio of main exposure (HIV exposure status) was altered by 10% or more. If the measure of effect was altered by 10% or more, then the variable was included in the

model. Other covariates were only included in the final model if they were considered important. Crude odds ratio and adjusted odds ratio were presented along with the 95% CI for univariate and multivariable analysis.

Post hoc analysis

To determine if birth weight modified the effect of HIV exposure on death, interaction between HIV exposure and birthweight was explored and this was included in the final model. This interaction was represented by the additive effect between HIV-exposed and birth weight in the final model. This product variable was added to the final model regardless of significance at $p<0.05$. To check the significance of models with and without the interaction term, we performed the Wald test.

3.9. Sample size

Power and sample size was computed using the chi-squared test comparing two independent proportions in Stata version 15.1. This was done only for objective three (iii). We had a sample size of 380 cases for the years 2016-2017, and only 330 had complete outcome and exposure information. Given our sample size, we were underpowered (65%) to detect difference in mortality at an alpha level of 0.05. To have sufficient power (80%) to detect 8-10% difference in mortality at an alpha level of 0.05, we needed a sample size between 788 and 1209 cases.

3.10. Ethical approval

Ethical approval for this study was obtained from the University of the Witwatersrand Human Research Ethics Committee (Medical) (HREC). The ethics clearance certificate (M1911195) is attached in Appendix A. Permission to use data was also obtained from NHLS CDW.

CHAPTER 4: Results

This chapter covers the results according to the three objectives of the study. We first describe the data cleaning process using a flow chart and the characteristics of all cases that were obtained. We then assess the susceptibility profile of all the viable isolates from the identified cases separately by looking at the antibiogram of each pathogen. Lastly, we compute the CFR and then determine whether HIV exposure status is associated with in-hospital mortality among neonates with the complete outcome and HIV PCR data.

4.1. Data management: flow chart

A total of 390 blood cultures meeting the case definition were retrieved from GERMS-SA (Figure 4.1). There were 380 cases (patients) of which 372 had only one pathogen isolated and 8 had two pathogens isolated. Of the 380 cases, 87% (330/380) had a known outcome and HIV exposure data.

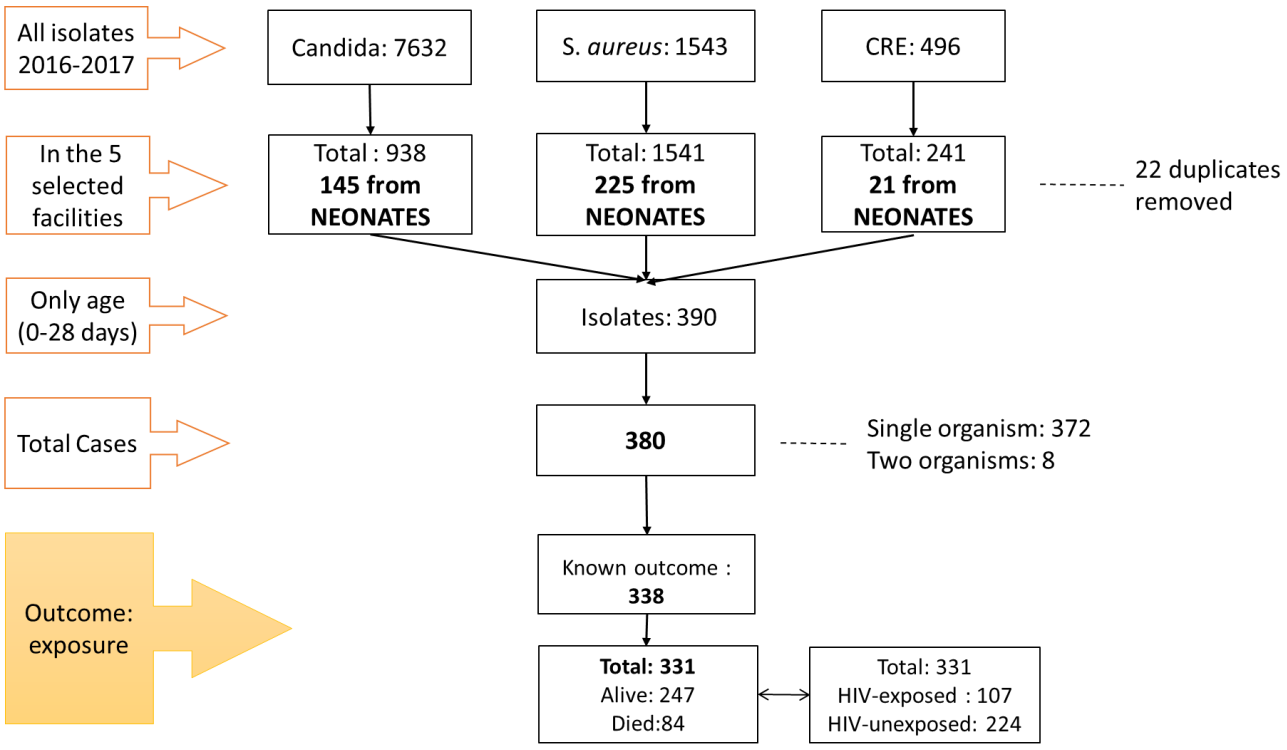

Figure 4.1.1: Flow chart of data management using the databases under the GERMS-SA. CRE-Carbapenem-resistant Enterobacterales.

