HIV-exposure as a risk factor for mortality among neonates with culture-confirmed bloodstream infection and meningitis in South Africa, 2019-2020.

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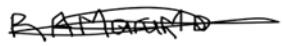
Declaration

I, Andani Ronel Marumo, declare that this research report is my original unassisted work. I am submitting this work for the Master of Science in Field Epidemiology degree at the University of the Witwatersrand, Johannesburg, South Africa. The research report has not been submitted before for any degree or examination at any other University.



(Candidate Signature)

I declare this on the 16 day of August 2024 in Johannesburg, South Africa.

Dedication

I dedicate this research report to my beautiful parents for their unwavering support, encouragement and always believing in the vision I have for myself, and to myself for not giving up and showing up even in the days I didn’t have strength.

Abstract

Background: HIV-exposed but uninfected neonates (HEU) are a growing population. Exposure to HIV has been associated with increased mortality and morbidity. We aimed to determine the effect of HIV-exposure as a risk factor for mortality in neonates admitted with bloodstream infections (BSIs) and/or meningitis at non-academic hospitals in South Africa.

Methods: We conducted a retrospective cohort study using data from the Baby GERMS-SA surveillance project of hospitalised neonates with culture-confirmed BSI and meningitis at six non-academic hospitals in South Africa from October 2019 to September 2020. A multivariable Cox proportional hazards regression was used to determine the effect of HIV-exposure regardless of HIV-status as a risk factor for mortality. We further examined the effect of HIV-exposure using a multivariable logistics regression.

Results: Of 697 neonates with a known maternal HIV status and in-hospital outcome, 34% (239/697) were exposed to HIV and 1% (4/239) were HIV PCR-positive. The HEU neonates had significant low gestational age (77% (184/239) vs. 66% (296/458), p=0.001) and very low birth weight (48% (115/239) vs. 40% (184/458), p=0.016) compared to HIV-unexposed uninfected (HUU) neonates. Exclusive breastfeeding was more common in HUU neonates (44% (202/458) vs. 32% (77/239)). We did not observe significant differences in age at the time of infection (median age 6 vs. 6 days p=0.14), and duration of hospitalisation (median length of 17 vs. 15 days p=0.12) between the HEU and HUU neonates. The crude in-hospital mortality among HIV-exposed neonates and HUU neonates was 26% (63/239) and 23% (104/458), respectively. After adjusting for relevant confounders such as birth weight, timing of infection, use of invasive devices, breastfeeding, and maternal age, there was no difference in the risk for mortality between HEU neonates and those who were HUU (HR 1.12, 95% CI: 0.76-1.67, p=0.549) at 28-days. The odds of mortality were 1.21 (95% CI 0.72–2.05,

p=0.467) times more among HEU neonates than among HUU neonates in the exploratory analysis.

Conclusions: We did not find a difference in mortality between HEU and HUU neonates with culture-confirmed invasive infections in our study. Regardless of their HIV exposure status, approximately a quarter of these neonates died in hospital.

Keywords: Neonates, HIV-exposure, bloodstream infections, meningitis, mortality, South Africa

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Ms. Rudzani Mashau

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Nomenclature/abbreviations

AHR	Adjusted hazard ratios
ART	Antiretroviral therapy
AMR	Antimicrobial resistance
BSI	Bloodstream infection
CoNS	Coagulase-negative staphylococci
EOS	Early onset sepsis
GBS	Group B *Streptococcus*
HEU	HIV-exposed but uninfected
HIV	Human Immunodeficiency Virus
HICs	High-income countries
HR	Hazard ratio
LOS	Late-onset sepsis
LMICs	Low and middle-income countries
SA	South Africa
UNAIDS	United Nations Program on HIV/AIDS
WHO	World Health Organization

Definitions

Variable	Definitions
Neonate	Newborn <28 days inclusive (first four weeks of birth)
Infant	A child under the age of 1 year; more specifically, newborn baby
BSI or meningitis	Isolation of pathogenic bacteria or fungi in culture from a blood or CSF specimen in a neonate
Early onset sepsis (EOS)	BSI/meningitis occurring within the first 3 days of life (0–2 days)
Late-onset sepsis (LOS)	BSI/meningitis occurring after 3 days of life (3–27 days)
HIV-exposed neonate	Neonates born to mothers with HIV (regardless of the HIV-infection status of the neonate)
Timing of infection	Time from birth date to date of specimen collection for a positive blood or CSF culture
Outcome	Death on or before 28 days after birth
Birth weight	Categorized into three groups: • Very low birthweight:<1500 grams • Low birthweight: 1500-2499 grams • Normal birthweight: >=2500 grams
Gestational age category	Categorized into four groups: • Premature: gestational age (GA) <37 weeks • Moderately premature: GA of 32-36 weeks • Very premature: GA of 28-31 weeks • Extremely premature: GA of <28 weeks

Chapter 1: Introduction

This chapter provides a detailed background and in-depth literature review on the role of HIV-exposure in neonates with and without laboratory-confirmed bloodstream infection and or meningitis and the impact of HIV exposure and known confounding risk factors for mortality. The gap of the study area, problem statement, and justification for our study are outlined along with the hypothesis for the research. This chapter concludes with the aims and objectives of this research analysis.

Background

Since 2010, new HIV infections in children under 15 years declined substantially by 58% globally between 2010 to 2022 from 310 00 (210 000- 490 000) to 130 000 (90 000-210 000), respectively.[1] In 2021, approximately 270 000 children between 0 and 14 years were living with HIV in South Africa (SA), while an estimated 12 000 had been newly infected according to UNAIDS estimates.[2] Despite a substantial decline in HIV-infections, the number of neonates who are HIV-exposed but uninfected (HEU) is rising even with marked success of prevention of vertical transmission interventions offered to pregnant women prenatally and postnatally.[3] In 2018, approximately 14.8 million children globally were HEU, and a majority (13.2 million) were reported from Africa and 23.5% (3.5 million) were estimated from South Africa.[4]

HIV exposure may be a risk factor for mortality in neonates with culture-confirmed neonatal infections. Several single studies from low and middle income countries (LMICs) have shown results of higher infectious morbidity and mortality risk in HEU neonates whether or not they remain HIV uninfected after delivery. [5,6] Moreover, HEU neonates were more likely to be born prematurely (defined as birth at maternal gestation of <37 weeks) and of low birth weight (birthweight <2500g). [7] Low birthweight and prematurity in turn may be significant

risk factors for infant mortality, morbidity, and other adverse outcomes, such as neurodevelopmental issues.[8]

The increased mortality and morbidity in HEU neonates could be explained by several risk factors that are unique to children born to mothers with HIV. These include exposure to HIV-affected in utero environment, maternal compromised immune system, reduced CD4+ T cells and exposure to ART thus increasing the risk of neonatal sepsis-related mortality.[7] According to previous reports, neonates born after April 1994 (ART prophylaxis era) had a higher risk of serious infections in comparison to neonates born in the pre-ART prophylaxis era.[6] With the various exposures linked to HIV-exposure and the growing population of HEU neonates, research in this field is required to determine the effect of HIV-exposure in neonates on the risk of mortality in South African non-academic hospitals over 12 months.

Literature review

HIV epidemic

The HIV/AIDS pandemic has been an ongoing public health issue since the 1990s in South Africa (SA).[3] In SA, the number of persons living with HIV rose from 3.68 million in 2002 to an estimated 8.45 million by 2022.[3] When the HIV epidemic occurred in the 1980s and 1990s, infant mortality rates increased considerably in some of the most badly affected areas in Southern Africa particularly SA, Zambia, Zimbabwe and others, because vertical transmission became a significant problem.[9] There are presently numerous ART regimens that have been scientifically demonstrated to lower HIV transmission including during breast-feeding to rates as low as 1%.[9] South Africa has adopted the WHO's recommended guidelines on ART for children, including prevention of vertical transmission aimed at ensuring that all HIV-seropositive pregnant and breastfeeding women should receive ART regardless of the clinical stage and/or CD4 count.[7] The overall national antenatal HIV prevalence in 2019 was approximately 30% (95% CI: 29– 31%).[10] The antenatal HIV sentinel survey (ANCHSS) found that 97% of pregnant women were well informed about their HIV status while 96% were already on ART demonstrating the success of this component of the ART programme in SA.[10] Despite significant challenges to comprehensive prevention of vertical transmission coverage in LMICs, we have witnessed over the last decade the amazing achievement of millions of neonates born without HIV infection who would otherwise have been infected through their mothers.[11] Some recognized challenges in the effort to reduce new infections to 0% include delayed diagnostic of HIV testing and initiation of treatment in neonates, inadequate adherence to antiretroviral therapy and poor linkage between mothers and access to healthcare facilities.[12]

HIV-exposed but uninfected population

The number of HIV infections in neonates is decreasing, but the population of HEU neonates is increasing. [13] The number of children exposed to maternal HIV during perinatal and postnatal periods who remain uninfected, known as HEU neonates, is increasing.[11] In 2018, there were approximately 14.8 million HEU children in the world, with 30% of these reported from the Southern African region (3.5 million (23.8%) reported in SA alone).[4,11] Children born to mothers with HIV, particularly in LMICs, are at greater risk of morbidity and mortality, mostly due to infectious diseases.[11] HEU neonates are exposed to a compromised maternal immune system, maternal infectious diseases and maternal ART. [8] HEU children receive reduced amounts of trans placental antibodies from the mother, and the lack of breastfeeding in this group may exacerbate the deficit in humoral immunity and increase their susceptibility to infection.[14] However, according to the WHO recommendations, "In settings where national health authorities are recommending breastfeeding for mothers with HIV: Mothers known to be HIV-infected (and whose infants are HIV uninfected or of unknown HIV status) should exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breast feeding."[15]

HIV-exposure and risk of infection

In 2020, approximately 2.4 million neonates died globally, with prematurity, intrapartum-related complications and infections including sepsis being among the top contributing causes of neonatal mortality. [16] The African continent reported the highest burden of neonatal mortality, with 27 deaths per 1000 live births in 2020 while SA neonatal mortality was 10.57 per 1000 live births in 2022. [16,17] [18] [19] Severe sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection"; clinical evidence of

dysfunction of organs and cultures of sterile specimens are the gold standard for sepsis diagnosis, though only some infections are culture-positive. [19,20] A bloodstream infection (BSI) refers to the presence of a pathogenic organism in the blood that is causing sepsis, and a sub-set of BSIs can be diagnosed by a positive blood culture (a culture-confirmed BSI).