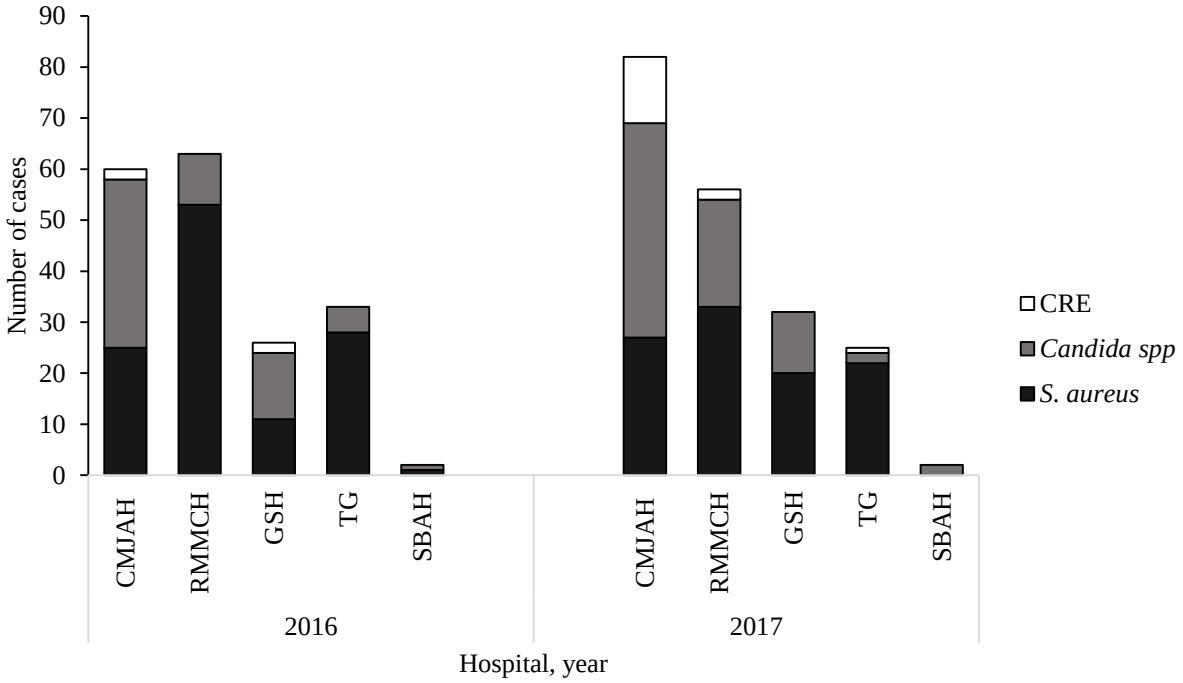

Figure 4.2.1: Total number of cases from each hospital included in the study from 2016-2017. CMJAH- Charlotte Maxeke Johannesburg Academic hospital, SBAH- Steve Biko Academic Hospital, RMMCH- Rahima Moosa Mother and Child Hospital, GSH- Groote Schuur Hospital and TH- Tygerburg Hospital (n=380).

The total number of cases from each hospital from 2016-2017 are summarised in Figure 4.21. In 2016 there were 184 cases and of these RMMCH accounted for 34.2% (n=63) followed by CMJAH (32.6%, n=60), TH (17.9%, n=33), GSH (14.1%, n=26) and lastly SBAH (<1%, n=2). In 2017, CMJAH accounted for 41.8% (82/196) followed by RMMCH (28.5%, [56/196]), GSH (16.3%, [32/196]), TH (12.8, [25/196]) and SBAH (0.01%, [2/196]). During the study period, S. *aureus* BSI accounted for the majority of cases and these were mostly from RMMCH (n=86). The majority of CRE cases (n=15) were from CMJAH. The majority of Candida cases in this study were from CMJAH (n=75).

4.2. Results showing analysis of each objective

4.2.1. Descriptive analysis of all cases

We describe 380 cases, of which 52.3% (195/373) were males. The median age (at positive blood culture) of the cases was 11 days (IQR 11-18 days) (Table 4.2.1). The percentage of HIV-exposed neonates was 33% (111/336) and those who died accounted for 25.7% (87/338). The median gestational age at birth was 29 weeks (IQR 27-32 weeks) and 26.8% (84/313) had normal birth weight (≥2.5 kg). Ninety-four percent received antimicrobial agents on current admission (324/345); 7% for CRE (21/324), 29% for *Candida* (95/324) and 64% for S. *aureus* (208/324). The first course of antibiotics was received within a median of 6 days of admission (IQR 3-13 days). The proportion of neonates with CRE infection given a combination of amoxicillin and gentamicin as first line empiric treatment was 76% (16/21). Among the cases of *Candida,* 82% (78/95) received amphotericin B and 17% (16/95) received fluconazole. Among the cases of S. *aureus,* 27% (58/208) were given meropenem, 25% (52/208) amoxicillin and 14% (19/208) vancomycin. Ninety-four percent (321/342) had an intravenous line inserted.

Table 4.2.1: Demographic and clinical characteristics of cases from the five hospitals, 2016-2017

	Variable (N*)	Frequency (n)	Percentage (%)
Demographic			
	Sex (N=373)		
	Female	178	47.7
	Male	195	52.3
	Median age (IQR), days (N=380)	11 (7-18)	
Clinical			
Main exposure	HIV-exposed (N=336)		
	No	225	67.0
	Yes	111	33.0
Outcome	Outcome status (N=338)		
	Died	87	25.7
	Alive	251	74.3
Confounders			
	Median LOS (IQR), days (N=337)	29 (15-51)	
	Median Gestational age (IQR) weeks, (N=216)	29 (27-32)	
	Birth weight (N=313)		
	<2.5 kg	229	73.2
	≥2.5kg	84	26.8
	Received ARV (N=325)		
	No	244	75.1
	Yes	81	24.9
	Antimicrobial use on current admission (N=343)		
	No	19	5.5
	Yes	324	94.5
	IV inserted (N=343)		
	No	22	6.4
	Yes	321	93.6
	CVC inserted (N=326)		
	No	213	65.3
	Yes	113	34.7
	Previous surgery (N=235)		
	No	208	88.5
	Yes	27	11.5
	Referred from another facility (N=348)		
	No	272	78.1
	Yes	76	21.8
	Organism isolated (N=380)		

	CRE	20	5.2
	Candida	142	37.4
	S. *aureus*	218	57.4

*****Total number is different for each variable due to missing data. Abbreviations: IQR=Interquartile range, LOS=Length of stay, ARV=Antiretroviral, IV=Intravenous line, CVC=Central venous catheter and CRE=Carbapenem-resistant Enterobacterales.

4.2.2. Description of antimicrobial susceptibility profile among cases with viable isolates

Of the 144 *Candida* isolates, the majority of isolates were *C. parapsilosis* (n=71) followed by *C. albicans* (n=57) and other spp. Only 60 *Candida* isolates were viable. Three were removed due to missing information on ASTs and species. Of the 57 viable isolates, 50.9% (29/57) were *C. parapsilosis* and 40.4% (23/57) were *C. albicans* (Table 4.2.2). The remaining 8.7% was distributed across C. *glabrata*, (2/57) *C. guilliermondii* (1/57) *C. krusei* (1/57) and *C. dubliniensis* (1/57). Only *C. albicans* and *C. parapsilosis* were included in the antibiogram because they accounted for most of the isolates evaluated. The proportion of fluconazole resistant *C. parapsilosis* isolates were as follows; 68.4% (13/19) at CMJAH and 15.7% (3/19) for both RMMCH and GSH. Eighty-three percent (5/6) of voriconazole resistant *C. parapsilosis* isolates were from CMJAH. All the *Candida* isolates (*C. albicans* and *C. parapsilosis*) tested against amphotericin B had an MIC of less than 2 µg/ml and were excluded from table 4.2.2. All the reported viable *C. albicans* and *C. parapsiolosis* isolates were susceptible to anidulafungin.

Table 4.2.2: *Candida* spp. antibiogram for cases with viable isolates, 2016-2017.

Antifungal agent by class	***Candida* spp.** **C. *albicans* (N=23)** **C. *parapsilosis* (N=29)**	**Susceptible n (%)**	**Intermediate n (%)**	**Resistant n (%)**
Azoles				
Fluconazole	C. *albicans*	22 (95.6)	0	1 (4.4)
	C. *parapsilosis*	10 (34.4)	0	19 (65.5)
Voriconazole	C. *albicans*	22 (95.6)	0	1 (4.3)
	C. *parapsilosis*	12 (41.4)	11 (37.9)	6 (20.6)
Polyenes				
Amphotericin B*	C. *albicans*	-	-	-
	C. *parapsilosis*	-	-	-
Echinocandins				
Anidulafungin	C. *albicans*	23 (100)	0	0
	C. *parapsilosis*	29(100)	0	0

***Epidemiologic cut-offs were reported for Amphotericin B**

There were 176 viable isolates of *S. aureus.* Of the 176 isolates of *S. aureus*, 70% (123/176) were MRSA and 30% (53/176) were MSSA (susceptible to oxacillin). Of the antibiotics listed, 100% of isolates were susceptible to vancomycin, linezolid and fosfomycin. Antibiotics with a large percentage of resistant isolates were erythromycin 71.0% (125/176), gentamicin 68.5% (121/176) and tobramycin 68.1% (120/176).