Neonatal sepsis can be classified into two groups; early-onset sepsis (EOS) and late-onset sepsis (LOS).[20] "EOS is defined as occurring within the first three days of life (0-2 days), while LOS is defined as occurring beyond day three (3-27 days)".[20] Predominance of invasive streptococcal infections, particularly GBS and *Streptococcus pneumoniae* has been reported in HEU compared to HIV unexposed neonates and beyond the neonatal period. [21,22] A hospital-based surveillance study in SA found an increased incidence risk of GBS EOS in infants (0-90 days) exposed to HIV in comparison to the HUU infants (2.10 vs. 1.24 cases per 1000 live births), and these infants did not differ in terms of birthweight, prematurity and mode of delivery. [23] Furthermore, HEU newborns had an overall greater risk of EOS and LOS in comparison to HUU newborns, suggesting that risk factors other than those linked to peripartum EOS (vertical bacterial transmission) play a role in the high susceptibility of invasive infections in HEU neonates. [23]

HEU children in the United States had more than twice the rate of first hospitalisation (8.27 compared to 3.67 per 1000 person-months) and first hospitalisation with an infectious disease diagnosis including sepsis (5.8 compared to 2.51 per 1000 person-months) compared to HUU children within the first two years of life. [14] HEU children also had rates of first hospitalization that were roughly 2-fold higher (incidence rate ratios (IRR): 2.25 (95% CI, 2.01-2.52)) and infection-related hospitalization (IRR: 2.31 (95% CI, 2.02–2.64)) hospitalisation, compared with HUU children. [14,24] A Zambian study by Kabwe *et al* found maternal HIV infection to have an association with a decreased risk of neonates contracting bloodstream infections (OR: 0.46; 95% CI 0.23–0.93 and p = 0.029). [25] HEU children

including neonates and infants seem to have more severe sepsis (that is when the infection causes damage to the organs) and other infections such as respiratory tract infections and an increased risk of impaired growth and development, treatment failure, especially in pneumonia and higher rates of surgical complications in those who require surgery. [6,21,26] HIV exposure greatly increases the likelihood of presenting with cyanosis, splenomegaly, hepatomegaly, or distension in neonates. [25] The pathogens causing pneumonia infections among HEU neonates are diverse and include *Pneumocystis jirovecii, Aspergillus* spp*, Pseudomonas aeruginosa* and *Escherichia coli.* [7,30]

Association between HIV-exposure and low birthweight

Low birthweight, intrauterine growth restriction, and prematurity all have a significant association with mortality and morbidity during the neonatal period as well as through the first year of life. [27] HIV-seropositive pregnant women on highly active ART may have a much higher risk (13–34%) of giving birth to a preterm child compared to HIV-negative pregnant women.[27] Xiao et al. reported in a meta-analysis that maternal HIV infection increases the risk of both low birthweight and prematurity and this association was not affected by the use of ART. [8] A propensity score matching study conducted in China in 2023 demonstrated the incidence rate of low birthweight and preterm birth to be 13.51% and 14.17% respectively in children born to mothers with HIV compared to 6.82% and 4.65% in the mothers who were HIV-seronegative. [28] In addition to infants exposed to HIV being at higher risk of being premature, having a lower birth weight, they were born to older women and these are in turn risk factors for mortality. [29] Recent research from Africa also shows that infants exposed to HIV have lower birthweights and slower growth rates than HUU neonates. [27,29] Although HEU neonates do not develop as poorly as HIV-infected neonates, they still have slower growth and higher rates of early mortality. [27]

Mortality of HEU versus HUU neonates

A retrospective review study conducted at an academic hospital in Johannesburg SA reported that exposure to HIV (25.6% of 181 were exposed), among other risk factors such as mechanical ventilation and birthweight, did not have an effect on the risk of mortality in neonates with BSI. [30] They found shock and necrotizing enterocolitis to be the main risk factors associated with sepsis-related death and this suggested that only neonates with severe illness regardless of HIV exposure die from sepsis.[30] A recent retrospective study of 506 neonates with sepsis at the same hospital found that HIV-exposure increased rates of sepsis which require intensive care/ respiratory support in HEU neonates (19.8% compared to 14.2% neonatal sepsis in HUU, p<0.001), mostly LOS (OR: 1.44 (95% CI 1.15–1.79).[7] They further indicated that HIV-exposure was associated with low birthweight and low gestational age (p<0.001).[7]

A study conducted in Zambia found similar results where HEU and HUU neonates with sepsis had no significant difference in mortality, but HEU infants were older than unexposed (3 days compared to 2 days; p=0.029) with lower mean birthweight (2.1 vs 2.7 kg, p=0.003).[25] Brennan *et al.* found a consistent increased risk of mortality for HEU group compared to HUU children.[31] In comparison to HUU children, HEU children had an all-cause mortality that was 70% higher (risk ratio (RR): 1.70; 95% CI: 1.30- 2.22) according to the summary measure of association. [31] Furthermore, "stratification by time results showed that HEU children had a statistically significant 60 - 70% increased risk of mortality at every age stratum (from birth to12 months – RR: 1.78, 95%CI: 1.14–2.78; 12 - 24 months RR: 1.58, 95% CI:1.10–2.27 and >24 months RR: 1.70, 95%CI:1.12–2.57)". [31]

Conceptual Framework

A representation of the relationship between the different factors/variables with HIV as the main exposure and in-hospital mortality as the outcome of interest in neonates with invasive infections (**Figure 1**). This was created to summarize the associations and findings from the literature review conducted.

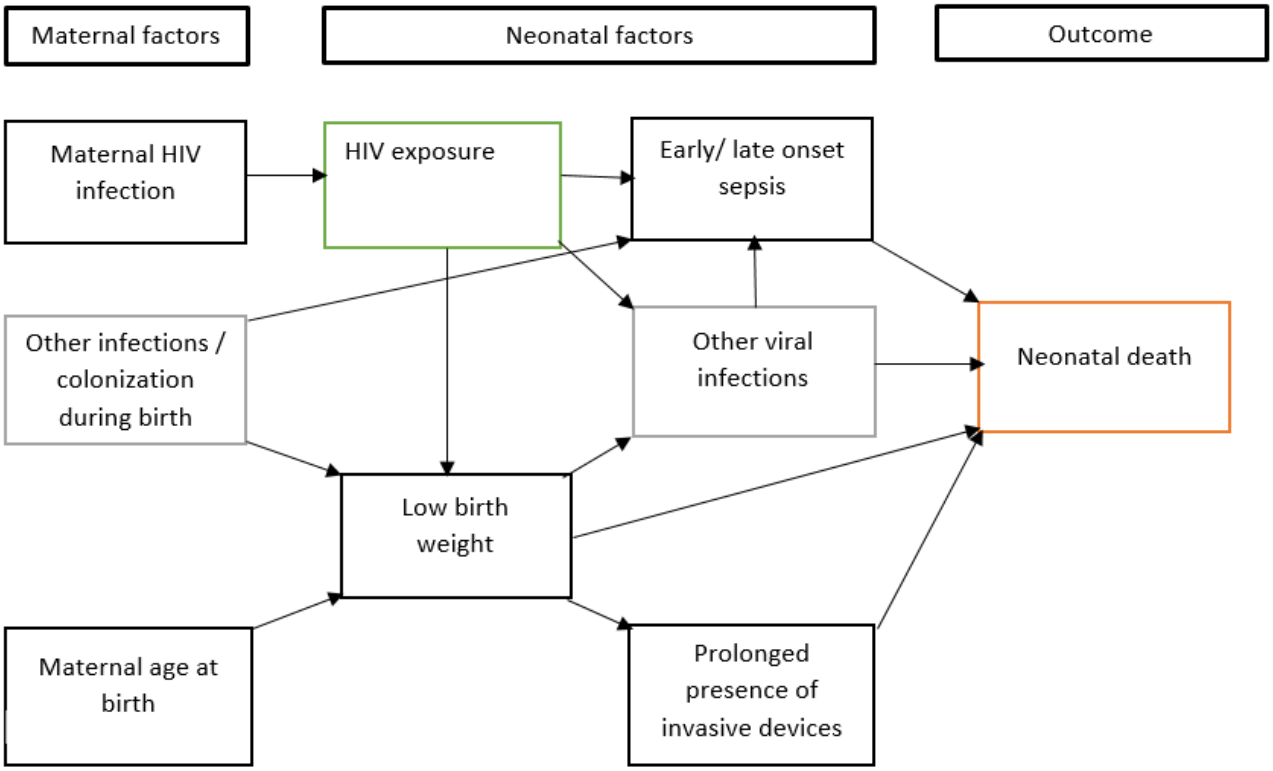

Figure 1: Conceptual framework of findings from previous studies.

Footnote: Green box: Main exposure; Orange box: Outcome; Grey box: Unmeasured variables; Black box: Measured variables/potential confounders

Problem statement

There is limited research on the effect of HIV-exposure among neonates with healthcare-associated bloodstream infection or meningitis. Previous studies have reported differing results about the association between maternal HIV infection, birthweight or gestation and mortality. These have also resulted in inconsistences with regards to the difference in mortality between the HEU and HUU neonates. Moreover, most of these findings are from HICs with low HIV prevalence and those conducted in South Africa were single studies conducted in academic/tertiary hospitals. Subsequently, there is limited research in neonates who acquire BSIs and meningitis in healthcare facilities and in settings with high rates of maternal HIV infection at non-academic hospitals including SA. We therefore have identified a gap in research of HIV-exposure in LMICs at non-academic hospitals particularly in neonates with BSIs and meningitis.