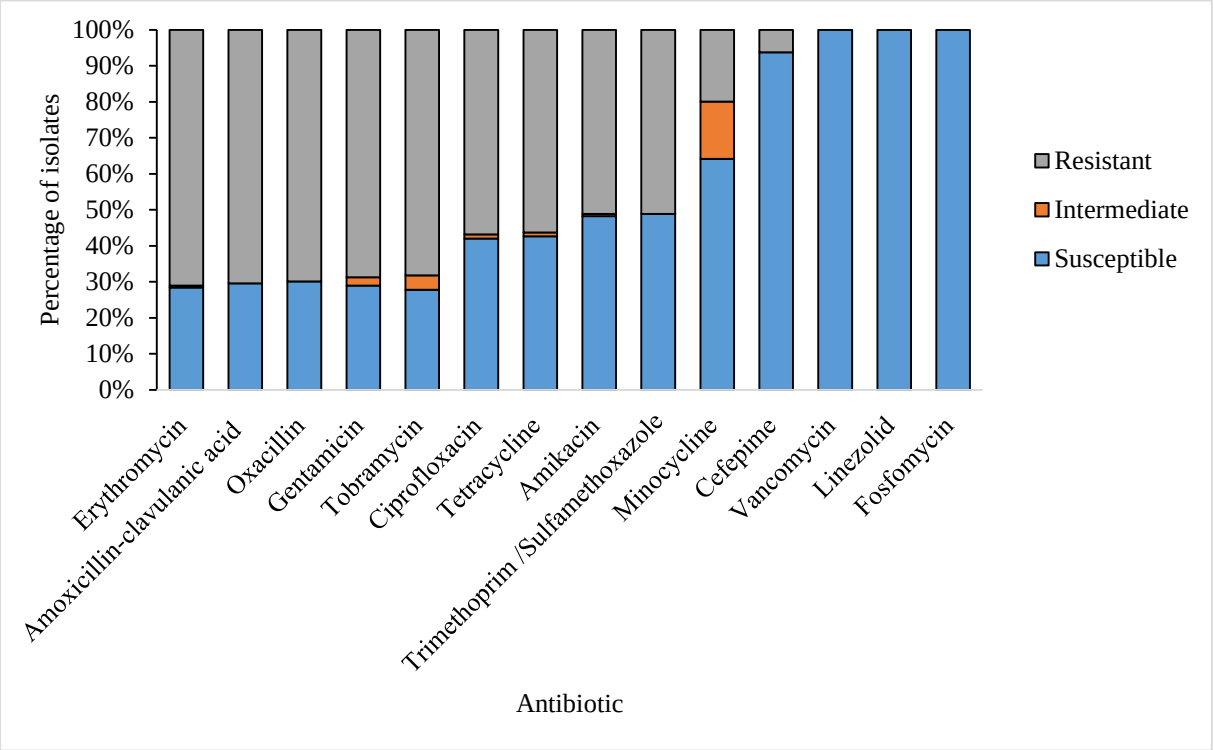

Figure 4.2.3: Antibiogram for *S. aureus* isolates (n=176) tested against the panel of antibiotics in the five hospitals, 2016-2017.

Twenty-one isolates from CRE were obtained, of which 90.4% (19/21) were identified as *K. pneumoniae*, the other 10% were *Enterobacter cloacae* complex (1/21) and *E. coli* (1/21). Of the 21 isolates of CRE identified in the database, all the isolates were resistant to at least one carbapenem. Ertapenem had the highest number 33.3% (7/21) of resistant isolates and the lowest being meropenem 14.3% (3/21). All isolates tested for colistin were susceptible and 90.4% (19/21) were susceptible to tigecycline (Table 4.2.4). All the *K. pneumoniae* isolates (n=19) were resistant to cefuroxime. The table below displays the AST profile of *K. pneumoniae* isolates.

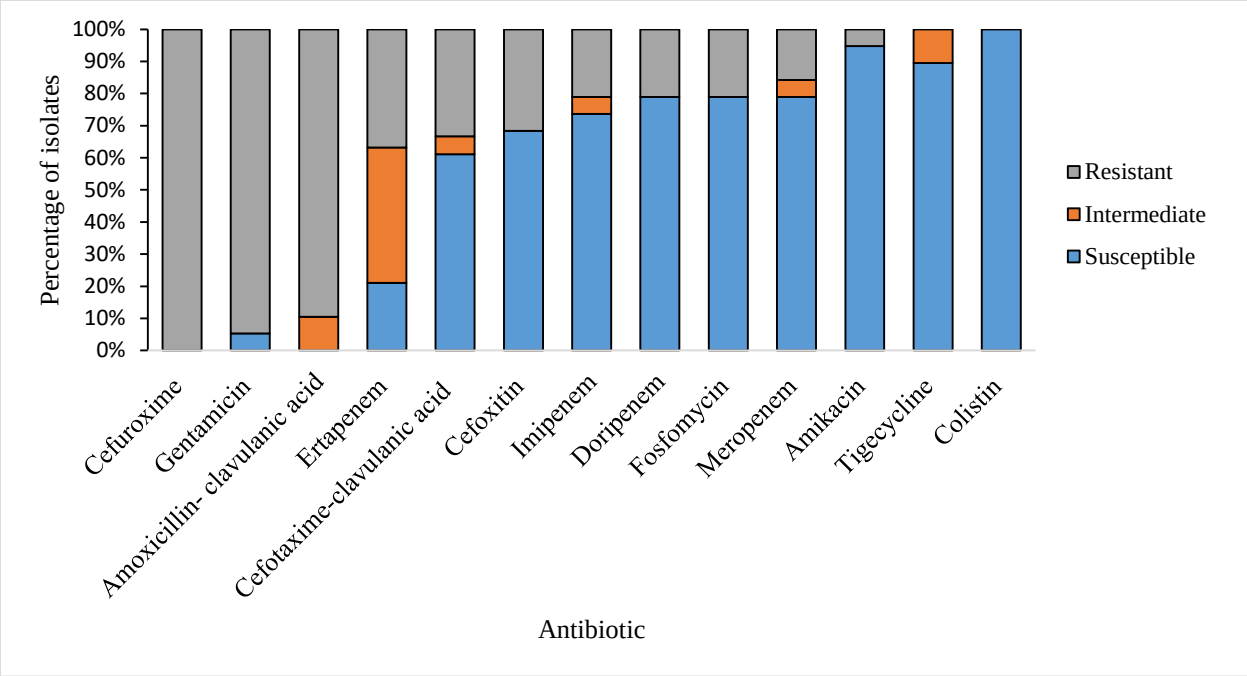

Figure 4.2.4: Antibiogram of carbapenem-resistant *K. pneumoniae* isolates (n=19) from the five hospitals, 2016-2017.

4.2.5. HIV exposure and other factors associated with mortality among cases

For this objective, only 86.8 % (330/380) of cases with a known outcome and HIV status (HIV PCR test result) were included (Table 4.2.6). The overall CFR was 25.4% (84/330; 95% CI: 0.21-0.31), and the CFR among HIV-unexposed neonates was 24.5% (55/224; 95% CI: 0.19-0.31). HIV exposed neonates had a higher CFR of 26.8% compared to HIV-unexposed neonates (29/108; 95% CI: 0.18-0.36).