Justification

Literature has highlighted that HEU neonates are at heightened risk of morbidity and mortality as a result of risk factors such as low birthweight, maternal infections and maternal immunocompromised system.[8,32,33] SA has one of the highest HIV prevalence rates globally, making it imperative to understand this group of HEU neonates who have existing risk factors (BSIs and/or meningitis) for mortality. South Africa has one of the largest preventions of vertical transmission programme. It is the combination of these factors that makes it possible to have a significant number of HEU neonates. Findings from this study would highlight the need for follow–up and specialized care programs to HEU neonates, which may help further reduce neonatal morbidity and mortality among HEU children.

Research question.

Is HIV-exposure amongst neonates with culture-confirmed BSI and meningitis a risk factor for mortality in non-academic hospitals in South Africa between 2019 and 2020?

Hypothesis

We hypothesise that HIV-exposure increases the risk of mortality in HEU neonates. Despite several studies done to find associations between HIV exposure and increased risk of infectious-related mortality in neonates, their results are inconsistent.

Study aim.

The study aimed to examine the effects of HIV-exposure as a risk for mortality among neonates with culture-confirmed bloodstream infections and meningitis in South Africa between 2019 and 2020.

Objectives:

1. To describe clinical and demographic characteristics of neonates with culture-confirmed BSI and meningitis in six sentinel sites in South Africa, 2019-2020.
2. To compare bacterial and fungal pathogen distribution in HIV-exposed and HIV-unexposed neonates with culture-confirmed BSI and meningitis
3. To determine the effects of HIV-exposure as a risk for mortality in neonates with culture-confirmed BSI and meningitis

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Chapter 2: Manuscript

This chapter presents the completed draft manuscript forming part of the submissible research report for examination. Manuscript was drafted with Public Health Bulletin of South Africa as the target journal. The guidelines of the journal can be accessed here: https://www.phbsa.ac.za/guidelines/ . This chapter includes an abstract which is a summary of Chapter 2, and introduction of the research area, the objectives of the study, the materials and methods used, results, discussion of findings and conclusions. Unlike Chapter 1, Chapter does not provide in-depth information on the background. This chapter shows the results and discusses the study findings in detail.

HIV-exposure as a risk factor for mortality among neonates with culture-confirmed bloodstream infection and meningitis in South Africa, 2019-2020.

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Abstract

Background: HIV-exposed but uninfected neonates (HEU) are a growing population. Exposure to HIV has been associated with increased morbidity and mortality. We aimed to determine the effect of HIV-exposure as a risk factor for mortality in neonates admitted with bloodstream infections (BSIs) and/or meningitis at non-academic hospitals in South Africa.

Methods: A retrospective cohort study was conducted using data from the Baby GERMS-SA surveillance project of hospitalised neonates with culture-confirmed BSI and meningitis at six non-academic hospitals in South Africa from October 2019 to September 2020. A multivariable Cox proportional hazards model was used to determine the effect of HIV-exposure as a risk factor for mortality.

Results: Of 697 neonates with known maternal HIV status and in-hospital outcome, 34% (239/697) were exposed to HIV and 1% (4/239) were HIV PCR-positive. The HEU neonates had significantly lower gestational age (77% (184/239) vs. 66% (296/458), p=0.001) and very low birth weight (48% (115/239) vs. 40% (184/458), p=0.016) compared to HUU neonates. Exclusive breastfeeding was more common in HUU neonates (44% (202/458) vs. 32% (77/239)). The crude in-hospital mortality among HIV-exposed neonates and HUU neonates was 26% (63/239) and 23% (104/458). After adjusting for relevant confounders such as birth weight, timing of infection, use of invasive devices, breastfeeding, and maternal age, there was no difference in the risk for mortality between HEU neonates and those who were HUU (HR:1.12, 95% CI: 0.76-1.67, p=0.549) at 28-days.

Conclusions: We did not find a difference in mortality between HEU and HUU neonates with culture-confirmed invasive infections in our study. Regardless of their HIV exposure status, approximately a quarter of these neonates died in hospital.

Keywords: Neonates HIV-exposure, bloodstream infections, Meningitis, Mortality, South Africa.

Introduction

Human Immunodeficiency Virus (HIV) is a continuing threat to children's health and wellbeing. Since 2004, the HIV prevalence among pregnant women has stayed relatively unchanged at around 27.5% in South Africa (SA).[1] Despite the high HIV burden in the country, antiretroviral therapy (ART) coverage for pregnant women living with HIV has shown significant progress with 98.8% reported to be on ART in 2022.[1] Due to the increasing awareness and accessibility of ART to pregnant women, rates have declined from 16% in 2010 to 3% in 2019.[2] The prevention of vertical transmission programmatic interventions have resulted in a rising population of HIV-exposed but uninfected (HEU) neonates.[3]

SA reduced the annual number of children acquiring new HIV infections through the prevention of vertical transmission programme by more than 70% from 2010 through 2020.[4] This resulted in the largest number of children (23.8%, 3.5 million) who were HEU in the world.[4] There are several risk factors that are unique to neonates born to HIV-seropositive mothers, these include exposure to an HIV-affected in-utero environment, lower maternal CD4+ cell counts and high viral load, and exposure to ART in utero which has been reported to possibly lead to increased morbidity and mortality.[27] However, these risks are significantly mitigated in HIV-seropositive mothers who achieve and maintain viral suppression through effective ART. HEU neonates are more susceptible to different infectious diseases as the maternal immune system is compromised, HEU are exposed to maternal viral and bacterial infections.[6,7] It has been reported that the risk of neonatal sepsis-related complications and mortality is elevated in HEU compared to HUU neonates .[5,6,28]

Most studies on HIV-exposure have been conducted in high income countries (HICs) with a low burden of maternal HIV infection while those in low middle income countries (LMICs) were mostly conducted in tertiary hospitals.[8,9] These studies are likely not comparable to our setting due to our high maternal HIV prevalence. We aimed to examine the effects of HIV-exposure on the risk of mortality within the first 28 days of life among neonates with culture-confirmed BSI and meningitis in regional and district hospital using data from six non-academic neonatal units in South Africa.

Methods

Study design and setting.

We conducted a retrospective cohort study involving patients with culture-confirmed BSIs and meningitis, using data from the Baby GERMS-SA surveillance project spanning from October 2019 to September 2020. The Baby GERMS-SA surveillance project was implemented at six non-academic neonatal units in South Africa.[10] These non-academic hospitals were selected due to their notably high incidence (16 630 (44%)) of number of laboratory-confirmed BSI in 2016 as reported by Mashau *et.al* in 2022. The hospitals are situated in Kwa-Zulu Natal, Mpumalanga, Eastern Cape, Northwest, Limpopo and Gauteng provinces. In our study we conducted a secondary analysis of the existing database, enrolling neonates with positive culture with and without HIV-exposure status.

Definitions

For this study we adopted case definitions as outlined in the Baby GERMS-SA project.[1] An episode of culture-confirmed BSI or meningitis was defined as pathogenic bacteria or fungi cultured from a cerebrospinal fluid or blood specimen in a neonate at any of the six sentinel sites. Multiple positive cultures from the same neonate with an identical pathogen within a 14-day period from the initial positive culture, were considered as a single episode. If a

second episode with the same pathogen occurred after 14 days, it was considered a separate episode.[22] Classification of cultured isolates as contaminants or pathogens was done according to the US Centres for Disease Control and Prevention (CDC). [22] "Coagulase-negative *Staphylococcus* was considered pathogenic only when two or more distinct cultures were isolated within 2 days of each other". For the purpose of our analysis, we included all neonates aged <28 days with a laboratory-confirmed BSI or meningitis and with a known maternal HIV status and known outcome. We defined EOS as infection occurring within the first 3 days of life (0–2 days) and LOS as infection occurring after day 3 of life (age 3–27 days). [11] We further subcategorised LOS into inborn and readmitted LOS, where inborn LOS pertained to neonates admitted since birth, and readmitted LOS referred to neonates readmitted from the community. HEU neonates were defined as neonates born to mothers with HIV infection, regardless of the neonate's HIV status. The outcome of interest was neonatal mortality, defined as mortality on or before 28 days after birth.

Data sources

The Baby GERMS-SA records were captured and stored on the Research Electronic Data Capture database (REDCap), hosted by the University of Witwatersrand.[26] REDCap is a secure, web-based application designed for data collection and management. It is often used in research settings for managing and organizing data related to research projects, it enables custom data collection, validation, and export, while supporting compliance with regulatory standards.[26] We extracted demographic and clinical information and laboratory results during admission. Demographic and clinical data included age, gestational age, sex, birth weight, underlying conditions, mode of delivery, clinical status information, maternal and neonate HIV status and in-hospital outcome. Laboratory data included culture results and antimicrobial susceptibility results.

Statistical analysis

All data analysis was performed on Stata/SE 17 (STATACORP, version 10.0, College Station, TX, USA) software. Proportions and counts were used to describe clinical and demographic characteristics of HEU and HUU neonates. The Kaplan-Meier method was used to plot the survival curves while the log-rank test was used to statistically compare the survival curves for HEU and unexposed neonates. Bi-variate and multivariable Cox proportional hazard regression models were used to examine the effect of HIV exposure on 28-day mortality at 5% significance level. We adjusted for confounders including, birthweight, timing of infection, breastfeeding, invasive devices and maternal age a priori and age in days at the time of positive culture. The decision to adjust for specific confounders was informed by their established associations with mortality risk in neonates with culture-confirmed bloodstream infections and meningitis. Random effects were incorporated to account for difference between the hospitals.

Ethical considerations

The Baby GERMS-SA study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (M190320). Ethics clearance for this secondary data analysis was also obtained from the Human Research Ethics Committee of the University of the Witwatersrand (clearance certificate number M230237).

Results

We retrieved 2658 positive CSF and blood cultures from neonates in the Baby GERMS-SA surveillance project. We excluded 336 records of babies with unknown maternal HIV status or outcome, 1254 records where single coagulase negative *Staphylococcus* was isolated, 265 cultures where contaminants were isolated (**Annex Table 1**), and 81 duplicate records. After all the exclusions, our cohort comprised of 697 cases, 239 (34%) of whom were HIV exposed (**Figure 1**).

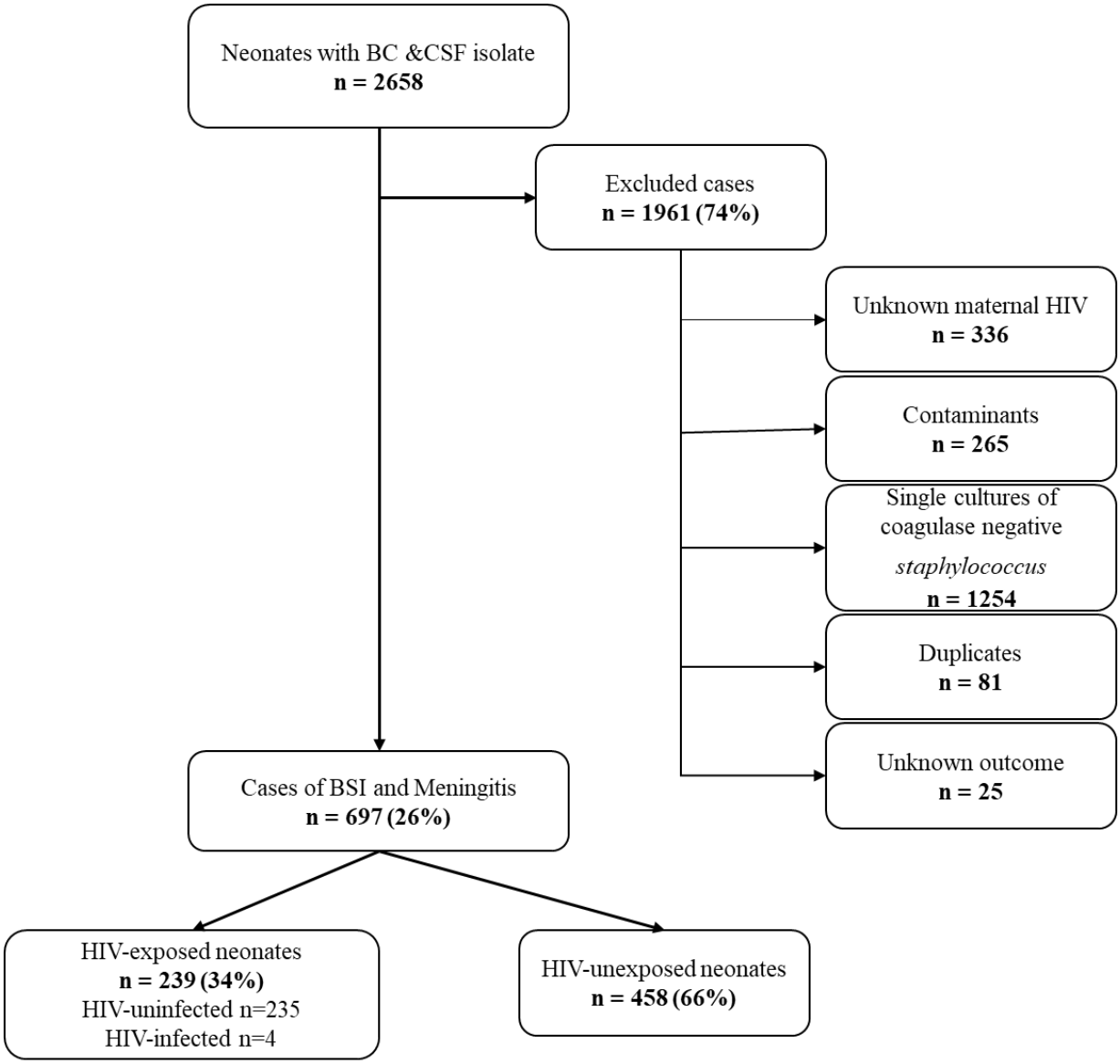

Figure 2: Flowchart of the neonates with laboratory-confirmed invasive infection in non-academic hospitals in South Africa, October 2019- September 2020

Footnote: BC= Blood culture; CSF= Cerebrospinal fluid; BSI=Bloodstream infection

The median age in days at the time of positive culture specimen collection was 6 days (interquartile range (IQR): 2-12 days) in the HEU group and 6 days (IQR 1-10 days) in the HUU group (p=0.14), (**Table 1**). The proportion of the neonates with very low birthweight (<1500 g) was 48% among HEU neonates and 40% among the unexposed neonates (p=0.02). HUU neonates were more likely to be breastfed (44%, 202/458) compared to HEU (32%, 77/239) (p=0.002). Respiratory distress syndrome 26% (165/697) and neonatal jaundice 17% (105/697) were the predominant underlying conditions amongst the two groups. Respiratory distress was more common among HEU group (32% (66/239) compared to 24% (99/458), p=0.03). The median length of hospital duration was 17 days (IQR: 7-32) in the HEU group and 15 days (IQR: 7 -31) in the HUU group (p=0.12). The overall crude in-hospital mortality was 24% (167/697). Twenty-six percent (63/239) deaths occurred among HIV-exposed neonates (3/4 of the HIV-exposed infected died), while 23% (104/458) occurred in the HUU group(p=0.28).

Table 1: Demographic and clinical characteristics of neonates with bloodstream infection and meningitis by HIV-exposure status, October 2019- September 2020.

Characteristics	Total n=697	HIV-exposed neonates n=239	HIV-unexposed neonates n=458	p-value
Hospital name				
Dora Nginza	97 (14%)	29 (12%)	68 (15%)	<0.01
Klerksdorp	90 (13%)	36 (15%)	54 (12%)	
Mankweng	146 (21%)	28 (12%)	118 (26%)	
Queen Nandi regional	122 (18%)	47 (20%)	75 (16%)	
Rob Ferreira	76 (11%)	40 (17%)	36 (8%)	
Tembisa	166 (24%)	59 (25%)	107 (23%)	
Median age in days (IQR)	6 (1-11)	6 (2-12)	6 (1-10)	0.14
Sex				
Male	367/692 (53%)	128/239 (54%)	239/453 (53%)	0.77
Female	303/692 (44%)	102/239 (43%)	201/453 (44%)	
Unknown	22/692 (3%)	9/239 (4%)	13/453 (3%)	
Median birthweight in grams (IQR)	1700 (1140-2680)	1565 (1100-2430)	1750 (1150-2790)	0.02
Birthweight				
<1500 g	299/693 (43%)	115/238 (48%)	184/455 (40%)	0.02
1500-2499 g	180/693 (26%)	66/238 (28%)	114/455 (25%)	
≥2500 g	214/693 (31%)	57/238 (24%)	157/455 (35%)	
Mode of delivery				
Vaginal	361/637 (57%)	126/215 (59%)	235/422 (56%)	0.48
Caesarean section	276/637 (43%)	89/215 (41%)	187/422 (44%)	
Invasive devices in-situ				
Central/umbilical line	95/608 (16%)	27/203 (13%)	68/405 (17%)	0.52
Peripheral line	491/608 (81%)	169/203 (83%)	322/405 (80%)	
No	22/608 (4%)	7/203 (3%)	15/405 (4%)	
Median time of infection from birth in days (IQR)	6 (1-11)	6 (2-12)	6 (1-10)	0.14
Timing of infection				
EOS	214 (31%)	68 (28%)	146 (32%)	0.44
Inborn LOS	391 (56%)	142 (59%)	249 (54%)	
Readmitted LOS	92 (13%)	29 (12%)	63 (14%)	
Exclusive breastfeeding				
No	417 (60%)	162 (68%)	255 (56%)	<0.01
Yes	279 (40%)	77 (32%)	202 (44%)	
Prior antimicrobial treatment 14 days before positive culture				
First line (ampicillin and gentamicin)	140/300 (47%)	48/107 (45%)	92/193 (48%)	0.81

Second line (piperacillin-tazobactam and amikacin)	48/300 (16%)	15/107 (14%)	33/193 (17%)	
Third line (carbapenem)	14/300 (5%)	6/107 (6%)	8/193 (4%)	
Third generation cephalosporin	4/300 (1%)	1/107 (1%)	3/193 (2%)	
Other	94/300 (31%)	37/107 (35%)	57/193 (30%)	
Underlying conditions				
Respiratory distress syndrome	165/624 (26%)	66/208 (32%)	99/416 (24%)	0.03
Neonatal jaundice	104/624 (17%)	24/208 (12%)	80/416 (19%)	
None	122/624 (20%)	44/208 (21%)	78/416 (19%)	
Other	233/624 (37%)	74/208 (36%)	159/416 (38%)	
Outcome at 28 days				
Alive	530 (76%)	176 (74%)	354 (77%)	0.28
Died	167 (24%)	63 (26%)	104 (23%)	
Median duration to discharge (IQR)	16 (7-31)	17 (7-32)	15 (7-31)	0.12
Median duration to death (positive culture)	10 (3-22)	10 (2-25)	10 (3-21)	0.83

IQR=Interquartile range; Other Underling conditions =Hypoxic ischaemic encephalopathy, Necrotising enterocolitis, Omphalocoele, Meningoencephaloceole, Cardic abnormalities, Congenital syndrome and Meconium aspiration syndrome.

Mothers of HIV-exposed neonates were older than mothers of HUU neonates (median age of 29 years versus 25 years; p<0.01) as shown in **Table 2**. Seventy-seven percent (184/239) of mothers with HIV delivered before 37 weeks of gestation in comparison to 66% (296/458) of the HIV-negative mothers (p<0.001). Additionally, mothers with HIV had an average of three pregnancies (IQR: 2-5) and parities of two (IQR: 2-3) compared to an average of two pregnancies (IQR :1-4) and parities of two (IQR: 1-3) of the HIV-negative mothers (p-values <0.01).