The characteristics of cases included in this analysis compared with those excluded due to missing data were tabulated (Table 4.2.5). The percentage of missing data for each variable varied from 12% to 45%, with gestational age at birth having the largest percentage of missing information.

Table 4.2.5: Assessing selection bias due to missing data for each variable based on HIV exposure status and outcome (survival or death).

Variables (N= 380)**	**Included cases (n*)**	**Excluded cases (n)**	**Proportion of cases with missing data (%) (n excluded/N)**
Sex	330	48	12.7%
Age	331	47	12.4%
Birth weight	302	65	17.2%
LOS (days)	320	58	15.3%
Gestational age (weeks)	206	172	45.3%
Antimicrobial use	326	52	13.8
CVC inserted	310	68	17.9%
Referred	330	48	12.7%
Previous surgery	225	153	40.5%
Received ARV	309	69	18.3%

Abbreviations: LOS=length of stay, CVC=central venous catheter and ARV= antiretroviral. *number of cases per variable, **Total number of cases.

Table 4.2.6 shows that there is an association (though not statistically significant) between HIV exposure and mortality (24.5% mortality among HIV-exposed neonates compared to 26.8% among HIV-unexposed neonates, p=0.61). Age and referral from another facility did not show a significant association with mortality. Sex was marginally significant with a higher percentage of death among males (29.6 % males vs 20.9% females, p=0.06). A higher number of cases with CVC *in situ* died (38.3% CVC inserted vs 17.9% no CVC, p<0.01). There was a significant association between antimicrobial use and mortality, fewer cases on antimicrobials died compared to those who did not use antimicrobials (23.0 vs 68.4, p<0.01). In terms of the organism isolated, a higher proportion (39/105) of cases where *Candida* was isolated died (30% CRE (6/20) vs. 37% (39/105) *Candida* spp. vs. 19% (42/213) *S. aureus*).

Table 4.2.6: Associations of HIV (and other factors) with mortality among cases from 2016-2017(n=380).

Variable	Alive n (%)	Died n (%)	p-value
Main exposure:			
HIV status **HIV-unexposed** **HIV-exposed**	 169 (75.5) 79 (73.2)	 55 (24.5) 29 (26.8)	 0.61
Co-variates			
Age (days)	251	87	0.76
LOS (days)	239	87	<0.01
Gestational age at birth (weeks)	145	62	<0.01
Birth weight **<2.5 kg** **≥2.5 kg**	 158 (71.2) 71 (85.5)	 64 (28.8) 12 (14.5)	 0.01
Sex **Female** **Male**	 125 (79.1) 126 (70.4)	 33 (20.9) 53 (29.6)	 0.06
Received ARV/ prophylaxis **No** **Yes**	 182 (75.8) 58 (72.5)	 58 (24.2) 22 (27.5)	 0.34
Antimicrobial use **No** **Yes**	 6 (31.6) 241(77.0)	 13 (68.4) 72(23.0)	 <0.01
IV inserted **No** **Yes**	 9 (40.9) 238 (76.8)	 13 (59.1) 72 (23.2)	 <0.01
CVC inserted **No** **Yes**	 165 (82.1) 66 (61.7)	 36 (17.9) 41 (38.3)	 <0.01
Previous surgery **No** **Yes**	 162 (79.8) 17 (63.0)	 41(20.2) 10 (37.0)	 0.05
Referred from another facility **No** **Yes**	 197 (74.9) 53 (71.6)	 66 (25.1) 21 (28.4)	 0.56
Organism isolated **CRE** ***Candida*** **S. *aureus***	 14 (70.0) 66 (62.9) 171(80.3)	 6 (30.0) 39 (37.1) 42 (19.7)	 <0.01

Abbreviations: LOS=Length of stay, ARV=Antiretroviral, IV=Intravenous line, CVC=Central venous catheter and

CRE=Carbapenem-resistant Enterobacterales

Among neonates with a BSI, HIV-exposed neonates had 1.14 times increased odds of death compared to HIV-unexposed neonates (OR 1.14, 95% CI: 0.68-1.93). Neonates who stayed in the hospital longer had a 3% lower odds of death (OR 0.97, 95% CI: 0.95-0.98) compared to those with shorter stay. Neonates who had a low birth weight had 2.39 times increased odds of death compared to neonates with normal birth weight (OR 2.39, 95% CI: 1.22-4.72). For each additional week in gestational age at birth, the odds of death were 19% less (OR 0.81, 95% CI: 0.72-0.91). Among the neonates receiving antimicrobials, the odds of death were 87% less compared with neonates not receiving antimicrobials. Neonates who had a CVC inserted had 2.98 times increased odds of death compared to neonates without a CVC inserted (OR 2.98, 95% CI: 1.76-5.02).

Table 4.2.7: Bivariate analysis showing the effect size (aOR) of HIV exposure on mortality adjusting for one covariate at a time among neonates at the five hospitals from 2016-2017

Exposure	Variable adjusted for	Mortality aOR	95% CI	p-value
HIV-exposed	Sex	1.19	0.70-2.02	0.51
	Age	1.14	0.67-1.93	0.62
	LOS	1.37	0.79-2.39	0.26
	Gestational age at birth	1.08	0.57-2.05	0.80
	Birth weight	1.06	0.60-1.86	0.85
	Referred	1.16	0.69-1.97	0.57
	CVC inserted	1.10	0.63-1.93	0.72
	Antimicrobial use	1.20	0.70-2.06	0.52

Abbreviations: aOR= adjusted odds ratio, CI= confidence interval, LOS=Length of stay, ARV=Antiretroviral and CVC=Central venous catheter

Table 4.2.8: Univariate and multivariable analysis of HIV exposure and mortality adjusting for all other factors at the same time among cases in the five hospitals from 2016-2017.