Table 2: Maternal characteristics for the neonates included in the study by HIV-exposure status, October 2019- September 2020

Variables	Total n=697	Mothers with HIV n=239	HIV-uninfected mothers n=458	p-value
Maternal age in years (IQR)	27 (22-32)	29 (25-33)	25 (21-31)	<0.01
Maternal age				
<24 years	206/581 (35%)	40/201 (20%)	166/380 (44%)	
25-35 years	319/581 (55%)	136/201 (68%)	183/380 (48%)	
≥35 years	56/581 (10%)	25/201 (12%)	31/380 (8%)	
Median gestational age in weeks (IQR)	33 (29-37)	32 (29-36)	33 (29-37)	0.01
Gestational age				
Term (≥37 weeks)	205/685 (30%)	52/236 (22%)	153/449 (34%)	<0.01
Preterm (<37 weeks)	480/685 (70%)	184/236 (78%)	296/449 (66%)	
Antenatal care				
Yes	173/629 (28%)	67/214 (31%)	106/415 (26%)	0.10
No	366/629 (58%)	112/214 (52%)	254/415 (61%)	
Unknown	90/629 (14%)	35/214 (16%)	55/415 (13%)	
Infections during pregnancy				
Urinary tract infection	4/35 (11%)	3/15 (20%)	1/20 (5%)	0.44
Syphilis	7/35 (20%)	3/15 (20%)	4/20 (20%)	
Premature rupture of membranes	20/35 (57%)	7/15 (47%)	13/20 (65%)	
Chorioamnionitis	4/35 (11%)	2/15 (13%)	2/20 (10%)	
None/Unknown	592/697 (85%)	198/697 (28%)	394/697 (57%)	
Median gravidity (IQR)	3 (2-5)	3 (2-5)	2 (1-4)	<0.01
Median parity (IQR)	2 (1-3)	2 (2-3)	2 (1-3)	<0.01

IQR=Interquartile range

Sixty-four percent of BSI and meningitis cases were caused by Gram-negative bacteria, followed by Gram-positive bacteria (31%) and then fungi (5%). Overall, there were no statistical evidence of differences in the distribution of pathogens amongst the HUU and HEU neonates. *Klebsiella pneumoniae* (36%), *Acinetobacter baumannii* (29%) *Enterobacter cloacae* (11%) were the most common in the Gram-negative bacteria group. *Staphylococcus aureus* (30%) and *Enterococcus faecium* (24%) and Group B *Streptococcus* (20%) were the

most common pathogen causing BSIs and meningitis in the Gram-positive bacteria group. Fungal infections were predominantly caused by *Candida parapsilosis* (41%), *Candida albicans* (18%) and *Candida auris* (18%).

Table 3: Pathogens isolated in neonates with culture-confirmed bloodstream infection and meningitis infection by HIV-exposure, October 2019- September 2020.

Variable	Total n=697	HIV-exposed neonates n=239	HIV-unexposed neonates n=458	p-value
Gram-negative bacteria	**448 (64%)**			
Klebsiella pneumoniae	163 (36%)	62 (39%)	101 (35%)	0.87
Acinetobacter baumannii	129 (29%)	49 (31%)	80 (28%)	
Enterobacter cloacae	51 (11%)	17 (11%)	34 (12%)	
Escherichia coli	28 (6%)	8 (5%)	20 (7%)	
Serratia marcescens	26 (6%)	9 (6%)	17 (6%)	
Pseudomonas aeruginosa	5 (1%)	1 (1%)	4 (1%)	
Other Gram negatives	46 (10%)	12 (8%)	29 (11%)	
Gram-positive bacteria	**215 (31%)**			
Staphylococcus aureus	64 (30%)	19 (28%)	45 (30%)	0.86
Enterococcus faecium	51 (24%)	17 (25%)	34 (23%)	
Group B *Streptococcus*	42 (20%)	11 (16%)	31 (21%)	
Enterococcus faecalis	37 (17%)	13 (19%)	24 (16%)	
Coagulase-negative *Staphylococcus*	12 (6%)	3 (4%)	9 (6%)	
Other Gram positives	9(4%)	23 (34%)	50 (34%)	
Fungi	**34 (5%)**			
Candida parapsilosis	14 (41%)	4 (33%)	10 (45%)	0.97
Candida albicans	6 (18%)	3 (25%)	3 (14%)	
Candida auris	6 (18%)	2 (17%)	4 (18%)	
Candida famata	3 (9%)	1 (8%)	2 (9%)	
Candida tropicalis	2 (6%)	1 (8%)	1 (5%)	
Other fungal pathogens	3 (9%)	1 (8%)	2 (9%)	

In our survival analysis, the time (hospital duration) was available for 661 neonates. The overall deaths within 28 days were 24% (158/661). More than 20% of all deaths occurred between day 1 and day 12 in both HEU and unexposed groups. On day one, 6 deaths occurred (83% were HUU). By day twelve, 112 deaths had occurred (38% were from HEU vs. 62% of HUU). The Kaplan-Meier survival curves did not diverge for HEU and HUU neonates until day 7. However, the curves converge at day 12 and then remained parallel to

each other until day 28. The survival proportion was similar and did not differ by HIV-exposure status (p=0.60) (**Figure 2**).

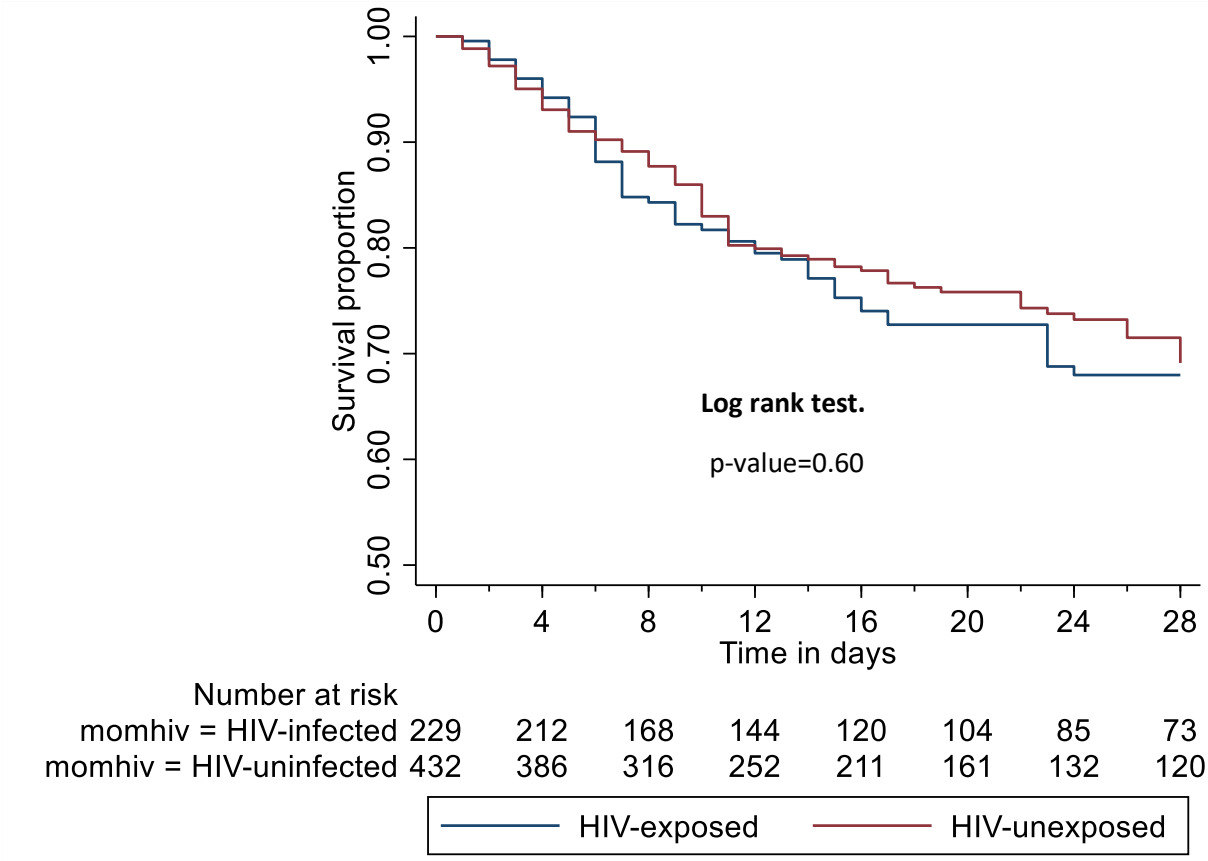

Figure 3: Kaplan Meir analysis of neonates with laboratory-confirmed bloodstream infection and meningitis during a 28-day period by HIV-exposure, October 2019- September 2020

In univariate Cox regression analysis, HIV-exposure was not associated with mortality (hazards ratio (HR): 1.08, 95%CI: 0.79-1.50, p=0.60) (**Table 4**). After adjusting for birth weight, timing of infection, use of invasive devices, breastfeeding and maternal age, the risk of death did not differ by HIV-exposure (aHR:1.12, 95%CI: 0.76-1.67, p=0.55).

Table 4: Random effects Cox regression analysis of the effect of HIV-exposure on mortality among neonates with bloodstream infections and meningitis, October 2019- September 2020

Variable	Survived	Died	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
HIV-exposure						
Unexposed	354/458	104/458	1 (Ref)		1(ref)	
Exposed	176/239	63/239	1.09 (0.79-1.50)	0.58	1.12 (0.76-1.67)	0.55
Age in days	530/697	167/697	0.94 (0.92-0.97)	<0.01		
Birthweight						
<1500 g	187/299	112/299	1 (Ref)			
1500-2499 g	146/180	34/180	0.61 (0.41-0.89)	0.01		
>=2500 g	194/214	20/214	0.36 (0.22-0.58)	<0.01		
Timing of infection						
Early onset sepsis	170/214	44/214	1 (Ref)			
LOS inborn	289/391	102/391	0.90 (0.62-1.31)	0.58		
LOS readmitted	71/92	21/92	0.91 (0.54-1.55)	0.73		
Invasive devices in situ						
Central/umbilical line	70/95	25/95	1 (Ref)			
Peripheral line	371/491	120/491	0.89 (0.58-1.38)	0.61		
None	20/22	2/22	0.72 (0.17-3.04)	0.65		
Exclusively breastfeed						
No	290/417	127/417	1 (Ref)			
Yes	239/279	40/279	0.49 (0.34-0.70)	<0.01		
Maternal age						
<24 years	137/178	41/178	1 (Ref)			
24-35 years	208/280	72/280	0.99 (0.69-1.43)	0.98		
>35 years	61/81	20/81	1.07 (0.58-1.97)	0.83		

Discussion

In this surveillance study of hospitalised neonates with neonatal invasive diseases admitted in non-academic hospitals, the prevalence of HIV-exposure was just over a third of the study population. In-hospital mortality rate at 28-days was similar between HEU neonates and HUU after adjusting for relevant confounders. Most HEU neonates had late-onset sepsis, were preterm, and had respiratory distress syndrome. Although this was not the primary exposure and was only included in the model as a confounder, we noted that being exclusively breastfed was protective against death.

The prevalence of HIV-exposure in neonates of 34% in our study is consistent with the HIV prevalence amongst pregnant women in SA reported at 30% in 2020 by the ANCHSS.[1] The consistent high prevalence of HEU is largely due to the high coverage of ART among pregnant women (96%) and viral suppression (>70%) in mothers with HIV-infection in South Africa showing success of the prevention of vertical transmission programme.[1] About one percent of our HIV exposed neonates tested HIV-positive on PCR highlighting some challenges of the prevention of vertical transmission programme in reaching its target of 0% infection. This suggests that effort to reduce vertical transmission of HIV in children via prevention of vertical transmission programme should remain. Although the prevention of vertical transmission programme aims for all pregnant women to receive ART regardless of CD4 count, mothers of the HIV-infected neonates likely had a high viral load although this was not confirmed in our study due to limited detailed maternal information. Despite progress made by the South Africa government towards elimination of vertical transmission of HIV in children, there are some issues that still require attention such as low adherence to ART, delayed HIV testing, delayed initiation of treatment and care for children and limited access to healthcare services. [12,13] A study conducted in SA demonstrated that ART exposure was linked with significant improvement in survival for both the mother and neonate.[17]

In our large study of neonates admitted with culture-confirmed infections, we found that all cause in-hospital mortality does not significantly differ between HEU and HUU neonates. This was in contrast to a neonatal study conducted in SA between 2017 and 2018 which found a mortality difference of 3% (10.8% in HEU and 13.3% in HUU, p=0.058).[5] In this study, an adjusted analysis for HIV-exposure was not performed. The lack of a statistically significant difference between the two groups was possibly due to inadequate power of our study. The presence of invasive infections in both our study groups could have also masked the true effect of HIV-exposure because neonatal infections result in high mortality in this population.[25] Our findings may also suggest that there are no unique exposures or risk factors for mortality in HEU during neonatal period. Nonetheless, our findings are in agreement with previous study conducted in South Africa, Botswana and Cameroon [16,19], which found no differences in mortality between HEU and HUU. In addition, a recent cross sectional study in SA also found that HEU were not at heightened risk for mortality (OR: 0.80, 95% CI: 0.31-2.09) but rather HIV infected neonates were at increased risk (OR: 4.79, 95% CI: 1.49-15.37) for mortality when compared to HUU neonates hospitalised with acute medical illness.[16] Our findings, along with universal maternal access of ART and improved access to healthcare services suggest that ART may have positively impacted neonates with HEU. Unfortunately, due to limited maternal detailed clinical information, we were unable to adjust for timing of maternal ART, virological levels and cd4 count. Timing of maternal ART plays in important role in the development of infant, especially when they are started prior pregnancy. In order to understand mortality risk among HIV-exposed infected, HEU and HUU children with invasive diseases, a substantially large prospective study beyond neonatal period is required.

Although our findings provide reassurance that neonates who are HEU do not have increased risk of in-hospital mortality compared with HUU neonates, the overall in-hospital mortality and mortality in HEI neonates was alarmingly high raising concerns regarding care of these

neonates. These results further suggest that regardless of the HIV exposure status, neonates with invasive infections are at increased risk of death. It is therefore imperative that the management of neonates with culture-confirmed neonatal infections be strengthened to reduce the observed mortality.

As previously reported, HEU neonates were more likely to be born prematurely and have lower birth weight than HUU neonates.[18,19] In our study we confirmed several risk factors among HEU neonates with neonatal BSI and meningitis infection including low birth weight, prematurity and respiratory distress. Although HEU neonates were more likely to have a lower birth weight, we still did not see a difference in mortality after adjustment for this; the resources available and the level of care given to the HEU neonates might have provided sufficient protection against adverse outcomes. The immune systems of preterm and term neonates, both HEU and HUU, are characterized by immaturity, which likely plays a significant role in the similar clinical outcomes observed between these groups during the neonatal period.[20,29] This immaturity includes diminished functionality in both innate and adaptive immune responses, as well as lower levels of maternal antibody transfer, particularly in preterm neonates. [20,29] These shared limitations in neonatal immunity may result in comparable vulnerabilities to infections and other morbidities, irrespective of HIV exposure status.

Increased low birthweight among HEU neonates could contribute to increased morbidity and mortality, our findings of more invasive device use, longer hospital stay shows the increased care needed in this group. A cohort study conducted between 2017 and 2019 in SA showed similar findings of very preterm HEU neonates requiring advanced care; ICU admission and longer hospital stay compared to unexposed neonates. [20] It has been previously reported that maternal HIV-infection was associated with low birthweight, that is children exposed to intrauterine HIV are more susceptible to being underweight and their birthweight is often

lower compared to unexposed neonates.[21] This HIV-associated low birthweight could be linked to low maternal CD4+ T cell counts and immunosuppression. [21] A prospective cohort study conducted in Zimbabwe in infants (4-12 weeks) found respiratory illness as one of the causes of mortality in HEU infants, although our study did not focus on the cause of mortality and infant age, the most common preceding conditions in our study align with those reported which included respiratory distress syndrome.[22]

According to our analysis, majority of HEU neonates had inborn LOS (59%, p=0.06), which was consistent with findings from prior studies conducted in SA tertiary level neonatal units, where LOS accounted for majority of the cases ranging from 83% to 93% of cases. [8,23] There were no notable differences in the pathogens isolated by HIV-exposure status. Although the distribution of pathogens was not different among the two groups, we did note *Acinetobacter baumannii, Klebsiella pneumoniae, Enterococcus faecium* and Group B *Streptococcus* (GBS) among the top pathogens causing BSI/meningitis in HEU neonates. Moreover, In SA *Klebsiella pneumoniae, Staphylococcus aureus* and *Acinetobacter baumannii,* are the major cause of HAIs.[11] Presence of HAIs suggest breach of infection prevention and control (IPC) and poor implementation of antimicrobial stewardship rather than exposure to HIV. A study conducted in Mozambique in 2021 also found *Klebsiella* species (50%) and *S. aureus* (12.5%) being the predominant Gram negative organisms causing community-acquired bacteraemia in hospitalised HEU children under three months. [24] Maternal HIV infection has been previously associated with a high prevalence of GBS colonization. This is in line with a previous study conducted in Soweto SA where the overall incidence of GBS disease in HEU infants was high at 2.72 cases per 1000 live births.[22] We found a slight increase in proportion of neonates with GBS in the exposed group even though the statistical evidence was weak.

A key strength of using the Baby GERMS-SA surveillance project was that detailed and good quality data were collected for the neonates. We were also able to confirm previously

described risk factor of HEU in high HIV burden setting in South Africa. However, there are some limitations due to the nature of the data collected in the Baby GERMS-SA surveillance study. We were unable to evaluate important risk factors associated with mortality in HEU neonates. For instance, maternal information such as ART use, CD4 count, and viral load were excluded in our multivariable cox regression analysis as important confounders due to missing data. In previous studies, maternal HIV-associated deficiency (such as nutrition, low CD4+ count, viral load) was found to be a significant contributing factor in the low birth weight and premature delivery of HEU neonates in LMICs as compared to mothers in HICs (OR: 2.12 (95%CI: 1.81-2.48) compared to OR:1.75 (95%CI: 1.44-2.12).[14] These variables were not collected because the Baby GERMS-SA study was set-up to image or scan medical charts of neonates only. Additionally, we did not have information on the date of symptom onset, which limited our ability to analyse the timing and progression of symptoms. This omission is significant because the timing of symptoms could influence the outcomes and severity of the conditions affecting HEU neonates. It is also important to note that data on antibiotic use during the current episode were incomplete, with a significant number of missing data points. This limited our ability to fully assess the impact of current antibiotic treatment on the outcomes, in contrast to the more complete data available for prior antibiotic treatment.

Social factors, beyond biological ones such as the death of a mother with HIV, were also not assessed or controlled for this could have changed over time as more treatment became available to pregnant women and this could also account for changes in outcomes of HEU with time. Other methods and study designs such as large prospective studies that follow-up HEU neonates beyond the neonatal period should be considered to incorporate the unmeasured factors that limited our analysis and to determine if the outcome changes as this was a retrospective study.

Conclusion

We found that the overall mortality rate of neonates with culture-confirmed neonatal infections did not differ by HIV exposure status, even though HEU neonates were more likely to be premature, of low birthweight and not exclusively breastfed. The similarly high mortality rate in both groups could be the cause of the lack of difference in mortality, therefore a very large sample size would be required to detect a difference, assuming one existed at all. Implementing and improving IPC with the aim of reducing the incidence of infections can have an impact on mortality rate in neonatal units. In addition, improve approaches for pregnant women missed during prevention of vertical transmission programme to reach 0% HIV-infection in HEU neonates.

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Annex

Table 5: List of commensals/contaminants

Positive bacilli	Mixed growth	Viridans *Streptococcus*
Negative bacilli	Mixed-3 dorsets	*Bacillus cereus*
Variable cocci	*Pseudomonas luteola*	*Aerococcus viridans*
Gram positive bacillus	*Streptococcus sanguinis*	*Bacillus firmus*
Gram positive cocci	*Streptococcus infantarius*	*Bacillus megaterium*
Micrococcus species	*Streptococcus constellatus*	*Bacillus pumilus*
Corynebacterium	*Corynebacterium jeikeium*	*Corynebacterium minutissimum*
Kocuria kristinae	*Microbacterium flavescens*	*Micrococcus luteus*
Micrococcus species	*Paenibacillus lautus*	*Rhodococcus rhodochrous*
Strep. Infantarius	*Streptococcus anginosus*	*Streptococcus gallolypticus subsp pasteurianus*
Streptococcus mitis/oralis	*Streptococcus parasanguinis*	*Streptococcus salivarius*
Streptococcus constellatus	*Streptococcus group f*	*Turicella otitidis*
Streptococococcus pseudopneumoniae	*Bacillus species*	*Brevibacillus species*
Streptococcus species	*Streptococcus alpha-haemolytic*	

Chapter 3: Extended Methodology

This section provides extended/different methods which were not included in Chapter 2 of the draft manuscript. It describes how we assessed if the study had the power to detect a difference in mortality between the HEU and HUU neonates. And also reported on the methods and post-hoc test done for the cox analysis presented in Chapter 2. We also outlined the bivariate and multivariate analyses conducted to assess the effect of HIV-exposure on mortality.

Power calculations

The sample size was derived for cases with a known maternal HIV status from the Baby GERMS-SA surveillance project. This was a secondary data analysis and we performed post hoc power calculations based on the final known number of study participants in the analysis cohort to determine if we have the power to detect a difference in mortality.

Power calculations were calculated using the chi-squared test comparing two independent proportions using Stata version 17. The final sample size after data management and cleaning was 697. The proportion of exposed was 34% and of the unexposed was 66%. A study conducted in SA "The effects of exposure to HIV in neonates at a referral hospital in SA" found the prevalence of HIV-exposure at **26%** in 2021. We calculated the statistical power for various percentages:

```
. power twoproportion 0.26, test(chi2) diff(0.05(0.01)0.15) n(697) nratio (0.521) continuity

Estimated power for a two-sample proportions test
Pearson's chi-squared test
H0: p2 = p1  versus  Ha: p2 != p1
```

alpha	power	N	N1	N2	nratio	delta	p1	p2	diff
.05	.261	697	458	238	.521	.05	.26	.31	.05
.05	.3555	697	458	238	.521	.06	.26	.32	.06
.05	.4587	697	458	238	.521	.07	.26	.33	.07
.05	.5639	697	458	238	.521	.08	.26	.34	.08
.05	.6638	697	458	238	.521	.09	.26	.35	.09
.05	.7526	697	458	238	.521	.1	.26	.36	.1
.05	.8266	697	458	238	.521	.11	.26	.37	.11
.05	.8845	697	458	238	.521	.12	.26	.38	.12
.05	.9269	697	458	238	.521	.13	.26	.39	.13
.05	.9561	697	458	238	.521	.14	.26	.4	.14
.05	.975	697	458	238	.521	.15	.26	.41	.15

Note: N1 and N2 have been rounded down.

Therefore, the data used was only sufficient to determine the effect of HIV-exposure on mortality if there was a big difference in mortality between the two groups of 10-20%. Our study was therefore underpowered.

Post-hoc analysis

We assessed proportionality of hazards using the Schoenfeld's global test and also performed a Kaplan–Meier curve to assess the observed survival curves in comparison to the Cox predicted curves for the same variable and effect.

Exploratory/additional statistical analysis

We conducted a univariate and multivariate logistic regression to determine the effect of HIV-exposure as a risk factor for mortality, the outcome of interest was mortality at/within 28 days. The potential confounders were identified a priori (through literature) and also included variables considered as important.

Chapter 4: Extended Results

This section provides the post-hoc tests results for the survival analysis performed in draft manuscript. We also outlined the results from the multivariate logistic regression analysis. The logistics regression results were done to determine if the effect of HIV-exposure would be the same even without the time component.

Post-hoc tests

We assessed the assumption of proportionality, in Figure 4. We see that the lines are not only non-parallel but also cross twice in the data region. Additionally, the global test showed a chi-square of 64.16, degrees of freedom was 8 and a p-value<0.001. In figure 5, we see that there are no significant differences between the observed and predicted values in both groups.

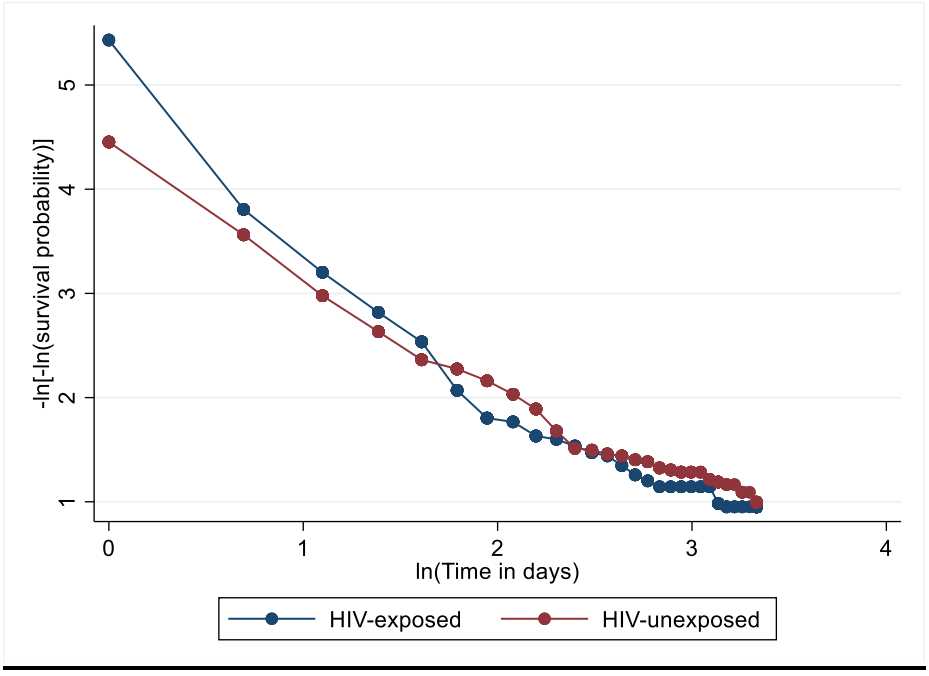

Figure 4: Graph of the test of assumption of proportionality (post-hoc test)

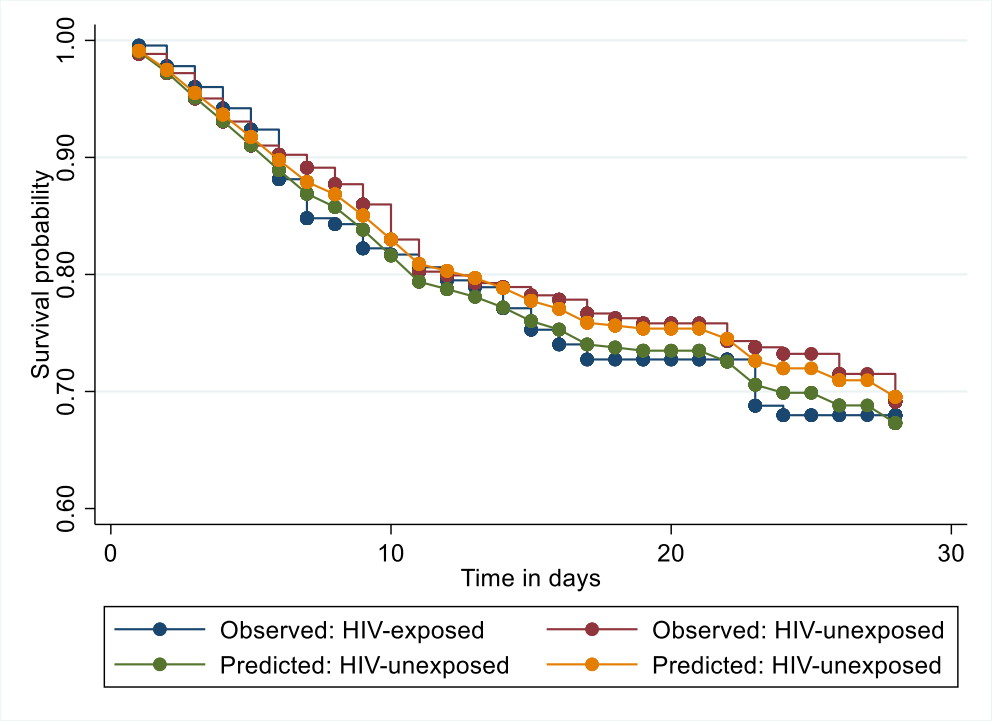

Figure 5: Kaplan–Meier curves of the observed survival curve compared to the cox predicted curves by HIV-exposure.

Logistic regression

After we adjusted for age in days, sex, enhanced surveillance sites, birthweight, timing and breastfeeding, the odds of death were 1.21 (95%CI:0.72–2.05, p=0.467) times more among HEU neonates than among HUU neonates. However, there was no significant statistical evidence for an association.

Table 6: Random-effects logistic regression to examine effects of HIV exposure in neonates, October 2019 to September 2020.

Variable	Survived	Died	Unadjusted OR	p-	Adjusted OR	p-value

			(95% CI)	value	(95% CI)	
HIV-exposure						
HIV-unexposed (Ref)	354/458	104/458	1.00			
HIV-exposed	176/239	63/239	1.22 (0.84-1.75)	0.284	1.21 (0.72-2.05)	0.467
Age in days	530/697	167/697	0.94 (0.91-0.96)	<0.001		
Birth weight.						
<1500g (Ref)	187/299	112/299	1.00			
1500g-2499 g	146/180	34/180	0.39 (0.25-0.60)	<0.001		
>=2500 g	194/214	20/214	0.17 (0.10-0.29)	<0.001		
Invasive devices						
Central/umbilical line (Ref)	70/95	25/95	1.00			
Peripheral line	371/491	120/491	0.91 (0.54-1.49)	0.698		
No	20/22	2/22	0.28 (0.06-1.28)	0.102		
Timing of infection						
Early onset (Ref)	170/214	44/214	1.00			
LOS Inborn	289/391	102/391	1.36 (0.91-2.04)	0.130		
LOS readmitted	71/92	21/92	1.14 (0.63-2.06)	0.657		
Exclusive breast feeding						
No (Ref)	290/417	127/417	1.00			
Yes	239/279	40/279	0.38 (0.26-0.57)	<0.001		
Maternal Age						
<24 years (Ref)	137/178	41/178	1.00			
24-35 years	208/280	72/280	1.01 (0.67- 1.52)	0.963		
>35 years	61/81	20/81	1.25 (0.64-2.41)	0.512		

Chapter 5: Extended discussions and conclusion

In this chapter, we discussed the findings from our extended analysis that could not fit in the manuscript discussion specifically the logistic regression findings. This chapter does not discuss any findings reported in Chapter 2. The chapter will conclude with study limitations and additional recommendations for care and future research in this field.

Discussion

Maternal HIV-infection has been reported to significantly predispose neonates and infants to elevated mortality, particularly in the first four months of life.[1] However in addition to our Cox regression, we found no significant difference in the mortality for HEU neonates when compared to HUU neonates with BSIs and meningitis. Several studies report on increased risk of mortality in infants and children exposed to HIV-infection in their first years or months, but few have reported on mortality during the neonatal period (first 28 days). In a retrospective cohort study conducted in Ethiopia, mortality rate was high in HEU neonates at 8.88 per 100 child a-year (95% CI: 6.36–12.36).[4] However, the ART coverage in Ethiopia has been at 76 % from 2010, with approximately 1.1 million pregnant women reported to be using ART in 2020.[5]

Neonatal mortality rate in our study was similar between the HEU and HUU neonates (OR: 1.21 95% CI: 0.72-2.05, p=0.467). We found that HEU neonates were 1.21 times more likely to die in the first 28-days of life compared to HUU neonates although this was not statistically significant. A Cameroon case-control study carried out in a neonatal unit also found no statistical difference in mortality between neonates born to mothers with HIV and HIV-uninfected mothers (p=0.52).[43] However, previous studies have reported HEU neonates born to mothers with HIV to experience mortality rates at least two times more likely than HUU infants regardless of HIV-status, a cohort study conducted in Malawi reported odds

ratios of 2.9 (95%CI: 1.1-7.2, p= 0.03).[2] This increased risk was associated with increased exposure to infections and poor healthcare for the infant.[2,3] The analysis of our study adds to the limited evidence on neonatal mortality exposed to HIV in LMICs. Despite the differences in clinical characteristics (we discussed in Chapter 2) between the HEU and HUU groups there was no statistical evidence of differences in mortality.

Conclusion and Recommendations

When compared to HUU neonates with neonatal infections, HEU neonates with infections were at a slight elevated risk for mortality if admitted to non-academic hospitals in South Africa although the association was not statistically significant. Promoting early and consistent antenatal care for pregnant mothers can aid in the identification, prevention and management of potential risk factors for newborn infections which include HIV exposure, infections, maternal CD4+ count and viral load. Clinical interventions and awareness of the elevated risk for neonatal sepsis are needed for improved survival outcomes this can be through educating healthcare workers in non-academic hospitals of the strategies to prevent infections in high-risk neonates. Non-academic hospitals are not as specialised and equipped as tertiary hospitals there is a need to enhance resources and support, further strengthening infection prevention and control measures.

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Appendices

Appendix A: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I ___Andani Ronel Marumo____ (Student number: 1664072 _) am a student registered for the degree of

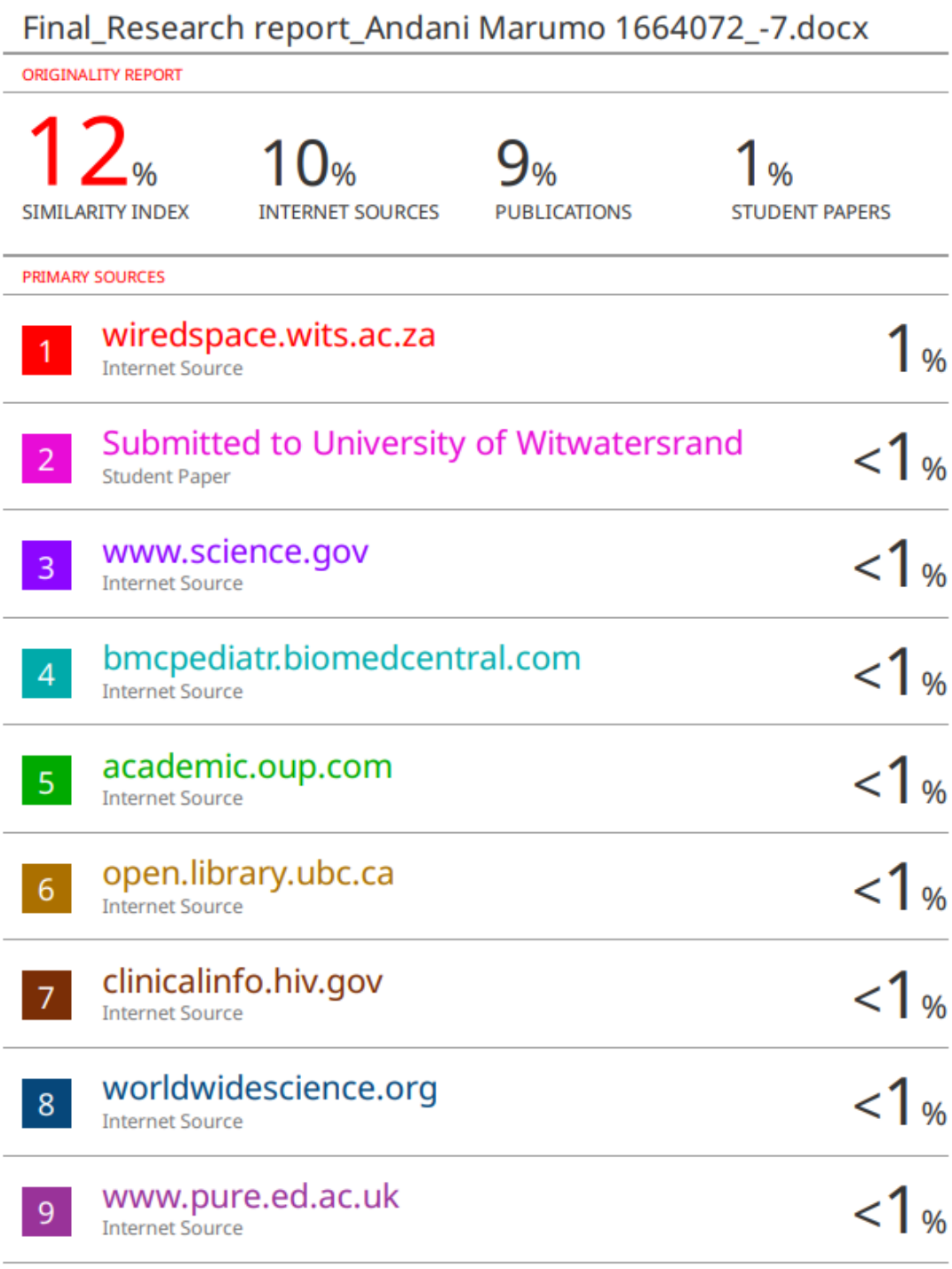
__Master of science Epidemiology in Field Epidemiology_in the academic year __2023_.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: ____ _____ Date: ___06 February 2024_____

Appendix B: Turnitin report



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Appendix C: Ethics certificate

M230237 MED23-01-077

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Andani Ronel Marumo

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M230237 MED23-01-077

NAME: (Principal Investigator)	Miss Andani Ronel Marumo
DEPARTMENT:	Division of Epidemiology and Biostatistics School of Public Health & NICD CHARM
PROJECT TITLE:	HIV-exposure as a risk factor for mortality among neonates with culture-confirmed bloodstream infection in South Africa, 2019-2020
DATE CONSIDERED:	24/02/2023
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof N Govender, Mrs R Mashau and Prof E Musenge
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/05/2023 **EXPIRY DATE:** 30/05/2028

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

DECLARATION OF INVESTIGATORS

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 resubmit the application to the Committee. **I agree to submit a yearly progress report** in February each year until study is closed. Failure to submit annual report will result in ethics clearance certificate suspension. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical). Login to upload signed copy of this ethics clearance certificate prior to commencing with the study https://www.witsethics.co.za/login.aspx

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix D: Permission letter

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Nelesh Govender
Head: Centre for HAI, AMR and Mycoses, NICD
1 Modderfontein Road, Sandringham, 2131
Tel: +27 (0) 11 555 0353
E-mail: neleshg@nicd.ac.za

7 February 2023

Ms Andani Marumo
National Institute of Communicable Diseases

Dear Andani,

RE: Approval to access Baby GERMS Tier 2 Data

Your application to undertake a research project "HIV exposure as a risk factor for mortality among neonates with culture-confirmed bloodstream infection in South Africa, 2019-2020" using data from the Baby-GERMS-SA Tier 2 surveillance project has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you to conduct the proposed study outlined in the submitted application.

Please note that the approval is granted subject to your adherence to the National Institute for Communicable Diseases (NICD) data protocols and policies. The study can only be undertaken provided that the following conditions have been met:

- Approval or a waiver is obtained from a recognised South African Research Ethics Committee
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidentiality information as described in the relevant NICD policies
- NICD and the Baby GERMS-SA surveillance is acknowledged appropriately in the final report of the research study and any published paper resulting from this study

Please note that this letter constitutes approval by the Principal Investigator of the Baby GERMS-SA surveillance project.

Kind regards,

Nelesh Govender