Variables	**Univariate**		**Multivariable (N=198)**	
	OR (95%CI)	p-value	aOR (95%CI)	p-value
HIV status: **HIV-unexposed** **HIV-exposed**	 Ref 1.14 (0.68-1.93)	 0.62	 1.19 (0.60-2.37)	 0.62
Age (days)	1.01 (0.97-1.03)	0.87	0.99 (0.95-1.04)	0.70
LOS (days)	0.97 (0.95-0.98)	<0.01	Excluded	
Gestational age at birth (weeks)	0.81 (0.72-0.91)	<0.01	0.84 (0.74-0.96)	0.01
Birth weight: **<2.5 kg** **≥2.5 kg**	 2.39 (1.22-4.72) Ref	 0.01	 2.12 (0.21 -20.93)	 0.52
Sex: **Female** **Male**	 Ref 1.56(0.97-2.63)	 0.07	 1.42 (0.71-2.83)	 0.32
Received ARV: **No** **Yes**	 Ref 1.27 (0.71-2.26)	 0.42	 Excluded	
Antimicrobial use on current admission: **No** **Yes**	 Ref 0.14 (0.06-0.44)	 <0.01	 0.05 (0.01-0.49)	 0.01
IV line inserted: **No** **Yes**	 Ref 0.20 (0.01-0.51)	 <0.01	 Excluded	
CVC inserted: **No** **Yes**	 Ref 2.98 (1.76-5.02)	 <0.01	 2.51 (1.23-5.15)	 0.01
Previous surgery: **No** **Yes**	 Ref 2.32 (0.99-5.45)	 0.05	 Excluded	
Referred from another facility: **No** **Yes**	 Ref 1.18 (0.66-2.11)	 0.57	 Excluded	
Stratum specific OR for interaction **Among <2.5kg** **Among ≥2.5k.g**	 0.91 (0.49-1.67) 2.42 (0.63-9.35)	 0.76 0.20	 1.12 (0.55-2.28) 4.01(0.14-113.7)	 0.75 0.42

Abbreviations: OR=odds ratio, CI= confidence interval, aOR=adjusted odds ratio, LOS=Length of stay, ARV=Antiretroviral, IV=Intravenous line and CVC=Central venous catheter. Due to a strong correlation (r=0.77) between HIV exposure and receipt of ARV, receipt of ARV was not added to the final model even though it was identified as a potential confounder. Additionally, a strong correlation existed between IV line insertion and antimicrobial use (r=0.93).Therefore, IV insertion was not included in the final model. LOS was removed from the final model as it was calculated in a similar way to age

Table 4.2.7 shows the change in the HIV-exposed effect when one variable was adjusted at a time. Adjusting for sex, HIV-exposed neonates had 19.0% increased odds of death compared to HIV-unexposed neonates (aOR 1.19, 95% CI: 0.70-2.02). Adjusting for LOS, the effect changed to 37.0% increased odds of death for HIV-exposed neonates compared to HIV-unexposed (aOR 1.37, 95% CI: 0.79-2.39). HIV-exposed neonates had 20.0%-increased odds of death compared to HIV-unexposed after adjusting for antimicrobial use. After adjusting for birth weight and gestational age, HIV exposed neonates had a 8.0% and 6.0% increased odds of death respectively compared to HIV-unexposed neonates (birth weight aOR 1.06, 95% CI: 0.60-1.86 and gestational age aOR 1.08, 95% CI: 0.57-2.05). When the interaction term between HIV exposure and birth weight was added to the model, the adjusted odds of death were 2.41 times higher for HIV-exposed neonates compared to HIV-unexposed neonates.

The variables included with HIV status in the final model were gestational age at birth, age, sex, birth weight, CVC inserted, use of antimicrobials on current admission and the interaction term between HIV-exposed and birthweight (Table 4.2.8). The adjusted odds of HIV-exposed neonates were 1.19 (95% CI: 0.60-2.37) in the presence of other factors excluding the interaction term. Among neonates with birthweight ≥2.5 kg, the adjusted OR for death was 4.01 (95%CI: 0.14-113.86) among HIV-exposed when compared to HIV-unexposed neonates. Among neonates with a low birth weight (<2.5 kg), the stratum-specific OR for the effect of HIV exposure on mortality was 1.12 (95%CI: 0.55-2.28).

CHAPTER 5: Discussion

In this chapter, we start by highlighting some important findings including the association of HIV exposure to in-hospital mortality and summarizing other key findings. We then discuss the pathogens and their AST results. We then move on to discuss the case fatality ratio and the influence of other factors on mortality. We also discuss the strengths in terms of how our findings fit into the existing knowledge of the subject. We close the chapter by highlighting the limitations of the study.

5.1. HIV exposure effect

HIV exposure was associated with mortality in our study though the 95%CI includes one; this could be due to several data limitations i.e., missing information and sample size. Theoretically, neonates exposed to HIV are born with other factors that increase the risk of mortality such as prematurity and low birth weight. This has been demonstrated in various studies that showed that neonates born to HIV-infected mothers are generally born prematurely or with low birth weight, Additionally, theses neonates were shown to have higher mortality mortality in the first year of life. In our unadjusted analysis, HIV-exposed neonates had a 14% higher crude odds of death than those HIV-unexposed (95%CI: 0.68-1.93; p=0.62). The adjusted odds ratio for death among HIV-exposed neonates was 1.19 (95% CI: 0.60-2.37; p=0.62) compared to HIV-unexposed neonates which was not very different from the crude.

5.2. Pathogens and ASTs

In this study from 2016-2017, there were 144 cases infected with *Candida* spp and a large number (n=76) was from CMJAH. Of the 144, 5.6% (8/144) were co-infected with bacterial pathogens (7 with *S. aureus* and 1 with CRE). The predominant *Candida* isolates were *C. parapsilosis* followed by *C. albicans*. Our results are consistent with published studies which indicated that *C. albicans* and *C. parapsilosis* are the most frequent causes of candidaemia among neonates.[27,30] Of the 29 isolates of C. *parapsilosis*, 65.5% (n=19) were resistant to fluconazole. This is consistent with results from a multicenter survey of candidaemia in South Africa which reported that in the public sector, the vast majority of cases of fungaemia are caused by a fluconazole-resistant *C. parapsilosis* isolates among neonates (90/142, 63.3%).[30]

Among *S. aureus* BSI cases with viable isolates, 69% (123/176) were MRSA, consistent with previously studies reporting MRSA as a predominant pathogen among neonates.[32] We could not establish if neonates with MRSA were given appropriate antibiotic treatment, as there was no way to link infection results with AST results. Neonates with *S. aureus* infection were given first-line treatment of amoxicillin and gentamicin and the AST results show high proportions of resistance to gentamicin and amoxicillin-clavulanic acid. MRSA has been reported to spread horizontally through contact with a healthcare worker. This might indicate a breach in IPC measures during the study period.[32,35]

CREs accounted for the least number (<10%) of cases in this study. *K. pneumoniae* has been reported as the most prevalent CRE among neonates with BSI and in our study, *K. pneumoniae* accounted for 90% (19/21) of the CRE isolates.[23] More than 90% were susceptible to tigecycline and colistin, considered effective therapy against CRE.[89]

5.3. Case fatality and the influence of other factors on mortality

The overall case fatality ratio in our study was 25% (95% CI: 0.19-0.29), which was higher than the previously reported case fatality of 15% (95% CI not reported) in a similar South African study.[46] Case fatality among HIV-exposed neonates was higher (27%) than the HIV-unexposed neonates (25%), similar to proportions reported in a study from Botswana.[52]

HIV exposure was associated with a 14% higher mortality among neonates with pathogen specific HABSI. After adjustment, the odds ratio for death among HIV-exposed neonates was 1.19 (95% CI: 0.60-2.37) compared to HIV-unexposed neonates. The effect of HIV exposure on mortality was not the same for neonates with low and normal birthweight with a stronger effect of HIV exposure on mortality among babies with a normal birthweight. Among normal birthweight neonates, the adjusted OR for death was 4.01 (95%CI: 0.14-113.86, p=0.42) among HIV-exposed versus HIV-unexposed neonates. The synergism between birthweight and HIV-exposure is a clinically important finding. Overall we expected HIV exposure to be a significant risk factor for death among these neonates as different studies have demonstrated.[45,47,52] We did not have sufficient evidence to conclude against our hypothesis that the risk of death is higher among HIV-exposed neonates. However, our study is subject to limitations that may have resulted in our results.

5.4. Strengths of the study

Our study adds to the current body of knowledge regarding hospitalized HIV-exposed neonates. This was one of the few multicentre studies reporting on pathogen-specific HABSI in South Africa. Additionally, our study focused only on neonates, which provides further evidence for the factors associated with death to clinicians dealing with BSIs from these pathogens. The hospitals used in this study can benefit from the AST information and reported risk factors to help guide IPC and antimicrobial stewardship. For future studies, more hospitals can be included over a longer and more recent period, as we have demonstrated that pathogens and their ASTs are different in neonatal units. Further studies can also look at additional maternal variables such as mother's viral load and CD4 count which are important predictors of mortality in HIV-exposed uninfected infants[69]. Further studies are required to assess the association of HIV exposure and these factors with neonatal death.

5.5. Limitations of the study

The study sample size was not large enough to detect a statistically significant difference in mortality among HIV-exposed neonates compared with HIV-unexposed neonates. The study used secondary surveillance data therefore the results are subject to issues related to incomplete information that was collected during medical chart review. Non-differential misclassification might have resulted from the inaccurate definition based on assumptions of HIV-exposed neonates because of missing maternal and neonate HIV status from the surveillance data. This misclassification might have resulted in underestimating the effect of HIV exposure on mortality. There were also missing data on important maternal variables relevant to our analysis i.e., viral load and CD4 count. Generalizability of our results is limited to the hospitals and pathogens we selected for this study, however, this provided insights into the pathogen specific AST profiles in these hospitals from 2016-2017. The study also sheds light on factors associated with mortality in the neonatal population of some of the biggest hospitals in South Africa.

CHAPTER 6: Conclusions and recommendations

In this chapter, we conclude by answering the research question and then summarize the most important findings of our research. We also highlight study limitations and the focus of future studies based on the findings. Finally, we provide a few recommendations to consider for future studies of this nature.

HIV exposure was a risk factor for death among neonates with pathogen-specific BSI namely CRE, *Candida* spp. and *S. aureus*. Based on the analysis, HIV-exposed neonates with a BSI had 19% higher adjusted odds of death compared to HIV-unexposed neonates, although the 95%CI spanned one. The effect of HIV exposure on mortality was different among neonates with low birth weight compared to those with normal birth weight. Our sample size may not have been sufficiently large and missing data may have resulted in selection bias, although it provided insights to the pathogens and AST profiles in the five selected hospitals. Future studies are still required to further explore these associations.

Recommendations include the following:

- A prospective cohort design for analysis of 30-day in-hospital mortality from the time of admission
- Selection of more facilities for inclusion
- Inclusion of more pathogens causing HABSI
- Inclusion of maternal variables i.e. maternal HIV status for all, viral load and CD4 count
- Medical chart review of missing data or a much more complete dataset be used
- Multiple imputation methods to account for data missing at random

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CHAPTER 8: Appendices

Appendix A: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Ms T Zwane

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M1911195

NAME: (Principal Investigator)	Ms T Zwane
DEPARTMENT:	School of Public Health Division of Epidemiology and Biostatistics Medical School University
PROJECT TITLE:	HIV exposure as a risk factor for death amongst neonates with healthcare-associated bloodstream infections in South Africa, 2016-2017
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M1809107
SUPERVISOR:	Professor N Govender
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/01/16

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

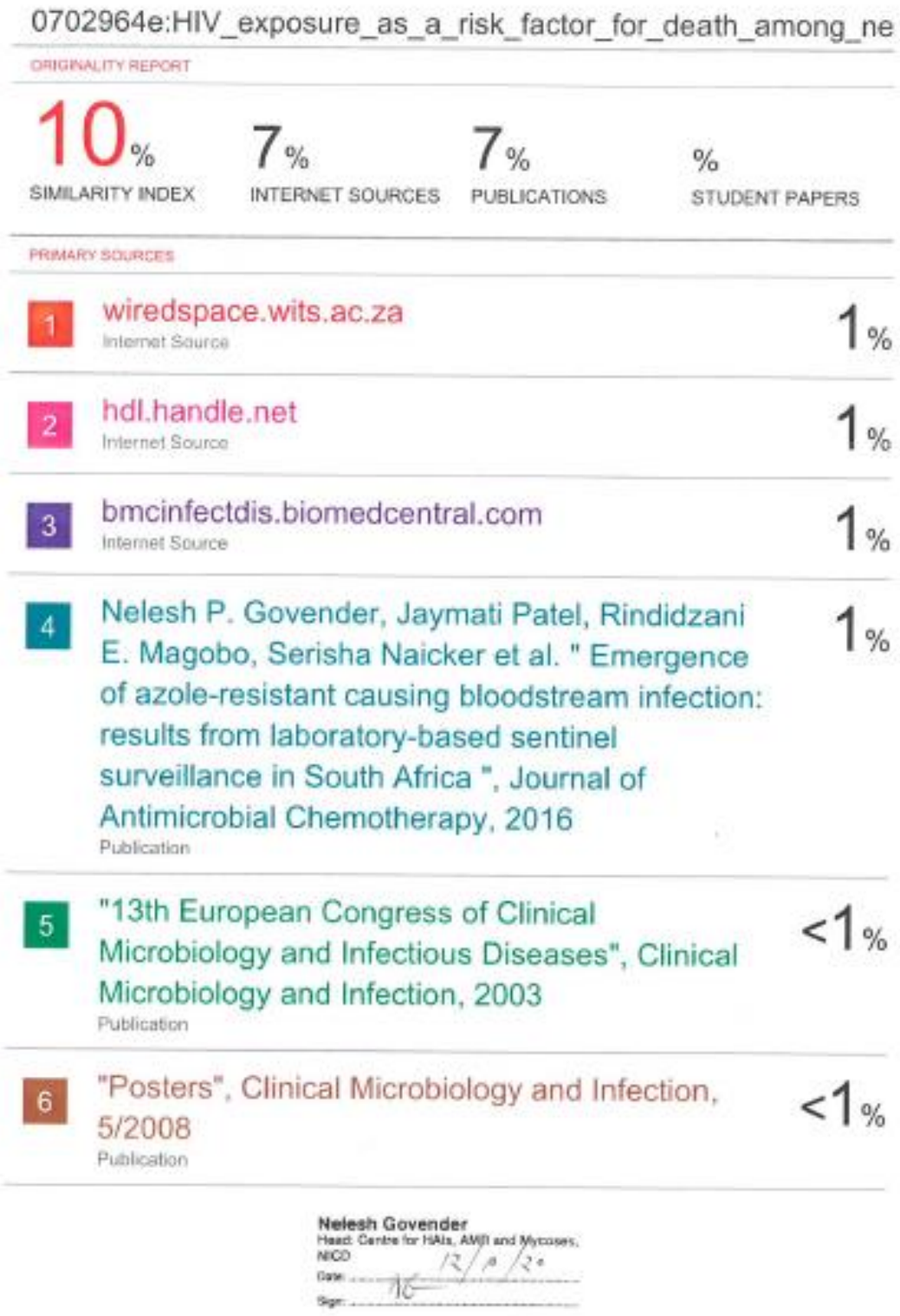
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **November** and will therefore reports and re-certification will be due early in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

Appendix B: Turnitin report



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PRIMARY SOURCES

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2	hdl.handle.net Internet Source	1%
3	bmcinfectdis.biomedcentral.com Internet Source	1%
4	Nelesh P. Govender, Jaymati Patel, Rindidzani E. Magobo, Serisha Naicker et al. " Emergence of azole-resistant causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa ", Journal of Antimicrobial Chemotherapy, 2016 Publication	1%
5	"13th European Congress of Clinical Microbiology and Infectious Diseases", Clinical Microbiology and Infection, 2003 Publication	<1%
6	"Posters", Clinical Microbiology and Infection, 5/2008 Publication	<1%

Nelesh Govender
Head: Centre for HAIs, AMR and Mycoses, NICD
Date: 12/8/20
Sign: