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Antimicrobial Resistance Patterns in Neonatal Sepsis Cases at Chris Hani Baragwanath Academic Hospital

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A research report submitted to the faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of the Master of Science in Medicine in the field of Vaccinology

Johannesburg, South Africa, 2021

Declaration

I, Ronald Opiecabo, declare that this research report is entirely my own work. It is intended for submission to the University of the Witwatersrand, Johannesburg, South Africa, for the award of Master of Science in medicine degree. It has never been submitted either in part or fully to this or any other university for examination or award of any academic qualification.

Signature: 

Date: 04th May 2021

Place: **Johannesburg, South Africa**

Dedication

I dedicate this work to my mother Mrs Edisa Amvucia Amandura, my brothers Mr Alex Candia, Mr Benard Masimale, Mr Ayaa Michael. This work is also dedicated to Mr Olungi Paul, and Mr Matua Asiyo Gard who have contributed immensely to my academic success todate.

Abstract

Problem and purpose

The purpose of this study was to describe the antimicrobial resistance (AMR) patterns of clinical isolates from neonatal sepsis cases admitted at Chris Hani Baragwanath Academic Hospital (CHBAH) from January 2016 to December 2019. Globally, 214 000 – 690 000 neonatal deaths (death in first 28 days after birth) are associated with sepsis, 31% of the deaths are potentially attributed to AMR, with the highest burden experienced in sub-Saharan Africa.

Methods

This study used descriptive data analysis which involved a retrospective review of the neonatal hospital database, drug susceptibility test results, and neonatal sepsis discharge summary at CHBAH.

Results

Bacteria and fungi were the two organism types identified among neonatal sepsis cases at CHBAH. Gram-negative bacteria 317(52.93%) were the most predominant bacterial isolates among neonatal sepsis cases compared to gram-positive bacteria 282(47.08%), but the difference was not statistically significant (P=0.3961). The leading gram-negative bacteria causing AMR sepsis at CHBAH were *Acinetobacter baumannii* 194(28.4%), *Klebsiella pneumoniae* 96(14.69%), and *Escherichia coli* 6(0.88%). The most prevalent gram-positive bacteria were *Staphylococcus aureus* 109(16.0%), and *Coagulase-negative staphylococci* 106(15.5%). With regards to fungi, *Candida parapsilosis* 27(3.95%), *Candida albicans* 18(2.64%), and *Candida tropicalis* 1(0.15%) being the most common causes of candidemia.

Acinetobacter baumannii was found to have very high resistance to amikacin (71.05%), meropenem (91.3%), and ceftazidime (82.46%). A high prevalence (73%) of extended spectrum beta-lactamase (ESBL) production was noted amongst the *Klebsiella pneumoniae.* A high prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE) were noted among the *Klebsiella pneumoniae* as demonstrated by the 46.7% resistance to meropenem. Thirty five percent of the *Staphylococcus aureus* were resistant to methicillin

Low susceptibility of the leading causes of AMR sepsis to the recommended empirical therapeutic options was noted. Overall, there was a 30% mortality rate among neonatal sepsis cases admitted..

Conclusion

Neonatal mortality associated with AMR sepsis remains significantly high at CHBAH and *Acinetobacter baumannii* was responsible for almost half the deaths. This was possibly attributed to poor therapeutic outcomes owing to the high resistance of the leading causes of neonatal sepsis to empiric treatment and the recommended therapeutic choices.

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Abbreviations

AMR	Antimicrobial resistance
CHBAH	Chris Hani Baragwanath Academic Hospital
WHO	World Health Organisation
ESBL	Extended spectrum beta-lactamase
CPE	Carbapenemase-producing *enterobacteriaceae*
MRSA	Methicilin-resistant *Staphylococcus aureus*
LMIC	Low middle-income countries
CDC	Centers for Disease Control and Prevention
FDA	Foods and Drugs Administration
FAO	Food and Agricultural Organisation
CSF	Cerebrospinal fluid
EOD	Early onset disease
LOD	Late onset disease

Study definitions

Antimicrobial resistance is defined as the ability of microorganisms to resist the effects of a drug.

A neonate is defined as a new-born infant under 28 days of age (< 28 days, where the day of birth is day 0).

Pre-term infant is defined as a newborn delivered before 37 weeks of gestation are completed

Neonatal sepsis is defined as a clinical syndrome consisting of nonspecific signs of infection accompanied by a positive culture from a normally sterile site including blood, cerebrospinal fluid (CSF), and urine. This study included isolates from blood and CSF only.

Early-onset disease (EOD) refers to neonatal sepsis with symptoms presenting between zero and six days after birth, indicating a high likelihood of maternal transmission of infection.

Late-onset disease (LOD) is sepsis with symptoms starting between seven and 90 days after birth, indicating hospital-acquired or community-acquired infection.

Empirical treatment is a treatment given based on a clinical educated guess in the absence of complete or perfect information. Clinicians use their expertise, intuition, and professional judgement to guess whether an infection is present and what is likely to be causing it and thus the most appropriate treatment.

First choice drug is classified as antimicrobials that can be prescribed without restrictions by any authority.

Restricted drugs are antimicrobials that can be prescribed for a specific indication as defined by a policy or with expert consultation.

Reserve drugs are drugs that can be prescribed only after permission from expert consultation or national expert committee.

Community acquired infection is an infection acquired in the community by someone who has not been recently hospitalised nor had a recent medical procedure.

Nosocomial infection is an infection acquired in the hospital up to 48 hours after hospital admission, or up to three days after discharge in a healthcare facility when someone was admitted for reasons other than the infection.

One health approach is a method of determining policies that bridge human, animal and environmental health.

Antimicrobial susceptibility test is a test used to determine to which specific antimicrobials a pathogen is sensitive.

Susceptible microbes are microbes that are vulnerable to the therapeutic effects of antimicrobials.

Multi-drug resistance is the phenomenon when a microorganism is resistant to the effects of more than one antimicrobial drug.

Antimicrobial stewardship involves coordinated interventions designed to promote, improve, monitor and evaluate the judicious use of antimicrobials so as to preserve their future effectiveness and promote and protect human and animal health.

Infection prevention and control are interventions intended to minimise the spread of pathogens, decrease the likelihood of infection in a healthcare setting, and reduce the overall need for antimicrobials.

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1. Chapter one: Introduction

This chapter contains the background and review of the literature for the study.

1.1 Background

Bacteria and fungi are the two leading invasive pathogen groups causing neonatal sepsis. Bacteria are the most predominant of the two pathogen groups as most neonatal fungal infections are associated with either a primary bacterial infection, prolonged use of antibiotics, or long hospital stay. Presented here in the background bacterial and fungal microbiology, treatment, the emergence of resistance, prevention of infection, and the relationship between antimicrobial resistance (AMR) and vaccines in public health.

1.1.1 Clinical microbiological overview and taxonomy of the bacteria

Bacteria can be classified as gram-negative or gram-positive based on the appearance of the bacterial cell wall on a gram stain which guides the susceptibility testing profile before a full identification is done. Gram stain further classifies bacteria based on their unique morphology such as the cocci and the rods. Some of the techniques used to identify bacteria include the culture methods, serological techniques, polymerase chain reaction (PCR), and genome sequencing [1].

1.1.2 Fungi of clinical importance in neonatal sepsis

Candida species are the most common group of fungi isolated in neonatal infections [2]. An investigation of a large outbreak of fungal and bacterial bloodstream infections in a neonatal unit in Gauteng province, South Africa, reported that *Candida species* as the only fungal isolates with *Candida krusei, Candida albicans,* and *Candida parapsilosis* being most prevalent [3]. At Charlotte Maxeke Hospital in Johannesburg, *Candida albicans* (6.6%), *Candida parapsilosis* (2.5%), and *Candida glabrata* (1.0%) were reported the most prevalent fungal isolates while at Tygerberg Children's Hospital in Cape town, *Candida albicans* (11%) was reported as the most prevalent fungal isolate in neonatal sepsis cases in a neonatal intensive care unit [4, 5].

1.1.3 Historical overview and introduction of antibiotics into clinical use

From an evolutionary perspective, antibiotics initially occurred naturally as secretions of environmental bacteria and fungi that help to protect each species from other environmental microorganisms [6]. It is therefore on this basis that a microorganism does not secrete a substance that causes it to self-destruct, which is a basis for an intrinsic mechanism that a bacterium uses to protect itself. Penicillin, the first antibiotic widely used in clinical practice,

was accidentally discovered and isolated from nature in 1928 and the discovery of other classes of antibiotics followed until the 1980s when the pipeline of discoveries dried up except for structurally modified derivatives of the parent antibiotics [7, 8]. The spectrum of activity of these antibiotics ranges from a narrow spectrum, which is specific to a species of bacteria, to a broad spectrum which is effective against a range of bacterial species [8].

1.1.4 Emergence of antimicrobial resistance

The advent of antibiotics made it possible to treat infections that caused significant mortality and morbidity in the pre-antibiotic era. However, the extensive use of antibiotics in human as medicine, agriculture, and veterinary medicine has since been associated with an increase in the emergence of antibiotic-resistant bacteria [9]. The clinical introduction of Penicillin in 1943 resulted in penicillin-resistant *Pneumococcus* becoming prevalent in 1965 while the clinical introduction of Methicillin in 1960 was followed by highly prevalent Methicillin-resistant *Staphylococcus* in 1962 [10]. This trend has persisted, including among the most recent antibiotics derivatives such as ceftaroline, a fourth-generation cephalosporin introduced in 2010 to treat Methicillin-resistant *Staphylococcus aureus* (MRSA) [11]. Antibiotic derivatives are developed on the understanding of the structure-activity relationships between a bacterial cell structure used as antibiotics target and the mechanism of action of that particular antibiotic. Inevitably, they do not remain effective for long. This is because mutation or acquisition of foreign genetic material can alter a particular structure of the organism or alter how it performs known functions, making the bacterial cell less susceptible to antibiotics.

Below is a tabular presentation of historical timelines of new antibiotics introduced for clinical use and the emergence of resistant clinical isolates for selected antibiotics [10, 11]

Table 1.1: Historical timelines of new antibiotics introduced.

Antibiotic	Year of clinical introduction	Year resistance is reported following clinical introduction	Time-point between clinical introduction and resistance (years)
Penicillin	1943	1965	22
Methicillin	1960	1962	2
Tetracycline	1950	1959	9
Erythromycin	1953	1968	15
Gentamicin	1967	1979	12
Vancomycin	1972	1988	16
Imipenem	1985	1998	13
Levofloxacin	1996	1996	0
Linezolid	2000	2001	1
Ceftaroline	2010	2010	0

Antimicrobial resistance occurs when microorganisms develop mechanisms to evade the therapeutic effects of drugs, making infections difficult to treat. The occurrence of resistance to antibiotics can be an intrinsic mechanism facilitated by innate natural adaptation processes or acquired through mutations, acquisition of genetic elements such as plasmids. Selective pressure from nature and antibiotic use can accelerate resistance while physical forces such as wind, run-off water, and biological forces like human migration, insect vectors, are responsible for its spread [13]. However, resistance can occur without selective pressure of antibiotics, implying that resistance genes pre-date human antibiotic use in clinical practice and the spontaneous loss of such genes following antibiotic withdrawal is rare [14, 15]. Once resistance genes are acquired by the bacteria, they can be passed within species through vertical transmission and to unrelated pathogens through horizontal gene transfer, making AMR containment difficult [16].

Resistance to antibiotics is already widely reported in neonatal sepsis cases in South Africa. A study from a neonatal intensive care unit (NICU) at Tygerberg Children's Hospital Stellenbosch University reported confirmed episodes of bloodstream infections (BSI) in 11% of admitted patients with 46% mortality, and 36% of deaths directly linked to the infection [5]. In this study, all *Staphylococcus aureus* was reported methicillin-resistant, 59% of *Klebsiella pneumoniae* were Extended-Spectrum-Beta Lactamase (ESBL) producers while *Acinetobacter baumannii* isolates demonstrated low susceptibility to Carbapenems, Aminoglycosides, and Cephalosporins. Furthermore, the reported overall mortality rate among neonates admitted with ESBL-producing *Klebsiella species* in the neonatal unit of CHBAH was 30% (16). This was based on the analysis of data from neonatal sepsis cases that received empirical antibiotic treatment, indicating that the recommended empiric therapeutic agents are not effective to treat ESBL-producing *Klebsiella pneumoniae* sepsis.

1.1.5 Prevention of Antimicrobial resistance

The World Health Organisation (WHO) recommends a 'one health' approach to combat the growing emergence and impact of AMR. This global, holistic, and multi-sectoral approach involves human health, animal health, and the environment because resistant bacteria which infect animals can infect humans and circulate in the environment [9]. The WHO executive board meeting of the 24th January 2014 recommended several action points to control AMR which include the following amongst others [17]:

- Increase political awareness, engagement, and leadership regarding AMR stewardship.

- Strengthen infection prevention and control (IPC) in communities and healthcare facilities.
- Strengthen international collaboration among global partners.
- Strengthen pharmaceutical management systems as well as laboratory infrastructure.
- Monitor the extent of antimicrobial resistance observed and intensify research.

The WHO also recommends AMR stewardship alongside clean water, hygiene, and sanitation (WASH), vaccines for more pathogens, and breastfeeding as measures to prevent neonatal sepsis [18]. However, the leading causes of AMR sepsis which include *Acinetobacter baumannii, Klebsiella pneumoniae* and *Escherichia coli* do not have licensed vaccines.

1.1.6 Recommended antibiotic treatment for neonatal sepsis

The WHO recommended first-line empiric antibiotic therapy for neonatal sepsis is Ampicillin and Gentamicin while third-generation Cephalosporins are the recommended second-line treatment [19]. In South Africa, the recommended first-line empiric antibiotic treatment for neonatal sepsis is Gentamicin and Ampicillin; Piperacillin/Tazobactam and Amikacin are the second-line treatment, while Amphotericin B is recommended for suspected fungal infections [20].

1.1.7 Challenges with the treatment of resistant pathogens

The introduction of resistance in predominant causes of neonatal sepsis – *Escherichia coli, Klebsiella* spp, *Staphylococcus aureus, Streptococcus* spp, *Acinetobacter* spp, and *Pseudomonas* spp – has resulted in poor outcomes from treatment with recommended antibiotics [21]. It is estimated that 50% of the bacterial causes of neonatal infections leading to sepsis are resistant to first-line and second-line WHO-recommended treatment regimens [22]. Pathogens such as *Klebsiella pneumoniae* which are resistant to first-line (inherent resistance) and second-line WHO-recommended treatment options require Carbapenems, but the emergence of CPE requires the use of Colistin, the last-line antibiotic not licensed in South Africa and yet has widely reported resistance patterns [17]. Potential last resort alternatives to colistin for neonatal sepsis include Fosfomycin and Tigecycline. Fosfomycin develops resistance rapidly when used as monotherapy while the use of tigecycline in neonates is limited due to inadequate safety data in neonatal populations [23, 24]. Lack of reliable safety data on potential last-line antibiotics for the treatment of multidrug-resistant infections, limits antibiotic choices for neonates [22, 24]. The Department of Health of South Africa reported susceptibility to Vancomycin by *Enterococcus faecium* ranging between 33 to 100%, three-quarters of *Klebsiella pneumoniae* isolated from hospitalised patients were ESBL producers while CPE

was reported to be widely spread throughout South Africa [17]. Vancomycin is a reserve antibiotic, and the last line treatment for many infections caused by gram-positive pathogens, therefore, emerging resistance to Vancomycin makes it extremely difficult to treat resistant gram-positive pathogens difficult.

Therefore, it is on this basis that strategies to prevent neonatal infections through vaccine development for pathogens resistant to antibiotics should be urgently explored to contain AMR.

1.1.8 Relationship between antimicrobial resistance and vaccines in public health

Vaccines are widely recognised to reduce the prevalence of resistant strains as observed with *Diphtheria* and *Pertussis,* which eventually promotes the use of narrow-spectrum antibiotics if needed as empirical therapy and this benefit is extended even to the unvaccinated through herd immunity [25]. Furthermore, the use of vaccines to prevent infections will result in less infections which leads to reduced use of antibiotics hence slowing down development of AMR. We, therefore, present evidence to support decisions on which vaccines in the development pipeline could be prioritised, as most pathogens causing neonatal sepsis do not have licensed vaccines

1.2 Literature review

This section provides a global, regional, country-specific perspective of AMR, and the AMR situation at CHBAH. Reviewed here is literature about community-acquired infections (CAI), and nosocomial infections. Covered areas include the epidemiology, hospital infection control practices, pathogenesis, factors associated with severe outcomes of neonatal sepsis, antibiotic prescriptions and consumption patterns in South Africa.

1.2.1 The global situation of AMR

Antimicrobial-resistant pathogens have a common occurrence across the globe. The global AMR surveillance report of the WHO indicates that *Escherichia coli, Klebsiella pneumoniae, Salmonella* spp*, Acinetobacter* spp*, Staphylococcus aureus, Streptococcus pneumoniae, Neisseria gonorrhea, and Shigella* spp are the most reported pathogens resistant to antibiotics [26]. A group of six AMR pathogens commonly termed 'ESKAPE' pathogens which includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter species* have been recognised as having growing multidrug resistance and increased virulence. Prevalence of these organisms can vary within a country, between countries, or between geographical regions. In Mali, *Klebsiella species (35%), Escherichia coli (27.5%),* and *Enterobacter cloacae (22.5%)* are the most

predominant AMR pathogens among neonatal sepsis cases(27). Meanwhile in Zambia, the prevalence of the AMR pathogens reported indicates *Klebsiella species (75%), Staphylococcus aureus (6%), a*nd *Escherichia coli (5%)* in that order(28).

1.2.2 Antimicrobial resistance in South Africa

The six leading causes of bacterial sepsis among young infants reported from CHBAH include gram-positive isolates (*Streptococcus agalactiae, Streptococcus pneumoniae, Staphylococcus aureus*) and gram-negative isolates (*Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp*) [29]. Published literature describing antimicrobial susceptibility profiles and case fatality rate in *Acinetobacter baumannii* sepsis at CHBAH has reported 17% extreme drug resistance [30]. This occurs when clinical isolates of the bacteria are susceptible to only one or two categories of antibiotics. Extreme resistance of *Acinetobacter baumannii* which is widely reported as a leading cause of neonatal deaths in sepsis cases is therefore a significant public health concern.

The sources of these pathogens may be community-acquired, or nosocomial. In South Africa, *Escherichia coli,* and *Staphylococcus aureus* are largely reported as community-acquired infections; *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterococcus faecalis, Enterococcus faecium,* and *Candida species* are reported as nosocomial infections[31, 32]. Understanding the source of infection can offer useful guidance for developing effective strategies to prevent these infections.

The drivers of AMR are well described in South Africa. The National Department of Health (NDoh) published a report 2018 which shows that South Africa has the highest rate of antibiotic consumption in Africa, and consumes between 1.3 to 3.3 more antibiotics than Brazil, Russia, India, and China (BRICS), whereas its consumption compared to the developed countries such as the UK and the USA also ranks higher by 0.8 [32]. Antibiotic exposure is well established as a driver of AMR, the higher the exposure, the bigger the risk of AMR. Successful containment of AMR in South Africa will therefore require reduction in the consumption of antibiotics even if this involves taking away some of the benefits.

The IPC both at home and in hospitals are established strategies to minimise the effects of AMR, and these require the full commitment of the healthcare workers and the community. The WHO has recommended strategies for healthcare facilities as infection control mechanisms which include hand hygiene, environment cleaning, disinfection, sterilisation, isolation of infected patients and standard precautions for the healthcare workers to protect themselves from blood borne infections [33]. However, published literature from the Gauteng region established

overcrowding (12% beyond capacity) in the neonatal ward, poor ventilation, and inadequate isolation facilities which all meet the criteria of risk factors for transmission of potential AMR pathogens [3]. Another study from four different hospitals in South Africa reported a breakdown in IPC practices resulting from low staffing of dedicated IPC nurses, few neonatal isolation rooms, low compliance of doctors (27.4%) and nurses (27.8%) compared to mothers (42.1%) in terms of hand hygiene as a means of infection control mechanism [34]. Poor hand hygiene among healthcare workers has been identified as a primary source for transmission of some commensal bacteria such as the *Coagulase-negative staphylococci*, implicating its most prominent family member, *Staphylococcus epidermidis* [35]. Poor IPC practices are well established risks for the spread of AMR pathogens, and thus IPC needs to improve in South Africa

Published findings of a study regarding prescribing practices and adherence to guidelines in a primary care setting in Cape Town metro city reported only 45.1% of prescriptions adhered to guidelines, 30.5% of diagnoses were undocumented, 21.6% of the antibiotics were prescribed without need, 12.5% antibiotics were prescribed in a wrong dose while 11.5% was an incorrect drug [36]. Irrational use of antibiotics results in unnecessary exposure to antibiotics which increases the risk of developing AMR. Promoting rational use of antibiotics will be crucial to address the development and impact of AMR.

1.2.3 Factors associated with severe outcomes of antimicrobial resistant infections in neonatal sepsis cases in Low and Middle Income Countries

In low- and middle-income countries (LMICs), there are established risk factors for AMR neonatal sepsis, and risk factors associated with prolonged hospital stay, or even increased chances of death. From CHBAH, preterm delivery and lower birth weight have been established as risk factors for increased chances for neonatal deaths following sepsis [37]. In Ethiopia, lower birth weight, preterm delivery, low Apgar score, and caesarean section delivery were all reported as risk factors for neonatal sepsis [38]. Whether these factors were associated with severe outcomes of sepsis was not reported. A study from India, a LMIC comparable to South Africa, reported risk factors for *candidemia*-associated neonatal sepsis as low birth weight, prolonged use of intravenous antibiotics, and the presence of central venous lines which are factors easily relatable to CHBAH, where prolonged use of antibiotics and long hospital stays are very common [39].

1.3 Problem statement

One of the primary causes of neonatal deaths in South Africa are infections which could be treated with effective antimicrobials. However, high AMR rates among the causal organisms have resulted in neonatal deaths from severe infections. In South Africa, data on AMR patterns among invasive strains remains largely unaddressed. Moreover, the paediatric antimicrobial stewardship in South Africa is largely confined to urban academic settings with limited expertise amongst doctors, pharmacists, nurses, and clinical associates, making routine AMR reporting difficult [40].

The WHO has recognised AMR among the top ten threats to public health in 2019 [41]. Global mortality from AMR is expected to rise from 700 000 deaths annually to 10 million by 2050 alongside financial losses exceeding USD10 trillion annually [42]. Every year, there are between 214 000 and 690 000 neonatal deaths globally due to sepsis, and AMR is estimated to account for 31% of the deaths [43]. There is also unexplained high morbidity and mortality during the first 12 months following treatment in LMICs (18). However, the paucity of data on the prevalence of AMR patterns in neonatal sepsis in LMICs including South Africa is a barrier to successful containment.

1.4 Justification for the study

There is significant progress in the reduction of mortality in children under the age of five years from leading causes such as preterm birth complications, pneumonia, malaria, and diarrhoea. However, mortality due to neonatal sepsis remains unacceptably high despite efforts aimed at its prevention, early diagnosis, and treatment. Antimicrobial-resistant pathogens contribute significantly to this high mortality despite the 'one health' approach to contain AMR. Antimicrobial resistance drives infection to sepsis conditions as a result of suboptimal or no response to antibiotic therapy. This study aims to add evidence required for standardised surveillance programs for collecting AMR data critical for the rational design of interventions, policy decisions, essential medicines list, antibiotics alternatives, and generate potential areas for exploring vaccine use for microorganisms causing serious infections.

1.5 Study aim and objectives

1.5.1 Aim

This study aimed to describe AMR patterns among pathogens isolated from admitted neonates with sepsis at CHBAH from January 2016 to December 2018.

Knowledge of prevalent bacteria and their antimicrobial susceptibility patterns will provide crucial evidence required to recommend effective interventions and provide a basis for ongoing research. This data is critical to advance the potential need to develop vaccines for AMR pathogens. There is a paucity of such prevalent data in neonatal sepsis cases at CHBAH.

1.5.2 Objectives

1. To describe the bacterial and fungal causes of neonatal sepsis at Chris Hani Baragwanath Academic Hospital from January 2016 to December 2018.
2. To describe the AMR profile of bacterial and fungal clinical isolates (culture proven blood and CSF isolates) from neonatal sepsis cases.
3. To describe the proportion of isolates that are resistant to recommended empiric treatment options stratified according to common causes of bacterial sepsis in neonates.

2. Chapter Two: Methods

2.1 Study design

This research is a descriptive retrospective study using results of AMR patterns of clinical isolates from neonatal sepsis cases at CHBAH. All admitted neonatal sepsis cases during the three-year period from January 2016 to December 2018 were included in the study. All bacterial and fungal clinical isolates from every considered case in the final sample were analysed.

Hospital laboratory records for the admitted cases were retrospectively reviewed. This data was matched with the sepsis surveillance data stored at the Vaccines and Infectious Diseases Analytics (VIDA) research unit formerly Respiratory and Meningeal Pathogens Research Unit (RMPRU) containing both the neonatal and maternal variables. This was linked to the hospital total neonatal cases database to ensure that the final data only included cases that are captured by the hospital database. Only patients with culture-confirmed bacterial and fungal sepsis were considered in the final dataset. This population had a matching laboratory and clinical data. The selected neonates had matching maternal data which was necessary to establish their risk for AMR sepsis.

The VIDA conducts surveillance of pathogens associated with sepsis in young infants admitted at CHBAH including risk factors. This data is collected and stored as excel sheets at the research unit. Samples collected for analysis of AMR profiles from the admitted neonates were analysed at the NHLS laboratory at CHBAH. This data was then stored at the NHLS Central Data Warehouse (CDW) at the Baragwanath hospital. Included in this data were all admitted neonatal sepsis cases. The data about the total hospital neonatal sepsis cases was also provided by the NHLS from the CDW, containing all suspected and confirmed cases of neonatal sepsis. All the data provided was for neonates admitted within the first 28 days of life.

The data collected amongst others included: basic non-identifying demographics, possible risk factors for AMR neonatal sepsis, AMR profile of isolated pathogens, outcome determination, etc. Some of the prenatal factors collected about the mother included maternal HIV status, HAART status, and gestational age while some of the postnatal data collected were neonatal birth weight, age of onset of sepsis, and mode of delivery.

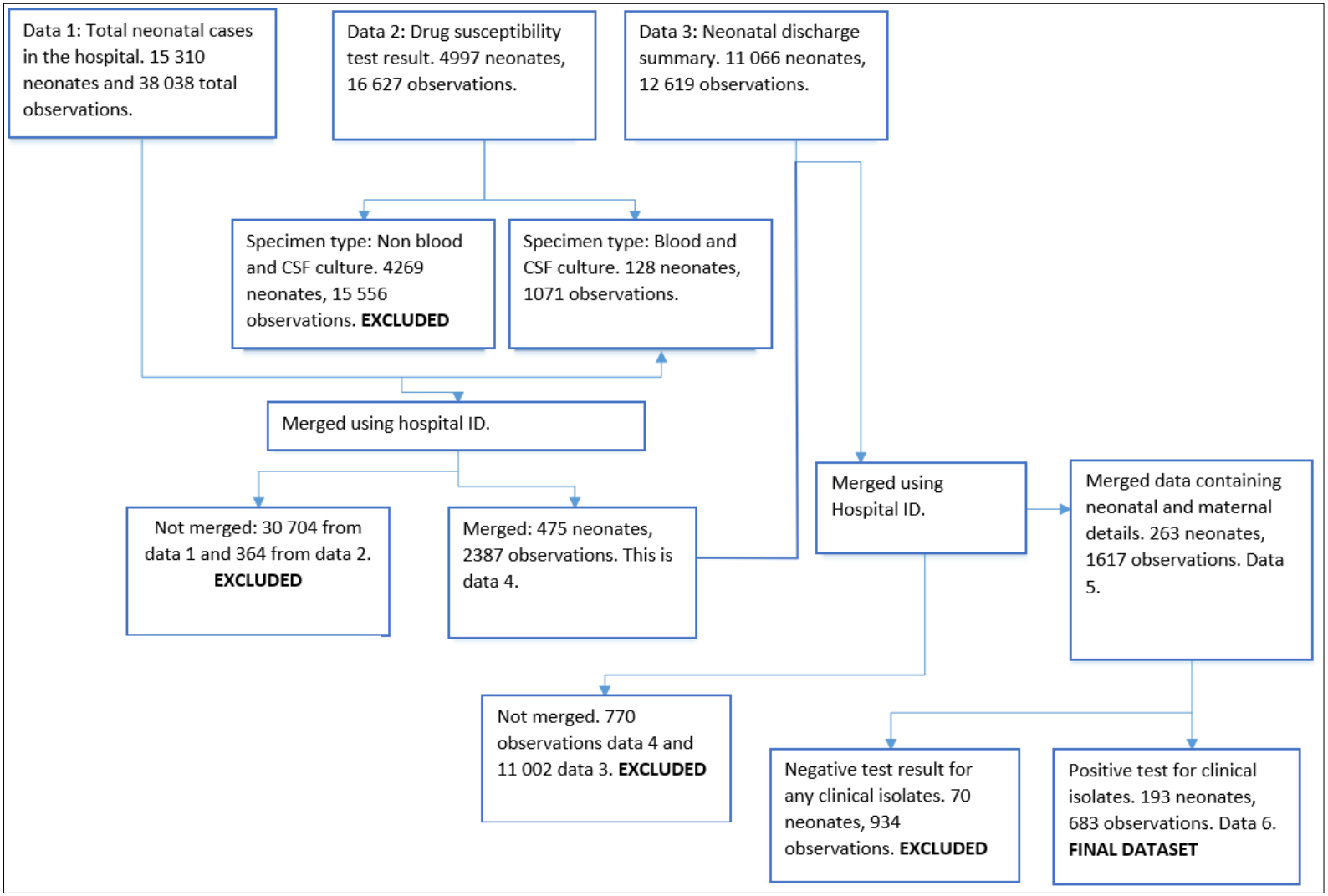
2.2 Study population

Admitted neonates at the neonatal unit of CHBAH between January 2016 and December 2018 diagnosed with sepsis were included in the study population.

2.3 Sample size

The study involved a three-year analysis of AMR patterns starting from January 2016 to December 2018. The sample size was determined as shown in the flow chart below.
In the final sample, only 192 cases out of 11,066 admitted cases had matching neonatal and maternal variables with corresponding drug susceptibility test results. All cases with missing data were not considered. And all admitted cases with culture negative test results were discarded. The total number of observations are the total number of tests done.

Figure 2.1: Sample selection process.



2.4 Data collection

Data was reviewed from NHLS microbiology reports that were recorded on MS Excel sheets and then analysed. The microbiology laboratory at CHBAH performs routine susceptibility testing of isolated bacteria. Blood and cerebrospinal fluid (CSF) samples were subjected to gram stain and the results guide on the choice of antimicrobial profile considered for susceptibility testing. The choice of what isolates were considered for antimicrobial profile was determined in the laboratory and results reported in excel sheets. Analysis was done using the CLSI guidelines for laboratory diagnosis of bacterial and fungal isolates [44].

A low-level resistance occurs when the clinical isolates exhibit a higher minimum inhibitory concentration (MIC) that is commonly recognised for the susceptible population, which is devoid of any acquired resistance mechanism. Intermediate resistance occurs when clinical isolates have minimum inhibitory concentrations which are similar to that attained in blood and tissues levels but for which response rates may be lower than for susceptible isolates. Meanwhile, high resistance implies that isolates are not inhibited by the concentrations of the antibiotics which are usually achievable with normal dosage schedules.

Demographic and clinical data for neonates identified to have positive isolates from normally sterile sites were abstracted from the neonatal discharge/death summary. Attending doctors complete paper summaries of the discharge/death summaries once a child has been discharged or has died were also reviewed. These data were entered into an electronic SQL database, which is managed by the staff at the Respiratory and Meningeal Pathogens Research Unit (RMPRU). Permission to utilise data was obtained from NHLS, RMPRU, the Department of Paediatrics, and Medical Advisory Committee of CHBAH.

2.5 Study setting

The study was conducted at CHBAH located in Soweto, Johannesburg. This is the second-largest hospital in the world and the largest in the Southern Hemisphere. It serves as the major tertiary level referral hospital serving the catchment areas of Soweto. Soweto is a densely populated urban setting largely comprising people with a low socio-economic status where unemployment rates are very high.

The CHBAH is the major teaching hospital for the medical school of the University of the Witwatersrand. The neonatal care services at CHBAH serve neonates both inborn and out born from the neighbouring medical facilities, including those delivered at home.

The neonatal inpatient care services consist of 179 beds containing 18 level IIIB ICU beds, 42 high care nursery beds, 100 beds general neonatal ward, and 19 Kangaroo mother care wards. CHBAH delivers 23 000 of the 32 000 annual birth cohort of the Soweto area.

Neonates admitted at either the neonatal wards or ICU receive empiric antibiotic therapy, and the blood samples collected are sent to the NHLS laboratory based at the hospital for culture and susceptibility testing. All positive cultures that yield microorganisms are subjected to antimicrobial susceptibility testing. Microbiologists review the AMR profiles and recommend the most appropriate drug of choice electronically or verbally to the attending physician. If no significant clinical improvement is observed in the neonate by the attending physicians, further tests are requested, and the sample is sent to the laboratory for analysis to recommend the most appropriate antimicrobials.

2.6 Inclusion criteria

All invasive culture-confirmed neonatal sepsis cases with corresponding results for antimicrobial susceptibility testing of pathogenic isolate/s available, collected between January 2016 and December 2018.

The neonates included had their data matched with maternal data to ascertain their risks for AMR sepsis.

All cases included in the final data were those that have been recorded in the hospital sepsis database, and had drug susceptibility test results that matched with the data in the discharge summary form.

2.7 Exclusion criteria

The study excluded all non-culture confirmed neonatal sepsis cases or cases whose corresponding results for antimicrobial susceptibility testing and/or clinical data were not available.

Neonates whose sample collected for analysis were not blood or CSF were excluded from the final data for analysis.

All results for neonates who were not registered sepsis cases on the hospital sepsis database were not included in the final analysis.

2.8 Data management

Data sets from the total hospital neonatal cases database, neonatal discharge summary, and the drug susceptibility testing, all available as MS Excel sheets, were merged and analysed using Stata.

Descriptive statistics, frequencies and proportions were used to summarise categorical variables. Pie charts, and bar charts were also used to give a visual presentation of some categorical data. Bivariate analysis between categorical variables was assessed using chi-square tests. Summarising continuous variables was done using median and interquartile range since the data was not normally distributed. Median differences between either the neonate discharged, or deceased were compared using the Mann-Whitney Test. Significance was set at 5%.

2.9 Ethics clearance

Ethics clearance was obtained from the Human Research Ethics Committee (HREC) – Medical School of the University of the Witwatersrand before commencing the study. The ethics clearance certificate number is M191071

2.10 Report dissemination

The final report is submitted to the postgraduate research office of the Faculty of Health Sciences, the University of the Witwatersrand in partial fulfilment of the requirements for the award of the Master of Science in Medicine (Vaccinology). Findings may be presented in scientific meetings, workshops, and published in online scientific journals.

3. Chapter 3: Results

3.1 Descriptive statistics of the data and outcome after admission

In this study, there were 1647 neonatal samples taken for laboratory analysis. Only 683 samples were culture-positive. These 683 samples (independent tests) correspond to 193 neonates, seen in Table 3.1. Some neonates either had negative culture results, missing AMR profiles, or mismatch between neonatal and maternal data sets. Where clinical and laboratory results did not match, they were also excluded. As shown in table 3.1, most neonates had multiple tests done (between 2 and 21) owing to the long hospital stay. These were not rule out sepsis evaluations but rather tests to establish AMR profile among cases admitted with culture confirmed neonatal sepsis.

Table 3.1: Frequency distribution of the total number of samples (independent tests) done per admitted neonate with culture confirmed sepsis to determine the AMR profiles of the clinical isolates from admission to outcome.

Number of samples (independent tests) done per neonate	Discharged after admission n (%)	Died after admission n (%)	Total n (%)
1	135 (27.66)	58 (29.74)	193 (28.26)
2	86 (17.62)	37 (18.97)	123 (18.01))
3	60 (12.3)	29 (14.87)	89 (13.03)
4	47 (9.63)	23 (11.79)	70 (10.25)
5	34 (6.97)	15 (7.69)	49 (7.17)
6	27 (5.53)	9 (4.62)	36 (5.27)
7	21 (4.3)	5 (2.56)	26 (3.81)
8	19 (3.89)	5 (2.56)	24 (3.51)
9	16 (3.28)	2 (1.03)	18 (2.51)
10	10 (2.05	1 (0.51)	11 (1.61)
11	7 (1.43)	1 (0.51)	8 (1.17)
12	6 (1.23)	1 (0.51)	7 (1.02)
13	4 (0.82)	1 (0.51)	5 (0.73)
14	4 (0.82)	1 (0.51)	5 (0.73)
15	4 (0.82)	1 (0.51)	5 (0.73)
16	3 (0.61)	1 (0.51)	4 (0.59)
17	3 (0.61)	1 (0.51)	4 (0.59
18	1 (0.2)	1 (0.51)	2 (0.29)
19	1 (0.2)	1 (0.51)	2 (0.29)
20	0	1 (0.51)	1 (00.15)
21	0	1 (0.51)	1 (0.15)
Total	**488 (100)**	**195 (100)**	**683 (100)**

Of the 193 neonates considered in this study, 58 (30%) died while 135 (70%) were either discharged home or transferred.

3.2 Baseline characteristics of the neonates

Table 3.2 shows the descriptive statistics of the neonates. The median age at admission was 0 days, as most of the neonates were admitted at birth. The median birth weight was 1435 grams with an interquartile range (IQR) of 1135-2180 grams. The neonates who died following admission had a lower median birth weight of 1225 grams compared to those who were discharged home with a median birth weight of 1550 grams. The median birth weights significantly differed between these two groups, with P=0.0036 compared using the Mann-Whitney Test. The median gestation age was 31 weeks with an IQR of 29-35 weeks which was also significantly different between those neonates who died after admission compared to those who were discharged, with P=0.0137. Lastly, the median agpar_5 score was 9 with an IQR of 7-10 scores, and this was not significantly different between the two groups of neonates, with P=0.1582.

Table 3.2: Baseline continuous characteristics of the neonates with culture-confirmed AMR neonatal sepsis stratified by the outcome status after admission.

Variable	Overall n=193	Died after admission n=58	Discharged after admission n=135	P-value
Age at admission (days) (n=193) **Median (IQR)**	0	0	0	0.5411
Birth weight (grams) (n=193) **Median (IQR)**	1435 (1135-2180)	1225 1045-2000)	1550 (1245-2277)	**0.0036**
Gestation age (weeks) **Median (IQR)**	obs=188 31 (29-35)	obs=133 30 (27-34)	obs=133 31(29-35)	**0.0137**
Apgar_1 score	obs=174	obs=49	obs=125	0.4006

Median (IQR)	6.5(5-8)	6(4-8)	7(5-8)	
Apgar_5 score	obs=174	obs=48	obs=126	0.1582
Median (IQR)	9(7-10)	8.5(7-9)	9(7-10)	

SD=standard deviation, IQR=interquartile range, bold-faced values=significant at 5%, obs=number of observations

Table 3.3 shows the baseline categorical factors of the neonates and the maternal factors exposed to them. There were more males (46.1%, n=89) in this study compared to females (45.6%, n=88). The females had a higher percentage of death (48.3%, n=28) compared to the males (44.8%, n=88); however, this was not significantly different (P=0.838). There were 13.9% (n=27) of neonates whose mothers were HIV positive at pregnancy and taking HAART; however, being exposed to HIV during pregnancy had no association with the admission outcome. The neonates were admitted to different wards during their hospitalisation period. Most of the neonates did not require admission to intensive care units (ICU) (n=142, 73.58%). The results show that there was no significant difference between being admitted in an ICU and the admission outcome status, with P=0.407.

Table 3.3: Baseline factor characteristics of the neonates with culture-confirmed AMR sepsis and maternal factors they were exposed to stratified by the outcome status after admission.

Variable	Overall n (%)	Died after admission n (%)	Discharged after admission n (%)	P-value
Gender				
Female	88 (45.6)	28 (48.28)	60 (44.44)	
Male	89 (46.11)	26 (44.83)	63 (46.67)	0.838
Unknown	16 (8.29)	4 (6.9)	12 (8.89)	
Method of delivery				
Caesarean	105 (54.4)	30 (51.72)	75 (55.56)	
Vaginal	84 (43.52)	25 (43.1)	59 (43.7)	0.138
Missing	4 (0.07)	3 (5.17)	1 (0.74)	
RVD				
Negative	124 (64.25)	36 (62.07)	88 (65.19)	0.639
Positive	63 (32.64)	21 (36.21)	42 (31.11)	

	Unknown	6 (3.11)	1 (1.72)	5 (3.7)	
HIV treatment status					
	On treatment	27 (13.99)	6 (10.34)	21 (15.56)	0.299
	Not on treatment	36 (18.86)	15 (25.86)	21 (15.56)	
	HIV negative	124 (64.25)	36 (62.07)	88 (65.19)	
	Unknown	6 (3.11)	1 (1.72)	5 (3.7)	
ICU					
	Yes	51 (26.42)	13 (22.41)	38 (28.15)	0.407
	No	142 (73.58)	45 (77.59)	97 (71.85)	

RVD=retroviral disease, HIV=Human immune deficiency syndrome, ICU=intensive care unit, bold-faced values=significant at 5%

Most of the neonates were delivered through caesarean section (n=105, 54.4%); however, there was no significant difference between the method of delivery and the admission outcome, as seen in Figure 3.2. Many of the neonates delivered through a caesarean section died after admission (51.7%) compared to those who had a vaginal delivery (43.1%).

With respect to the Retroviral disease (RVD) status of the mothers, 32.12% (n=62) of neonates had mothers who were RVD positive while 64.3% (n=124) of neonates had RVD negative mothers, as seen in Figure 3.3. There was no significant difference between RVD and admission outcome status, with p=0.517.

Of the Bacteria and the fungi positive clinical isolates identified, most of the clinical isolates were bacteria (88%, n=599) compared to fungi (12%, n=84), seen in Figure 3.1.

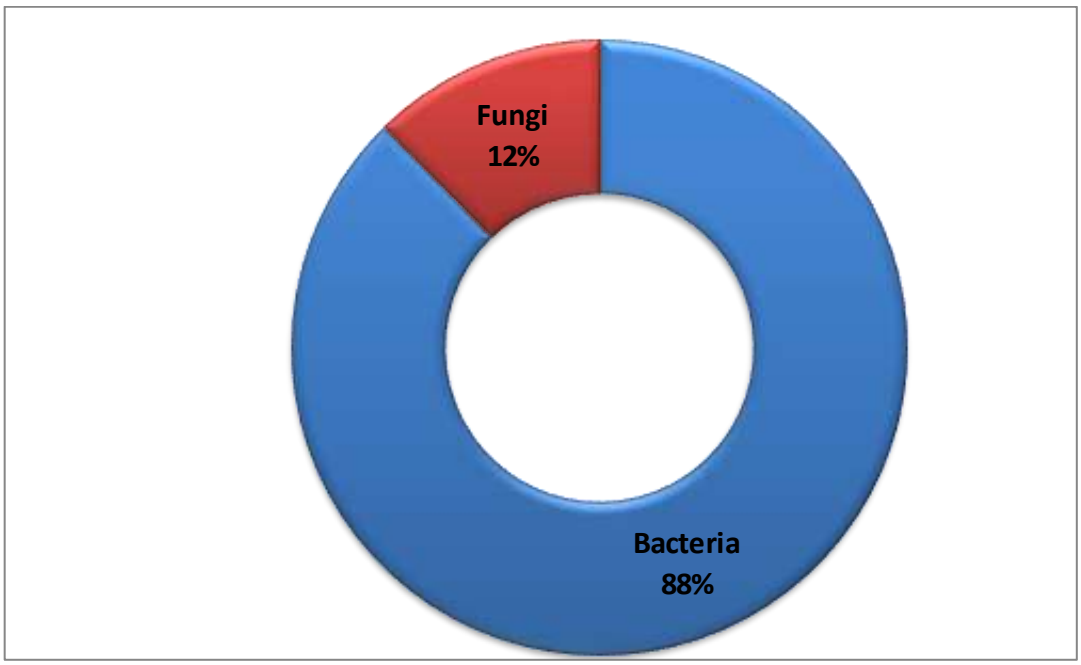


Figure 3.1: Pie chart distribution of the clinical isolates.

Of the 599 neonatal samples with bacterial isolates, the majority were gram-negative (n=317, 52.9%), however, this difference was not statistically significant (P=0.3961) with the gram positive- bacteria as seen in Figure 3.2.

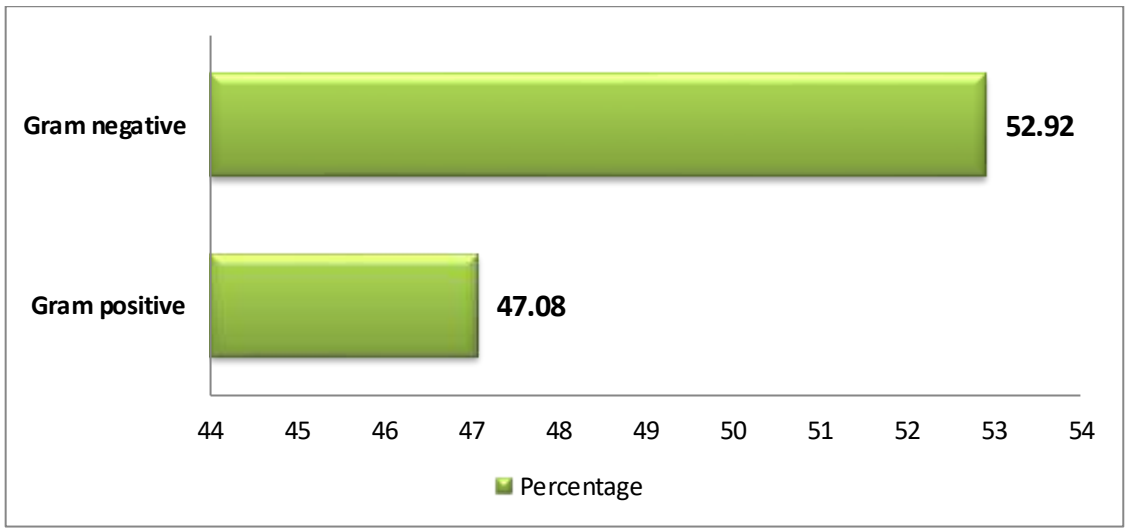


Figure 3.2: A horizontal bar chart of the classification of the isolated bacteria.

3.3 The frequency distribution of clinical isolates from blood cultures and CSF

The frequency distribution of all identified clinical isolates are summarised in Table 3.4. The most predominant isolates were the *Acinetobacter baumannii* (n=194, 30.1%), *Staphylococcus aureus* (n=109, 16.9%), *Staphylococcus epidermidis* (n=50, 7.3%), Coagulase-negative *staphylococci* (n=106, 16.4%) and *Klebsiella pneumoniae* (n=96, 14.9%),

Table 3.4: The frequency distribution of the isolated organisms.

Organism name	Frequency	Percentage	Death Associated n (%)
Acinetobacter baumannii	194	30.1	92 (47.18)
Staphylococcus aureus	109	16.9	26 (13.0)
Coagulase-negative *Staphylococci (CONS)*	106	16.4	20 (10.0)
Klebsiella pneumoniae	96	14.9	13 (6.67)
Candida parapsilosis	27	4.2	6 (3.18)
Enterococcus faecium	24	3.7	5 (2.56)
Candida albicans	18	2.8	5 (2.56)
Enterococcus faecalis	11	1.7	3 (1.54)
Bacillus species	9	1.3	1 (0.51)

Streptococcus salivarius	8	1.2	0
Viridans streptococcus	8	1.2	0
Escherichia coli	6	0.9	2 (1.03)
Enterobacter cloacae	5	0.8	3 (1.54)
Bacteroides corrodens	4	0.6	1 (0.51)
Serratia marcescens	4	0.6	0
Pseudomonas aeruginosa	3	0.4	0
Corynebacterium species	2	0.3	1 (0.51)
Streptococcus species	2	0.3	0
Burkholderia cepacian	1	0.1	0
Candida tropicalis	1	0.1	0
Corynebacterium confusum	1	0.1	1 (0.51)
Klebsiella species	1	0.1	0
Listeria monocytogenes	1	0.1	0
Proteus mirabilis	1	0.1	1 (0.51)
Providencia stuartii	1	0.1	0
Serratia liquefaciens	1	0.1	0
Streptococcus agalactiae	1	0.1	0
Total	**645**	**100**	**201(100)**

Coinfections were a common occurrence as summarised in Table 3.5 and Figure 3.6. Whereas most of the neonates had only a single isolate (52.85%, n=102) followed by those who had two isolates only (19.2%, n=37), the rest had multiple isolates with one neonate having eight different clinical isolates.

Table 3.5: The cumulative number of clinical isolates (co-infections) per neonate stratified by admission outcome.

Number of pathogens	Discharged after admission n (%)	Died after admission n (%)	Total n (%)
1	70 (51.85)	32 (55.17)	102 (52.85)
2	25 (18.52)	12 (20.69)	37 (19.17)
3	20 (14.81)	7 (12.07)	27 (13.99)
4	12 (8.89)	5 (8.62)	17 (8.81)
5	5 (3.7)	1 (1.72)	6 (3.11)
6	2 (1.48)	1 (1.72)	3 (1.55)

8	1 (0.74)	0	1 (0.52)
Total	**135 (100)**	**58 (100)**	**193 (100)**

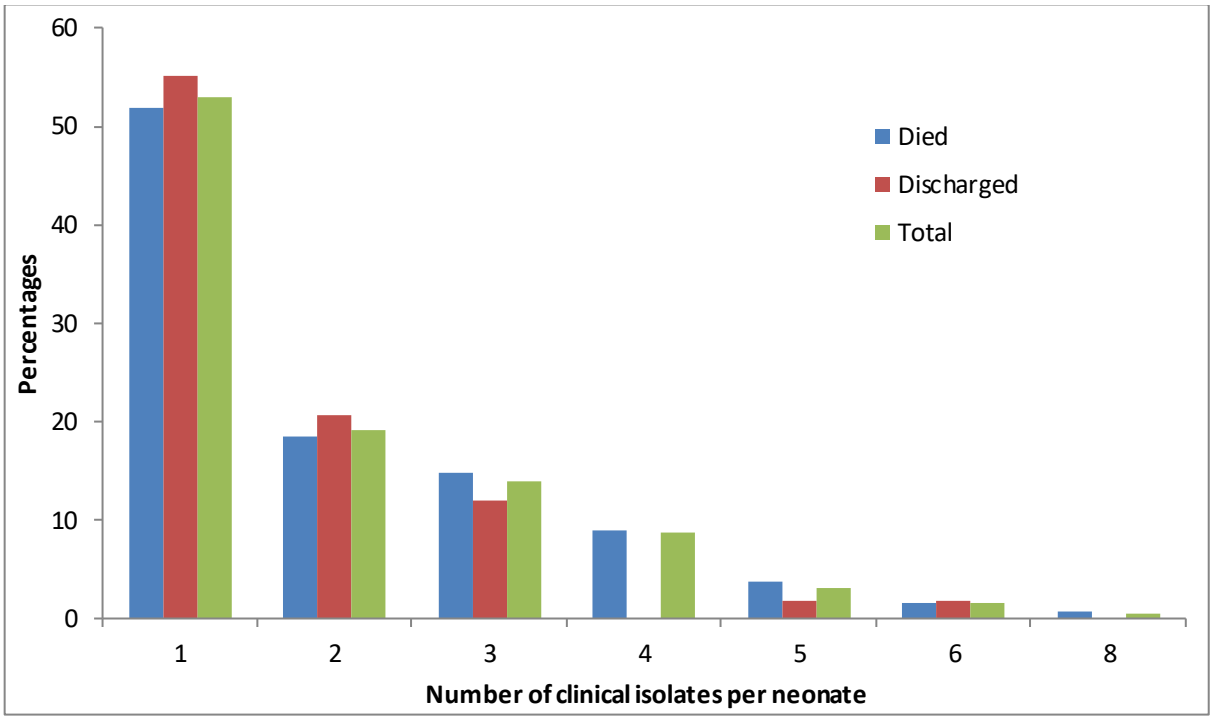


Figure 3.3: A cylindrical chart of the distribution of the number of clinical isolates per neonate.

Figure 3.6 above shows the distribution of the total number of clinical isolates per neonatal sepsis case as summarised in Table 3.5. As the number of clinical isolates per neonatal sepsis case increases, the chances of the admission outcome being death increases as well.

3.4 Antibiotic susceptibility tests against the isolated organisms

The antimicrobial susceptibility patterns of the clinical isolates are stratified in the tables below according to gram-negative bacteria, gram-positive bacteria and the fungi.

3.4.1 Antimicrobial susceptibility patterns of gram-negative bacteria

The series of tables below summarise the AMR profiles of the gram-negative bacteria isolated in neonatal sepsis cases.

Pseudomonas aeruginosa, and *Acinetobacter baumanni* AMR patterns

The resistance patterns of, *Acinetobacter baumanni*, and *Klebsiella pneumoniae* are summarised in Table 3.6. *Acinetobacter baumanni* was 87.5% susceptible to colistin. However, *Acinetobacter baumanni* was resistant to gentamicin (85.9%), cefepime (83.1%), imipenem (90.7%), ceftazidime (82.5%), and meropenem (93.1%).

Pseudomonas aeruginosa was 100% resistant to most of the antibiotics tested against including gentamicin, piperacillin/tazobactam, meropenem, imipenem, Cefepime, Ceftazidime, and amikacin.

Table 3.6: Antimicrobial susceptibility patterns of *Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii*.

	Pseudomonas Aeruginosa		*Acinetobacter baumannii*	
Antibiotic name	**S**	**R**	**S**	**R**
Gentamicin	-----	3 (100)	25 (14.12)	**152 (85.88)**
Ciprofloxacin	1 (33.33)	2 (66.67)	44 (25.73)	115 (67.25)
Trimethoprim-sulfamethoxazole	-----	3 (100)	0	19 (10.67)
Cefepime	-----	1 (100)	20 (16.95)	**98 (83.05)**
Imipenem	-----	1 (100)	11 (9.32)	**107 (90.68**
Ceftazidime	-----	1 (100)	20 (17.54)	**94 (82.46)**
Meropenem	-----	1 (100)	8 (6.9)	**108 (93.1)**
Amikacin	-----	1 (100)	33 (28.95)	**81 (71.05)**
Chloramphenicol	-----	2 (100)	38 (50.0)	38 (50.0)
Piperacillin/ Tazobactam	-----	1 (100)	6 (5.26)	**107 (93.86)**
Tobramycin	1 (100)	-----	16 (14.04)	98 (85.96)
Rifampicin				
Cefotaxime/ Ceftriaxone	-----	-----	1 (14.29)	6 (85.71)
Ertapenem	-----	-----		
Cefazolin	-----	-----	0(0)	**6 (100)**
Cefoxitin	-----	-----	0(0)	**6 (100)**
Nalidixic acid	-----	-----	0	
Gentamicin	-----	-----	5 (45.45)	6 (54.55)
Cefuroxime oral	-----	-----		
Colistin	22 (55)	**18 (45)**	7 (87.5)	1 (12.5)

R=resistant, S=susceptible, I=intermediate

***Klebsiella species, Klebsiella pneumoniae, Escherichia coli* and *Proteus Mirabilis* AMR patterns**

The resistance patterns of *Klebsiella species*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis* are summarised in Table 3.7. The results show a 78% resistance of *Klebsiella pneumoniae* to gentamicin, 97.1% resistance to ampicillin and ampicillin/amoxicillin, 90.6% resistance to cefazolin, cefoxitin and cefuroxime parenteral, 84.4% resistance to amoxicillin/clavulanic acid, 46.67% resistant to meropenem, 45.9 resistant to imipenem.

Escherichia coli had moderate susceptibility and resistance patterns. It was 75% susceptible to imipenem, meropenem, amikacin, and piperacillin tazobactam. However, *Escherichia coli* was 66.67% resistant to ampicillin, 66.67% resistant to gentamicin, and only 12.5% resistant to amikacin. It had 25% resistance to meropenem and piperacillin/tazobactam each, while there was also observed a 33.33% resistance to ciprofloxacin, 66.67% resistance to cefoxitin, and 83.3% resistant to trimethoprim-sulfamethoxazole.

Proteus mirabilis was either 100% susceptible or 100% intermediate to the antibiotics tested against. Some of the antibiotics that were 100% susceptible include gentamicin, ampicillin, amikacin, piperacillin/tazobactam, and meropenem.

Table 3.7: Antimicrobial susceptibility patterns of *Klebsiella species, Klebsiella pneumoniae, Escherichia coli and Proteus Mirabilis*.

	Klebsiella species		*Klebsiella pneumoniae*		*Escherichia coli*		*Proteus Mirabilis*
Antibiotic name	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Gentamicin	1 (100)	0(0)	16 (17.02)	**78 (82.98)**	2(33.33)	**4(66.67)**	1 (100)
Ciprofloxacin	1 (100)	0(0)	35 (37.23)	**40 (42.55)**	**4(66.67)**	2(33.33)	1 (100)
Trimethoprim-sulfamethoxazole	1 (100)	0(0)	22 (23.66)	71 (76.34)	1(16.67)	5(83.33)	1 (100)
Cefepime	-----	-----	16 (26.23)	**45 (73.77)**	1(25)	**3(75.0)**	1 (100)
Imipenem	-----	-----	25 (40.98)	**28 (45.9)**	3(75.0)	1(25.0)	1 (100)
Ceftazidime	-----	-----	16 (26.23)	**45 (73.77)**	1(25.0)	**3(75.0)**	1 (100)
Meropenem	-----	-----	24 (40.0)	**28 (46.67)**	3(75.0)	**1(25.0)**	1 (100)
Amikacin	-----	-----	23 (37.7)	**36 (59.02)**	**3(75.0)**	1(25.0)	1 (100)
Chloramphenicol	1 (100)	0(0)	35 (53.03)	31 (46.97)	2(40.0)	3(60.0)	-----
Piperacillin/Tazobactam	-----	-----	15 (24.59)	41 (67.21)	**3(75.0)**	1(25.0)	1 (100)
Tobramycin	-----	-----	5 (8.2)	56 (91.8)	1(25.0)	3(75.0)	-----
Rifampicin	1 (100)	0(0)	20 (58.82)	14 (41.18)	1(33.0)	2(66.67)	-----
Cefotaxime/Ceftriaxone	-----	-----	4 (12.12)	29 (87.88)	1(33.33)	**2(66.67)**	1 (100)
Ertapenem	-----	-----	25 (75.76)	8 (24.24)	3(100)	0(0)	1 (100)
Cefazolin	-----	-----	3 (9.38)	29 (90.63)	1(33.33)	**2(66.67)**	-----
Cefoxitin	-----	-----	3 (9.38)	29 (90.63)	1(33.33)	**2(66.67)**	-----
Amoxicillin/Clavulanic acid	-----	-----	5 (15.63)	27 (84.38)	2(66.67)	1(33.33)	1 (100)
Cefuroxime Parenteral	-----	-----	3 (9.38)	29 (90.63)	1(50.0)	1(50.0)	1 (100)
Nalidixic acid	-----	-----	13 (44.83)	14 (48.28)	2(66.67)	1(33.33)	1 (100)
Gentamicin	-----	-----	1 (100)	0(0)	-----	-----	-----
Colistin	-----	-----	1 (100)	0(0)	22(55.0)	18(33.33)	-----

R=resistant, S=susceptible, I=intermediate ----- = Not reported

***Bacteroides corrodens*, *Burkholderia cepacia*, and *Enterobacter cloacae* AMR patterns**

Burkholderia cepacia was generally resistant to most of the selected antibiotics tested against but it was 100% susceptible to tobramycin.

Enterobacter cloacae showed some moderate resistance (25%) to ciprofloxacin, imipenem, meropenem, and amikacin. It was 100% susceptible to Ertapenem, Nalidixic acid, and Colistin among others. On the contrary, it was highly resistant (100%) to Cefoxitin, Amoxicillin/Clavulanic acid, and Cefuroxime Parenteral.

Table 3.8: Antimicrobial susceptibility patterns of *Bacteroides corrodens, Burkholderia cepacia*, and *Enterobacter cloacae*

	Bacteroides corrodens		*Burkholderia cepacia*		*Enterobacter cloaca*	
Antibiotic name	**S**	**R**	**S**	**R**	**S**	**R**
Gentamicin	0(0)	3 (100)	0(0)	1 (100)	2 (50.0)	2 (50.0)
Ciprofloxacine	2 (50.0)	2 (50.0)	0(0)	1 (100)	3 (75.0)	1 (25.0)
Trimethoprim-sulfamethoxazole	0(0)	4 (100)	0(0)	1 (100)	2 (50.0)	2 (50.0)
Cefepime	0(0)	2 (100)	0(0)	1 (100)	2 (50.0)	2 (50.0)
Imipenem	1 (50.0)	1 (50.0)	0(0)	1 (100)	3 (75.0)	1 (25.0)
Ceftazidime	0(0)	2 (100)	0((0)	1 (100)	2 (50.0)	2 (50.0)
Meropenem	1 (50.0)	1 (50.0)	0(0)	1 (100)	3 (75.0)	1 (25.0)
Amikacin	1 (50.0)	1 (50.0)	0(0)	1 (100)	3 (75.0)	1 (25.0)
Chloramphenicol	1 (33.33)	2 (66.67)	_____	_____	2 (66.67)	1 (33.33)
Piperacillin/Tazobactam	1 (50.0)	1 (50.0)	0(0)	1 (100)	3 (75.0)	1 (25.0)
Tobramycin	0(0)	2 (100)	1 (100)	0(0)	_____	_____
Penicillin/Ampicillin	0(0)	3 (100)	_____	_____	_____	_____
Ampicillin/Amoxicillin	0(0)	1 (100)	_____	_____	1 (25.0)	3 (75.0)
Cefotaxime/Ceftriaxone	0(0)	1 (100)	_____	_____	2 (66.67)	1 (33.33)
Ertapenem	1 (100)	0(0)	_____	_____	3 (100)	0(0)
Cefazolin	0(0)	1 (100)	_____	_____	0(0)	3 (100)
Cefoxitin	___0(0)__	1 (100)	_____	_____	___0(0)__	3 (100)
Amoxicillin/Clavulanic acid	0(0)	1 (100)	_____	_____	0(0)	3 (100)
Cefuroxime Parenteral	0(0)	1 (100)	_____	_____	0(0)	3 (100)
Nalidixic acid	1 (100)	0(0)	_____	_____	3 (100)	0(0)
Gentamicin	_____	_____	_____	_____	1 (100)	0(0)
Streptomycin	_____	_____	_____	_____	1 (100)	0(0)
Colistin	_____	_____	_____	_____	1 (100)	0(0)

R=resistant, S=susceptible, ____ = Not reported

Providencia stuartii, Serratia liquefaciens and Serratia marcescens patterns

The resistance patterns of *Providencia stuartii, Serratia liquefaciens,* and *Serratia marcescens* are summarised in Table 3.9.

A single sample for *Serratia liquefaciens* was 100% susceptible to all the antibiotics tested against including Gentamycine, Chloramphenicol, and Rifampicin. No resistance was observed.

Serratia marcescens was 100% resistant to Ceftazidime, Tobramycin, Ampicillin, Cefepime, Ertapenem, and Colistin. It was 100% susceptible to Gentamicin, Clindamycin, and Vancomycin.

Table 3.9: Antimicrobial sensitivity patterns of *Providencia stuartii, Serratia liquefaciens* and *Serratia marcescens*

	Providencia Stuartii		*Serratia liquefaciens*	*Serratia marcescens*	
Antibiotic name	**S**	**R**	**S**	**S**	**R**
Gentamicin	0(0)	1 (100)	1 (100)	1 (100)	_____
Ciprofloxacin	0(0)	1 (100)	1 (100)	1 (25.0)	3 (75.0)
Trimethoprim-sulfamethoxazole	0(0)	1 (100)	1 (100)	1 (25.0)	3 (75.0)
Cefepime	_____	_____	_____	0(0)	4 (100)
Imipenem	_____	_____	_____	1 (25.0)	3 (75.0)
Ceftazidime	_____	_____	_____	0(0)	4 (100)
Meropenem	_____	_____	_____	1 (25.0)	3 (75.0)
Amikacin	_____	_____	_____	1 (25.0)	3 (75.0)
Chloramphenicol	0(0)	1 (100)	1 (100)	1 (50.0)	1 (50.0)
Piperacillin/Tazobactam	_____	_____	_____	1 (25.0)	3 (75.0)
Tobramycin	_____	_____	_____	0(0)	4 (100)
Penicillin/Ampicillin	0(0)	1 (100)	1 (100)	0(0)	1 (100)
Ampicillin/Amoxicillin	_____	_____	_____	0(0)	1 (100)
Cefotaxime/Ceftriaxone	_____	_____	_____	0(0)	1 (100)
Ertapenem	_____	_____	_____	1 (100)	0(0)
Cefazolin	_____	_____	_____	0(0)	1 (100)
Cefoxitin	_____	_____	_____	0(0)	1 (100)
Amoxicillin/Clavulanic acid	_____	_____	_____	0(0)	1 (100)
Cefuroxime Parenteral	_____	_____	_____	0(0)	1 (100)
Nalidixic acid	_____	_____	_____	1 (100)	0(0)
Colistin	_____	_____	_____	0(0)	2 (100)

R=resistant, S=susceptible ____=Not reported

3.4.2 Antimicrobial susceptibility patterns of gram-positive bacteria

The series of tables below describe the AMR profiles of the isolated gram-positive bacteria.

Corynebacterium confusum* and *Corynebacterium species patterns

The resistance patterns of *Corynebacterium confusum* and *Corynebacterium species* isolated organisms are summarised in Table 3.10.

The single sample tested for *Corynebacterium confusum* against different antibiotics showed either 100% sensitivity or 100% resistance to the antibiotics. The antibiotics which were 100% sensitive to the single sample include Chloramphenicol, Clindamycin, Vancomycin, and Rifampicin while it was 100% resistant to Gentamicin, Ciprofloxacin, and Ampicillin among others.

Other *Corynebacterium species* had similar AMR profiles to the identified *Corynebacterium confusum* as shown in the table below.

Table 3.10: Antimicrobial sensitivity patterns of *Corynebacterium confusum* and *Corynebacterium species.*

	Corynebacterium confusum		*Corynebacterium species*	
Antibiotic name	**S**	**R**	**S**	**R**
Gentamicin	0(0)	1 (100)	0(0)	2 (100)
Ciprofloxacin	0(0)	1 (100)	0(0)	1 (100)
Trimethoprim-sulfamethoxazole	0(0)	1 (100)	0(0)	2 (100)
Cefepime	____	____	0(0)	1 (100)
Imipenem	____	____	0(0)	1 (100)
Ceftazidime	____	____	0(0)	1 (100)
Meropenem	____	____	0(0)	1 (100)
Amikacin	____	____	0(0)	1 (100)
Chloramphenicol	1 (100)	0(0)	1 (100)	0(0)
Piperacillin/Tazobactam	____	____	0(0)	1 (100)
Tobramycin	____	____	0(0)	1 (100)

Penicillin/Ampicillin	0(0)	1 (100)	____	____
Fusidic acid	1 (100)	0(0)	1 (100)	0(0)
Rifampicin	1 (100)	0(0)	1 (100)	0(0)

R=resistant, S=susceptible ____ = Not reported

Staphylococcus aureus, Staphylococcus haemolyticus, and *Staphylococcus hominis* antimicrobial resistance patterns

The AMR profiles of *Staphylococcus aureus, Staphylococcus haemolyticus,* and *Staphylococcus Hominis* are summarised in Table 3.11.

Staphylococcus aureus has 100% susceptibility to linezolid and vancomycin, fusidic acid (90.6%), and Rifampicin (81.3%). However, it was resistant to Cloxacillin (70%), Gentamicin (89.5%), ciprofloxacin (86.2%), trimethoprim-sulfamethoxazole (96.3%), and cefepime and ceftazidime both at 80.6% resistance. A 100% resistance was observed for Imipenem, Meropenem, and Piperacillin/Tazobactam.

Staphylococcus haemolyticus was 100% susceptible to vancomycin, linezolid, and ertapenem but 100% resistant to, ampicillin, and ampicillin/amoxicillin.

Staphylococcus Hominis was 100% susceptible to Vancomycin, Linezolid, and Clindamycin but 100% resistant to Gentamicin, Cloxacillin, and Meropenem among other antibiotics.

Table 3.11: Antimicrobial susceptibility patterns of *Staphylococcus aureus, Staphylococcus haemolyticus* , and *Staphylococcus hominis.*

	Staphylococcus aureus		*Staphylococcus haemolyticus*		*Staphylococcus hominis*	
Antibiotic name	**S**	**R**	**S**	**R**	**S**	**R**
Gentamicin	6 (10.53)	51 (89.47)	3 (50.0)	3 (50.0)	0(0)	3 (100)
Ciprofloxacin	8 (13.79)	50 (86.21)	4 (66.67)	2 (33.33)	2 (66.67)	1 (33.33)
Trimethoprim-sulfamethoxazole	2 (3.7)	52 (96.3)	1 (16.67)	5 (83.33)	0(0)	3 (100)
Cefepime	5 (19.23)	21 (80.77)	1 (25.0)	3 (75.0)	1 (100)	0(0)
Imipenem	0(0)	**26 (100)**	3 (75.0)	1 (25.0)	0(0)	1 (100)

Ceftazidime	5 (19.23)	21 (80.77)	1 (25.0)	3 (75.0)	1 (100)	0(0)
Meropenem	0(0)	**16 (100)**	2 (66.67)	1 (33.33)	0(0)	1 (100)
Amikacin	12 (46.15)	14 (53.85)	-----	-----	0(0)	1 (100)
Chloramphenicol	12 (35.29)	22 (64.71)	4 (66.67)	2 (33.33)	0(0)	2 (100)
Tobramycin	4 (15.38)	22 (84.62)	1 (33.33)	2 (66.67)	0(0)	1 (100)
Vancomycin	37 (100)	0(0)	4 (100)	0(0)	2 (100)	0(0)
Linezolid	36 (100)	0(0)	4 (100)	0(0)	2 (100)	0(0)
Clindamycin	26 (74.29)	9 (25.71)	4 (100)	0(0)	2 (100)	0(0)
Erythromycin/Azithromycin	5 (14.29)	30 (85.71)	2 (50.0)	2 (50.0)	0(0)	2 (100)
Penicillin/Ampicillin	1 (2.86)	**34 (97.14)**	0(0)	4 (100)	0(0)	2 (100)
Fusidic acid	29 (90.63)	3 (9.38)	2 (50.0)	2 (50.0)	1 (50.0)	1 (50.0)
Rifampicin	26 (81.25)	6 (18.75)	2 (50.0)	2 (50.0)	1 (50.0)	1 (50.0)
Cloxacillin	6 (20.0)	**24 (80.0)**	2 (50.0)	2 (50.0)	0(0)	1 (100)
Ampicillin/Amoxicillin	0(0)	**4 (100)**	0(0)	3 (100)	-----	-----
Cefotaxime/Ceftriaxone	1 (11.11)	8 (88.89)	1 (33.33)	2 (66.67)	-----	-----
Cefazolin	-----	-----	1 (33.33)	2 (6.67)	-----	-----
Cefoxitin	-----	-----	1 (33.33)	2 (66.67)	-----	-----
Nalidixic acid	-----	-----	1 (100)	0(0)	-----	-----
Gentamicin	2 (50.0)	2 (50.0)	-----	-----	-----	-----
Streptomycin	2(50.0)	2(50.0)	-----	-----	-----	-----

R=resistant, S=susceptible ----- = Not reported

Enterococcus faecalis and *Enterococcus faecium* antimicrobial resistance patterns

Table 3.12 shows the antimicrobial sensitivity tests for *Enterococcus faecalis* and *Enterococcus faecium* against the listed antibiotics. There were some similarities in response to antibiotics between the two pathogens as shown below.

Table 3.12: Antimicrobial susceptibility patterns of *Enterococcus faecalis* and *Enterococcus faecium.*

	Enterococcus faecalis			*Enterococcus faecium*		
Antibiotic name	**S**	**R**	**I**	**S**	**R**	**I**
Ciprofloxacin	6 (66.67)	2 (22.22)	1 (11.11)	5 (31.25)	10 (62.5)	1 (6.25)
Trimethoprim-sulfamethoxazole	1 (11.11)	8 (88.89)	0(0)	1 (6.25)	15 (93.75)	0(0)
Cefepime	1 (33.33)	2 (66.67)	0(0)	1 (9.09)	10 (90.91)	0(0)
Imipenem	2 (66.67)	1 (33.33)	0(0)	1 (9.09)	10 (90.91)	0(0)
Ceftazidime	1 (33.33)	2 (66.67)	0(0)	1 (9.09)	10 (90.91)	0(0)
Meropenem	2 (66.67)	**1 (33.33)**	0(0)	1 (9.09)	10 (90.91)	0(0)
Amikacin	2 (66.67)	**1 (33.33)**	0(0)	3 (27.27)	**8 (72.73)**	0(0)
Chloramphenicol	3 (75.0)	1 (25.0)	0(0)	3 (60.0)	2 (40.0)	0(0)
Vancomycin	8 (100)	0(0)	0(0)	12 (100)	0(0)	0(0)
Linezolid	8 (100)	0(0)	0(0)	12 (100)	0(0)	0(0)
Penicillin/Ampicillin	4 (66.67)	**2 (33.33)**	0(0)	5 (83.33)	**1 (16.67)**	0(0)

Rifampicin	2 (100)	0(0)	0(0)	0(0)	1 (100)	0(0)
Cloxacillin	5 (83.33)	1 (16.67)	0(0)	4 (80.0)	1 (20.0)	0(0)
Ampicillin/Amoxicillin	0(0)	4 (100)	0(0)	1 (11.11)	8 (88.89)	0(0)
Cefotaxime/Ceftriaxone	1 (50.0)	1 (50.0)	0(0)	1 (33.33)	2 (66.67)	0(0)
Nalidixic acid	1 (50.0)	1 (50.0)	0(0)	1 (50.0)	1 (50.0)	0(0)
Gentamicin high level resistance	1 (50.0)	1 (50.0)	0(0)	2 (28.57)	5 (71.43)	0(0)
Streptomycin high level resistance	1 (50.0)	1 (50.0)	0(0)	5 (71.43)	2 (28.57)	0(0)

R=resistant, S=susceptible, I=intermediate

Both pathogens showed a 100% susceptibility to vancomycin and linezolid and an 83.3% susceptibility to clindamycin. Contradicting results were observed for rifampicin to which *Enterococcus faecalis* was 100% susceptible while *Enterococcus faecium* was 100% resistant. Individually, *Enterococcus faecalis* was 75% susceptible to chloramphenicol, and cloxacillin (83.3%) while 100% resistant to ampicillin/amoxicillin. Individually, *Enterococcus faecium* showed resistance to trimethoprim-sulfamethoxazole (93.8%) and a 90.9% resistance to cefepime, imipenem, ceftazidime, and meropenem.

***Streptococcus species*, *Streptococcus salivarius*, Methicillin-resistant *Staphylococcus*, and *Streptococcus agalactiae* antimicrobial resistance patterns**

Table 3.13 shows the antimicrobial sensitivity tests for *Streptococcus species*, *Streptococcus salivarius, Viridans streptococcus,* and *Streptococcus agalactiae*.

Table 3.13: Antimicrobial susceptibility patterns of *Streptococcus species, Streptococcus salivarius, Viridans streptococcus,* and *Streptococcus agalactiae*

	Streptococcus species		*Streptococcus salivarius*		*Viridans streptococcus*		*Streptococcus agalactiae*
Antibiotic name	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Gentamicin	0(0)	1 (100)	0(0)	8 (100)	2(50)	2(50)	____
Ciprofloxacin	0(0)	1 (100)	0(0)	8 (100)	1(25)	3(75)	____
Trimethoprim-sulfamethoxazole	1 (100)	____	____	____	1(25)	3(75)	____
Cefepime	____	____	____	____	0(0)	2(100)	____
Imipenem	____	____	____	____	0(0)	2(100)	____
Ceftazidime	____	____	____	____	0(0)	2(100)	____
Meropenem	____	____	____	____	0(0)	2(100)	____
Amikacin	____	____	____	____	0(0)	2(100)	
Chloramphenicol	1 (100)	0(0)	8 (100)	0(0)	1(50)	1(50)	1 (100)
Piperacillin/Tazobactam	____	____	____	____	0(0)	3(100)	1 (100)

Tobramycin	____	____	____	____	0(0)	2(100)	____
Vancomycin	1 (100)	0(0)	8 (100)	0(0)	4(100)	0(0)	1 (100)
Linezolid	0(0)	2 (100)	0(0)	8 (100)	4(100)	0(0)	1 (100)
Clindamycin	1 (50.0)	1 (50.0)	0(0)	8 (100)	2(66.67)	1(33.3)	1 (100)
Erythromycin/Azithromycin	0(0)	2 (100)	0(0)	8 (100)	1(33.33)	2(66.6)	1 (100)
Penicillin/Ampicillin	0(0)	2 (100)	____	____	____	____	____
Fusidic acid	0(0)	1 (100)	0(0)	8 (100)	1(50)	1(50)	____
Rifampicin	0(0)	1 (100)	0(0)	8 (100)	1(50)	1(50)	____
Cloxacillin	0(0)	1 (100)	____	____	1(50)	1(50)	____
Ampicillin/Amoxicillin	0(0)	1 (100)	____	____	0(0)	2(100)	____
Cefotaxime/Ceftriaxone	____	____	____	____	1(50)	1(50)	1 (100)
Ertapenem	____	____	____	____	____	____	____
Cefazolin	____	____	____	____	____	____	____
Cefoxitin	____	____	____	____	____	____	____
Amoxicillin/Clavulanic acid	____	____	____	____	____	____	____
Cefuroxime Parenteral	____	____	____	____	____	____	____
Nalidixic acid	____	____	____	____	____	____	____
Gentamicin high-level resistance	0(0)	1 (100)	____	____	0(0)	2(100)	____
Streptomycin high-level resistance	0(0)	1 (100)	____	____	1(100)	0(0)	____

R=resistant, S=susceptible ____= Not reported

Streptococcus species was 100% resistant to most antibiotics including gentamycin, ampicillin, and ciprofloxacin while it was 100% susceptible to chloramphenicol, vancomycin, and trimethoprim-sulfamethoxazole.

Streptococcus salivarius was 100% resistant to gentamycin, ampicillin, clindamycin, and ciprofloxacin but 100% susceptible to chloramphenicol and vancomycin which were the only two antibiotics with activity against the pathogen.

The *Viridans streptococcus* was 100% susceptible to vancomycin, and linezolid,

.

Streptococcus agalactiae was 100% susceptible to all the antibiotics it was tested against.

***Coagulase-negative staphylococcus, Staphylococcus epidermidis* and *Methicilllin-resistant staphylococcus aureus* antimicrobial resistance patterns**

Table 3.14 shows the AMR profiles of *Coagulase-negative staphylococcus, Staphylococcus epidermidis,* and *Viridans streptococcus* against the listed antibiotics.

Coagulase-negative staphylococcus was 100% susceptible to vancomycin, linezolid, nitrofurantoin, cefoxitin, and amoxicillin/clavulanic acid. It had 57.1% resistance to meropenem and imipenem while showing 71.43% resistance to piperacillin/tazobactam and 42.86% resistance to amikacin.

Staphylococcus epidermidis was 100% susceptible to linezolid and streptomycin high-level resistance while it showed 100% resistance to ampicillin/amoxicillin and gentamicin high-level resistance antibiotics.

Methicillin-resistant Staphylococcus was susceptible to most of the antibiotics; however, it showed 100% resistance to amikacin, ampicillin/amoxicillin, and chloramphenicol

Table 3.14: Antimicrobial susceptibility patterns of *Coagulase-negative staphylococcus, Staphylococcus epidermidis,* and *Viridans streptococcus.*

	Coagulase-negative Staphylococcus			*Staphylococcus epidermidis*			*Methicillin-resistant Staphylococcus*	
Antibiotic name	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**
Gentamicin	16 (40.0)	24 (60.0)	____	4 (9.52)	**38 (90.48)**	____	1(100)	0(0)
Ciprofloxacin	11 (28.21)	27 (69.23)	1 (2.56)	12 (26.67)	27 (60.0)	6 (13.33)	1(100)	0(0)
Trimethoprim-sulfamethoxazole	4 (10.3)	35 (89.7)	____	5 (11.11)	40 (88.89)	____	1(100)	0(0)
Cefepime	2 (14.29)	**12 (85.71)**	____	4 (15.38)	22 (84.62)	____	1(100)	0(0)
Imipenem	6 (42.9)	**8 (57.1)**	____	7 (26.92)	**17 (65.38)**	2 (7.69)	1(100)	0(0)
Ceftazidime	3 (21.43)	11 (78.57)	____	4 (16.0)	21 (84.0)	____	1(100)	0(0)
Meropenem	6 (42.9)	**8 (57.1)**	____	7 (26.92)	**17 (65.38)**	2 (7.69)	1(100)	0(0)
Amikacin	8 (57.14)	6 (42.86)	____	13 (50.0)	**13 (50.0)**	____	0(0)	1(100)
Chloramphenicol	17 (53.13)	15 (46.88)	____	12 (41.38)	17 (58.62)	____	0(0)	1(100)
Piperacillin/Tazobactam	4 (28.57)	**10 (71.43)**	____	6 (23.08)	**20 (76.92)**	____	1(100)	0(0)
Tobramycin	4 (28.6)	10 (71.4)	____	5 (19.23)	21 (80.77)	____	1(100)	0(0)
Vancomycin	34 (100)	0(0)	____	23 (95.83)	**1 (4.17)**	____	____	____
Linezolid	34 (100)	0(0)	____	23 (100)	____	____	____	____
Clindamycin	17 (58.62)	12 (41.38)	____	17 (77.27)	5 (22.73)	____	____	____
Erythromycin/Azithromycin	3 (10.3)	26 (89.7)	____	7 (31.82)	15 (68.18)	____	____	____
Penicillin/Ampicillin	1 (3.57)	**27 (96.4)**	____	1 (4.55)	**21 (95.45)**	____	____	____
Fusidic acid	16 (57.1)	12 (42.9)	____	18 (81.82)	4 (18.18)	____	____	____
Rifampicin	17 (60.7)	11 (39.29)	____	9 (40.91)	13 (59.09)	____	____	____
Cloxacillin	7 (26.92)	**19 (73.1)**	____	2 (8.7)	**21 (91.3)**	____	____	____
Ampicillin/Amoxicillin	4 (40.0)	**6 (60.0)**	____	0(0)	**10 (100)**	____	0(0)	1(100)
Cefotaxime-ceftriaxone	2 (33.33)	4 (66.67)	____	1 (7.69)	**8 (61.54)**	4 (30.77)	1(100)	0(0)

Ertapenem	____	5 (100)	____	7 (77.78)	**2 (22.22)**	____	1(100)	0(0)
Cefazolin	____	**5 (100)**	____	1 (11.11)	**8 (88.89)**	____	1(100)	0(0)
Cefoxitin	____	**5 (100)**	____	1 (11.11)	**8 (88.89)**	____	1(100)	0(0)
Amoxicillin/Clavulanic acid	____	5 (100)	____	1 (12.5)	7 (87.5)	____	1(100)	0(0)
Cefuroxime Parenteral	1 (25.0)	3 (75.0)	____	1 (11.11)	8 (88.89)	____	1(100)	0(0)
Nalidixic acid	2 (40.0)	3 (60.0)	____	5 (71.43)	1 (14.29)	1 (14.29)	1(100)	0(0)
Gentamicin high-level resistance	5 (83.3)	1 (16.7)	____	0(0)	1 (100)	____	____	____
Streptomycin high-level resistance	5 (83.3)	1 (16.7)	____	1 (100)	0(0)	____	____	____
Nitrofurantoin	3 (100)	0(0)	____	____	____	____	____	____

R=resistant, S=susceptible, I=Intermediate, ____= Not reported

***Listeria Monocytogenes* and *Bacillus species* AMR patterns**

The AMR profiles of *Listeria Monocytogenes* and *Bacillus species* are summarised in (Table 3.15). The single sample of *Listeria Monocytogenes* pathogen was generally resistant against all the considered antibiotics it was tested against including Gentamicin, Cefepime, and Meropenem. The *Bacillus species* was susceptible to Vancomycin, Rifampicin, Linezolid, and Ertapenem while showing 100% resistance to Ampicillin/Amoxicillin, Cefazolin, and Cefoxitin among other antibiotics.

Table 3.15: Antimicrobial susceptibility patterns of *Listeria Monocytogenes* and *Bacillus species*.

	Listeria Monocytogenes	*Bacillus species*	
Antibiotic name	**R**	**S**	**R**
Gentamicin	1 (100)	5 (55.56)	4 (44.44)
Ciprofloxacin	1 (100)	6 (66.67)	2 (22.22)
Trimethoprim-sulfamethoxazole	1 (100)	1 (11.11)	8 (88.89)
Cefepime	1 (100)	1 (33.33)	2 (66.67)
Imipenem	1 (100)	2 (66.67)	1 (33.33)
Ceftazidime	1 (100)	1 (33.33)	2 (66.67)
Meropenem	1 (100)	2 (66.67)	1 (33.33)
Amikacin	1 (100)	2 (66.67)	1 (33.33)
Chloramphenicol	_____	3 (75.0)	1 (25.0)
Piperacillin/Tazobactam	1 (100)	1 (33.33)	2 (66.67)
Tobramycin	1 (100)	1 (33.33)	2 (66.67)
Vancomycin	_____	8 (100)	0(0)
Linezolid	_____	8 (100)	0(0)
Clindamycin	_____	5 (83.33)	1 (16.67)
Erythromycin	_____	4 (66.67)	2 (33.33)
Penicillin/Ampicillin	_____	4 (66.67)	2 (33.33)
Fusidic acid	_____	5 (83.33)	1 (16.67)
Rifampicin	_____	2 (100)	0(0)
Cloxacillin	_____	5 (83.33)	1 (16.67)
Ampicillin/Amoxicillin	_____	0(0)	4 (100)

Cefotaxime/Ceftriaxone	_____	1 (50.0)	1 (50.0)
Ertapenem	_____	2 (100)	0(0)
Cefazolin	_____	0(0)	2 (100)
Cefoxitin	_____	0(0)	2 (100)
Amoxicillin/Clavulanic acid	_____	0(0)	2 (100)
Cefuroxime Parenteral	_____	0(0)	2 (100)
Nalidixic acid	_____	1 (50.0)	1 (50.0)
Gentamicin	_____	1 (50.0)	1 (50.0)
Streptomycin	_____	1 (50.0)	1 (50.0)

R=resistant, S=susceptible, ____ = Not reported

3.4.3 Antimicrobial susceptibility patterns of *candida* species

Below is a summary of the AMR profiles of the three identified candida species

***Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*, AMR patterns**

The *candida* species had relatively good susceptibility to the available antifungals. *Candida albicans* were 100% susceptible to Fluconazole and Voriconazole. *Candida parapsilosis* were 100% susceptible to Amphotericin B, 80.7% susceptible to Voriconazole, but 73.5% resistant to Fluconazole. *Candida tropicalis* were 100% sensitive to Fluconazole.

Table 3.16: Antimicrobial susceptibility patterns of *Candida albicans, Candida parapsilosis, Candida tropicalis*

	Candida Albicans		*Candida parapsilosis*		*Candida Tropicalis*	
Antibiotic name	**S**	**R**	**S**	**R**	**S**	**R**
Amphotericin B	____	____	2 (100)	0(0)	____	____
Fluconazole	10 (100)	0(0)	5 (14.71)	**25(73.53)**	1 (100)	0(0)
Voriconazole	**2 (100)**	0(0)	**25(80.7)**	1 (3.23)	____	____

3.5 Summary

High prevalence of AMR was observed among the leading causes of neonatal sepsis and known risk factors were common among the study population. Below, we discuss these findings.

4. Chapter 4: Discussion

The purpose of this retrospective descriptive study was to describe AMR patterns among pathogens isolated from admitted neonatal sepsis cases at CHBAH from January 2016 to December 2018. This chapter includes a discussion of major findings as related to the baseline characteristics of neonatal risks for AMR sepsis, the prevalence of the causative invasive pathogens causing sepsis, AMR profile of all the clinical isolates, and the AMR profile of the clinical isolates to recommended empiric treatment stratified according to the common causes of AMR sepsis. The chapter concludes with a discussion of the limitations of the study, areas for future research, and general conclusions of the chapter.

4.1 Baseline characteristics of the neonates and the admission outcome

The overall mortality rate from the admitted neonatal sepsis cases with AMR was 30%. This is in keeping with the findings from the Tygerberg's Children's Hospital that reported 36% mortality among admitted neonates in the ICU directly linked to the infection, however, the findings are consistent with a previous study at CHBAH which reported an overall mortality rate of 30% among the ESBL producing *Klebsiella spp,* [5, 12]. The findings are also consistent with the global neonatal sepsis mortality estimate of 30% due to AMR [45]. This highlights the enormity of the impact of AMR in neonatal sepsis and suggests that the impact of the AMR does not greatly differ within a country or across geographical regions.

Most neonates were admitted on day 0 of birth. Age at admission did not have any significant contribution to the admission outcome (P=0.5411) since most neonates were admitted the same day they were born. Apgar's score did not also have any significance on the admission outcome (P=0.4006, Apgar-1-score) and (P=0.1582, Apgar-5-score). Lower birth weight (P=0.0036) and preterm delivery (P=0.0137) were associated with an increased risk of death. These appear to be established risk factors and have been widely reported including at CHBAH [37]. Preterm delivery, low birth weight, caesarean section, and low Apgar score were reported as risk factors for neonatal sepsis, however, the effect of lower Apgar score on the admission outcome following infection with resistant pathogens appears not to be widely studied [38]. These findings show that the admission outcome following infection with AMR sepsis can only be improved by effective antibiotics.

Death among the females in the study was 28(48.28%) following admission compared to their male counterparts 26(44.83%). The difference in mortality between males and females was not

statistically significant (P=0.838). Higher male susceptibility to neonatal sepsis is well reported in LMICs and reasons for this occurrence warrant further research [46, 47].

The HIV status was not associated with AMR as the difference in mortality was not statistically significant among the HIV exposed and unexposed (P=0.639). Most HIV exposed neonates had mothers already on HAART. Equally, HAART treatment did not appear to significantly alter the mortality rates among the HIV exposed or the HIV exposed and non-exposed neonates (P=0.299). It has been previously established at CHBAH that the proportion of neonatal sepsis cases did not significantly differ by HIV exposure status [47]. This is consistent with this study's findings which goes on to suggest that HIV exposure does not significantly alter the outcome of AMR sepsis in the absence of effective antibiotics.

Fewer neonates (26.4%) required ICU admission. ICU admission is often a proxy for the severity of the disease. However, in this study there were more deaths (77.6%) among neonates not admitted in the ICU compared to 26.4% admitted in the ICU. This was because most neonates enrolled in the study were not admitted to the ICU. The admission outcome was not statistically different between the two groups (P=0.407). It, therefore, suggests that admission outcomes can only be improved by effective antimicrobials regardless of the place of admission.

4.2 Discussion on Objective 1

The first objective of the study was to describe the bacterial and nonbacterial causes of neonatal sepsis at CHBAH from January 2016 to December 2018. This was achieved through a quantitative analysis of all clinical isolates and the numbers converted to percentages to obtain their frequencies. This objective was achieved, and the results are shown from pages 20 to 23. Included in the discussions of this objective is the frequency of occurrence of all clinical isolates which includes both the gram-negative and gram-positive bacterial isolates and the fungi.

Acinetobacter baumanni (30.1%) and *Klebsiella pneumoniae* (14.06%) were the most prevalent gram-negative bacteria while *Staphylococcus aureus* (16.9%), and Coagulase-negative *staphylococci* (16.4%) were the most prevalent gram-positive bacterial isolates. *Acinetobacter baumannii* and *Klebsiella pneumoniae* being the most prevalent gram-negative isolates is consistent with previous findings from CHBAH which reported *Staphylococcus aureus* as the most prevalent gram-positive isolate [29]. High prevalence of CNS considered a contaminant, is an indication of failure in infection control practices during sample collection or patient care. Poor infection control practices among care providers can result in the introduction of commensal bacteria into sterile sites leading to sepsis.

Some of the ESKAPE pathogens had lower prevalence and these include the *Pseudomonas aeruginosa* (0.44%), *Escherichia coli* (0.88%), *Enterococcus faecium* (3.51%), and *Enterococcus faecalis* (1.61%). This study's findings of *Pseudomonas aeruginosa* are similar to previous findings from CBHAH that reported a 1% prevalence in neonates while *Escherichia coli* has previously been highly reported with a prevalence of 8% contrary to this study's findings [47]. *P. aeruginosa* is largely a healthcare-associated pathogen; its low prevalence indicates that the practices at CHBAH have been successful in preventing its infection. *Escherichia coli* is widely reported as a community-acquired infection in South Africa. The sample population being largely inborn at the hospital and admitted immediately after birth (day 0) means a high frequency of community-acquired infections was not expected, unless vertical transmission from the mothers is proven.

Listeria monocytogenes, Proteus mirabilis, Providencia stuartii, Serratia liquefaciens, and *Streptococcus agalactiae (GBS)* were the least isolated pathogens with a prevalence of 0.15% each. Though very low in prevalence, the changing epidemiology of these clinical isolates requires constant surveillance to avert possible widespread future outbreaks. Our findings of the low prevalence of *Listeria monocytogenes* are consistent with published literature that reported only 0.4% *Listeria monocytogenes* in neonatal sepsis cases at Charlotte Maxeke hospital in Johannesburg [4]. This, therefore, indicates that *Listeria monocytogenes* is generally not a very common clinical isolate in neonatal sepsis cases. There is a paucity of published data on the prevalence of *Providentia stuartii*, *Proteus mirabilis*, and *Serratia liquefaciens* in neonatal sepsis cases. These isolates have not been well studied in neonatal sepsis cases owing to their limited clinical significance in neonatal sepsis. Our results on GBS are consistent with published literature from Charlotte Maxeke Hospital in Johannesburg where the prevalence of Group B *streptococcus* was reported at 1.7%, but Group B *streptococcus* has been established at CHBAH as the leading cause of neonatal sepsis contrary to our data [4, 47]. Unlike our data which is based on hospital and ICU treated AMR sepsis, the previous findings were based on population-based estimates of all-cause neonatal sepsis where AMR was not part of the inclusion criteria. Another contributing factor could be the fact that the circulating serotypes of *Streptococcus agalactiae* have low AMR profiles, and therefore they were not expected to have a very high prevalence in the context of AMR sepsis.

The fungal isolates identified to species level were the *Candida parapsilosis* (3.95%), *Candidate albicans* (2.64%), and *Candida tropicalis* (0.15%) in that order. These findings are comparable to published literature from a neonatal unit in Gauteng province that found *Candida krusei* (35%), *Candida albicans* (29%), and *Candida parapsilosis* (16%) as the most prevalent

[3]. These high calculations were based on causes of candidemia sepsis while our calculation is based on the prevalence of each species in all causes of AMR sepsis. Furthermore, the literature from Gauteng province reported data from an outbreak situation, which explains the big difference with our findings.

4.3 Discussion on Objective 2

The second objective was to describe the AMR profile of all clinical isolates from neonatal sepsis cases. This was achieved through a quantitative analysis of the antimicrobial susceptibility test results of all the clinical isolates from the 192 neonates included in the final analysis. This objective was achieved, and the results are as shown between pages 23 and 39 Included in the discussions are the most important findings and results that have serious clinical implications for treatment.

4.3.1 The AMR of clinically significant bacterial isolates

Acinetobacter baumannii was highly resistant to gentamycin (85.88%), Cefepime (83.05%), imipenem (90.68%), ciprofloxacin (67.25%), ceftazidime (82.46), meropenem (93.1%), and showed low resistance to colistin (12.5%). The low susceptibility to carbapenems, aminoglycosides, and cephalosporins is consistent with published data from two hospitals which are Tygerberg Children's Hospital in Cape Town and Charlotte Maxeke Hospital in Johannesburg [4, 5]. According to the Department of Health of South Africa's guidance on surveillance and consumption of antibiotics, treatment options for multidrug-resistant *Acinetobacter baumannii* includes Colistin, Tigecycline, or combination treatment of the two [32]. Colistin is not available in public health facilities as it is an unregistered drug in South Africa, hence not a feasible option to the poor who access healthcare services from the public sector and yet bear the biggest burden of neonatal sepsis. This notwithstanding, Colistin has a proven safety concern of nephrotoxicity which requires that its use in neonates should only be a last resort. Tigecycline as monotherapy is ineffective because organisms easily develop resistance against it. Furthermore, Tigecycline is bacteriostatic; neonatal sepsis being a medical emergency is better treated using agents that are bacteriocidal to improve treatment outcomes as fast as possible.

Carbapenem-resistant *Acinetobacter* is considered an urgent threat by the Centers for Disease Control and Prevention (CDC) and estimated to have caused 8500 hospitalisations and 700 deaths in 2017 in the United Stated of America, yet there is no *Acinetobacter baumannii* vaccine under clinical development at the moment [48, 49]. However, reverse vaccinology and other techniques have been used to identify vaccine candidates, a few of which have shown promising

immunogenicity in mice [50–53]. The wait for a vaccine against this pathogen appears much longer. Given the complexities involved in the vaccine discovery and development process coupled with high failure rates, antimicrobial stewardship and other IPC appear to be the only hope to contain this extremely drug-resistant pathogen.

Klebsiella pneumoniae was highly resistant to Gentamycin (78%), Amikacin (59.2%), Ciprofloxacin (42.55%), Cefepime (73.77%), Ceftazidime (73.77%), Meropenem (46.67%), Imipenem (45.9%), and had moderate resistance to Ertapenem (24.24%). This shows a 73% ESBL production (measured by resistance to third-generation cephalosporins) among *Klebsiella pneumoniae*. The high ESBL prevalence has been well reported in South Africa, such as a 59% ESBL prevalence among neonatal sepsis cases at Tygerberg Children's Hospital in Cape Town [5]. The Department of Health of South Africa estimates ESBL prevalence in South Africa to be between 63% and 70% [32]. Here we have shown that ESBL prevalence in neonatal sepsis at CHBAH is about 73%.

Carbapenems are the antibiotics with the best activity against *Klebsiella pneumoniae,* but our findings suggest that only Ertapenem has good activity against *Klebsiella pneumoniae,* as the high CPE prevalence observed makes other Carbapenems less effective. A systematic review and meta-analysis results of AMR sepsis data for the Southern Africa region shows that *Klebsiella species,* of which *Klebsiella pneumoniae* is the most predominant and clinically significant, is resistant to Carbapenems (3%), Amikacin (21%), Ciprofloxacin (58%), Ceftazidime (96%), and Gentamicin (83%). Our data shows that South Africa, owing to its higher consumption rate of antibiotics, is expected to have higher resistance rates compared to the rest of the Southern African region. Ciprofloxacin is the only quinolone with good activity against *Klebsiella pneumoniae,* but our findings shows that the high resistance to Ciprofloxacin makes it ineffective to treat AMR sepsis caused *by Klebsiella pneumoniae* at CHBAH.

The very high resistance to Gentamicin and Amikacin also indicates that Aminoglycosides are equally ineffective treatment options for AMR sepsis caused by *Klebsiella pneumoniae* at CHBAH. This leaves Tigecycline as the potential last resort antibiotic to treat *Klebsiella pneumoniae* caused AMR sepsis. However, Tigecycline has been reported to be less effective as a monotherapy and being bacteriostatic, it is not a good choice for treatment of neonatal sepsis which is a medical emergency.

Carbapenem-resistant *Enterobacteriaceae* is classified as an urgent threat by the CDC [49]. There are three vaccine candidates for *Klebsiella pneumoniae* in pre-clinical development, however, vaccine development is complicated owing to the many virulence factors of the

pathogen [48, 54]. The development of these vaccines ought to be prioritised to reduce the burden of AMR *Klebsiella pneumoniae.*

Staphylococcus aureus was susceptible to Linezolid (100%), Vancomycin (100%), and Fusidic acid (89%). There was high resistance of *Staphylococcus aureus* to Ampicillin (97.14%), Gentamicin (89.5%), Ciprofloxacin (86%), Ceftazidime (80%), Cloxacillin (80%). Our findings of MRSA is lower than two separate reports from Tygerberg Children's Hospital where the prevalence in one study was 66% while another reported all *Staphylococcus aureus* MRSA [5, 55]. Compared with a similar setting in sub-Saharan Africa, our findings are lower than those from Ethiopia where the prevalence of MRSA was reported at 69% [56]. We have therefore shown that resistance to methicillin in neonatal sepsis cases at CHBAH is still unacceptably high but lower than estimates from other provinces and similar settings in sub-Saharan Africa.

Staphylococcus aureus has been reported highly susceptible to Cloxacillin at a tertiary level referral hospital in South Africa and susceptible to Ampicillin at CHBAH, all contrary to our findings [29, 31]. This suggests that resistance patterns change over time, and the two drugs are no longer effective to treat AMR sepsis caused by *Staphylococcus aureus.* Vancomycin, therefore, remains the most effective therapeutic option to treat MRSA alongside adhering to infection control practices, as *Staphylococcus aureus* is a healthcare-associated pathogen.

The CDC classifies MRSA as a serious threat, and yet there has been no successful vaccine trial beyond phase three against *Staphylococcus aureus* [49, 57]. There are a total of 27 *Staphylococcus aureus* vaccines under development, with 23 in preclinical studies, two in phase 1 and two in phase 2 clinical development [48]. One of the leading vaccine candidates, V710 that made it to phase 3 was stopped because those who got vaccinated and had immunity were twice as likely to have multiple organ failure if infected and five times more likely to die from the infection compared to the unvaccinated control group [58]. The high failure rates discourage development of new vaccines for *Staphylococcus aureus* due to the high financial loss, time wasted and opportunity costs involved. New strategies that employ the latest advances in vaccine development technology should be urgently deployed to facilitate faster development of a vaccine. In the absence of an effective vaccine, it appears that resistance of *Staphylococcus aureus* will continue to rise against the existing antibiotics.

Staphylococcus epidermidis, the most predominant CNS was 100% susceptible to Linezolid, and Streptomycin high-level resistance. It was 100% resistant to ampicillin/amoxicillin, and Gentamycin high-level resistance. It was also resistant to Cloxacillin (91.3%), Meropenem

(65.38%), Piperacillin tazobactam (76.92%), Ampicillin (95.4%), Cefazolin (88.89%), Cefoxitin (88.89), Imipenem (22.22%), Amikacin (50%), and Vancomycin (4.17%). This shows that penicillins, Aminoglycosides, and third-generation Cephalosporins are not susceptible enough to treat AMR sepsis caused by *Staphylococcus epidermidis*. Emerging resistance to Vancomycin was observed. There is a paucity of literature describing AMR profiles of *Staphylococcus epidermidis* in sub-Saharan Africa, despite being increasingly isolated in neonatal sepsis cases. However, data from India, an LMIC similar to South Africa, indicates that *Staphylococcus epidermidis* is resistant to Ampicillin, Gentamycin, and Ciprofloxacin but susceptible to Cefotaxime and Amikacin [59]. South Africa, with proven higher consumption rates of antibiotics than India, justifiably has a higher AMR profile as shown in our data. Vancomycin should be preferred treatment option due to the lower resistance observed, but its use should be closely monitored for abuse to prevent escalation of resistance.

There is paucity of data describing any vaccine-related projects against CNS. The IPC activities should be improved to reduce its transmission.

Escherichia coli had overall moderate susceptibility to most antibiotics. It was 75% susceptible to imipenem, meropenem, amikacin, and piperacillin/tazobactam. It was 75% resistant to Ceftazidime and 33% resistant to Ciprofloxacin. It was 100% resistant to ampicillin/amoxicillin, and vancomycin. The 75% resistance to ceftazidime in our study is higher than the 68% established by a systematic review and meta-analysis for the Southern African region, while the 33% resistance to ciprofloxacin from our data is less than the 64% reported by the same systematic review for the Southern Africa region [60]. Quinolones are therefore still effective for the treatment of AMR sepsis caused by *Escherichia coli*, but regular surveillance is required to establish changes in AMR profile to maintain clinical efficacy.

There are several vaccines under development against *Escherichia coli* but none have been licensed against the invasive disease [48, 61]. With increasing prevalence of resistance of *Escherichia coli* against the available antibiotics, the need to develop a vaccine for this pathogen is very urgent.

Pseudomonas aeruginosa was 100% susceptible to tobramycin. However, resistance to most antibiotics was 100% and these include piperacillin/tazobactam, meropenem, and amikacin. This is a huge concern because of the extreme resistance to recommended therapeutic options, yet the drugs that show good effectiveness are not regularly used to treat neonatal sepsis. They

are expensive and not easily accessible to the poor that bear the biggest burden of neonatal sepsis.

Our findings are contrary to the 2017 report of the National Department of Health of South Africa that established non-susceptibility to Piperacillin/Tazobactam at 19% and Carbapenems at 23%, and the two are the first and second-line therapeutic options respectively [32]. *Pseudomonas aeruginosa* is known for low prevalence but extremely resistant to antibiotics; our small sample size may therefore not limit the generalisation of our findings of extreme resistance in *Pseudomonas aeruginosa* at CHBAH.

Multidrug-resistant *Pseudomonas aeruginosa* is classified as a serious threat by the CDC [49]. There are four vaccine candidates under development against *Pseudomonas aeruginosa*, all of which are in pre-clinical phase [48]. This therefore means the race to an effective vaccine against *Pseudomonas aeruginosa* is still a long one, despite the pathogen being extremely resistant to antibiotics. Its therefore necessary that efforts to find a vaccine against *Pseudomonas aeruginosa* be enhanced.

Enterococcus faecium and ***Enterococcus faecalis*** were both 100% susceptible to vancomycin and linezolid while showing 83% susceptibility to clindamycin. *Enterococcus faecium* had lower resistance to ampicillin (16.67%) than *Enterococcus faecalis* (33.33%). Ampicillin is the first therapeutic option for *Enterococcus* infections in South Africa and vancomycin is used to treat ampicillin-resistant *Enterococcus* infections. Our findings show that ampicillin is still effective to treat susceptible *Enterococcus* spp caused sepsis at CHBAH despite observed resistance. This is in keeping with the 33% resistance of *Enterococcus species* observed in Ethiopia, while unlike Ethiopia, where resistance to vancomycin was reported 16.67%), this was not observed at CHBAH [56].

However, treatment of neonatal sepsis cases with vancomycin and ampicillin may still result in a longer hospital stay because the two drugs exhibit bacteriostatic activity against *Enterococci*. Bactericidal activity with these agents can be achieved by the addition of an aminoglycoside, but high resistance especially of *Enterococcus faecium* to gentamicin (75%) and amikacin (72.73%) makes this therapeutic benefit difficult to achieve.

There are currently no vaccines under development against *Enterococcus faecium* and *Enterococcus faecalis* [62]. Antimicrobial stewardship activities and IPC should be promoted to contain the infections of the two pathogens.

4.3.2 The AMR profiles of the isolated fungi

Three species of *candida* were identified to species level. These were *Candida parapsilosis* (3.94%), *Candida albicans* (2.64%), and *Candida tropicalis* (0.15%).

Candida albicans showed high sensitivity (100%) to Fluconazole and Voriconazole while non-albican *candida* showed relative sensitivity with high resistance (73.5%) to Fluconazole in *Candida parapsilosis*, while showing high susceptibility (80.7%) to Voriconazole. *Candida tropicalis* was 100% susceptible to Fluconazole. Susceptibility to Amphotericin B was generally 100% across all *Candida species*. These findings are comparable to previous studies done in South Africa where *Candida albicans* were reported 100% susceptible to Fluconazole at Tygerberg Children's Hospital while non-albican *Candida species* were 50% resistant to fluconazole [5, 63]. Our findings suggest that resistance of *candida species* to Amphotericin B was not observed at CHBAH and that Fluconazole is still effective for treatment of *Candidemia*, however, Voriconazole has a better effect.

The CDC classifies drug-resistant *candida* species as a serious threat [49]. The past decade has seen progress in the development of anti-*candida* vaccines, however, very few have made it to the phase 1 and phase 2 of clinical development [64]. To date, no vaccine has been licensed against any fungal infection. The wait for an anti-*candida* vaccine therefore remains a long one.

4.4 Discussion on Objective 3

The third objective was to describe the proportion of isolates that are resistant to recommended empiric treatment options stratified according to common causes of bacterial sepsis in neonates. The first empirical first-line treatment recommended in South Africa is gentamicin plus ampicillin, while the recommended empirical second-line treatment is piperacillin tazobactam plus Amikacin. The rationale behind the combination therapy is to achieve synergy and theoretically, this is expected to reduce resistance to antibiotics as they act through different mechanisms to achieve desired therapeutic outcomes. However, this in practice may end driving AMR higher due to increased exposure to antibiotics simultaneously. The discussions here include evaluation of the resistance or susceptibility profile of the leading causes of sepsis to the recommended first- and second-line empiric treatment options in South Africa against the leading bacterial causes of sepsis. This objective was achieved, and the results discussed here are available between pages 23 and 33.

From our findings, *Acinetobacter baumanii* is highly resistant to gentamicin (85.88%), ampicillin (88.89%), piperacillin/tazobactam (93.86%), and amikacin (71.05%). This implies

that the recommended first- and second-line empirical antibiotic therapy in South Africa is not effective against *Acinetobacter* AMR neonatal sepsis. Our findings are consistent with published literature from CHBAH where susceptibility of *Acinetobacter baumannii* to gentamicin was only 6%, 1% to Piperacillin/tazobactam, and 5% to amikacin. The recommended empiric therapeutic options are therefore not effective against AMR sepsis caused by *Acinetobacter baumannii* at CHBAH.

Klebsiella pneumoniae is highly resistant to Gentamicin (82.98%), Ampicillin (97.06%), Piperacillin/Tazobactam (67.21%), and Amikacin (59.02%). This is not in keeping with a recent systematic review and meta-analysis which showed that in the Southern part of Africa, the resistance of *Klebsiella species* is 21% to Gentamicin, 21% to Amikacin, 89% to Ceftriaxone, and 9% to Piperacillin/Tazobactam [60]. The higher rate in South Africa relative to the rest of the region is possibly due to the higher consumption of antibiotics in South Africa compared to the other Southern Africa countries which are yet to introduce some antibiotics already widely used in South Africa. The recommended empiric therapeutic options are not effective to treat AMR sepsis caused by *Klebsiella pneumoniae* at CHBAH.

Escherichia coli is highly resistant to gentamicin (66.67%) and ampicillin (66.67%), but shows low resistance to Piperacillin/Tazobactam (25.0%), and amikacin (25.0%). This is consistent with the average estimate of resistance profile for *Escherichia coli* from a recent systematic review and meta-analysis where resistance was reported against Ampicillin is 84%, Gentamicin is 48%, Amikacin 8%, Piperacillin/Tazobactam is 9%, and Cefotaxime 34% for the Southern Africa region [60]. The second-line empiric treatment option shows better effectiveness in the treatment of *Escherichia coli* caused AMR sepsis compared to the first-line treatment.

Pseudomonas aeruginosa is 100% resistant to Gentamicin, Amikacin, and Piperacillin/Tazobactam. This extreme resistance of *Pseudomonas aeruginosa* in neonatal sepsis has been well described in Nepal where 100% resistance was reported against Ampicillin/Sulbactam, Amikacin, and Piperacillin/Tazobactam(65). Both the first-line and second-line recommended therapeutic options have no activity against *Pseudomonas aeruginosa*.

Staphylococcus aureus is highly resistant to Gentamicin (89.47%), Ampicillin (97.14%), Piperacillin/Tazobactam (100%), and Amikacin (53.85%). This is consistent with previous findings from CHBAH where Ampicillin (92%) and Gentamicin (28.8%) were reported resistant while AMR profiles of Amikacin and Piperacillin/Tazobactam were not established

[29]. Our findings, therefore, confirm that the recommended empiric therapeutic options are not effective to treat AMR sepsis caused by *Staphylococcus aureus*.

Enterococcus faecalis and *Enterococcus faecium* show varying responses to both the first- and second-line empirical therapeutic options. *Enterococcus faecalis* had moderate resistance to gentamycin (44.44%) while *Enterococcus faecium* had very high resistance to gentamicin high level resistance(71.43%). *Enterococcus faecalis* had moderate resistance to ampicillin (33.33%) while *Enterococcus faecium* showed low resistance to ampicillin (16.67%). They both showed high resistance to piperacillin/tazobactam, 66.67% for *Enterococcus faecalis*, and 90.91% for *Enterococcus faecium*. *Enterococcus faecalis* showed moderate resistance to amikacin (33.33%) while *Enterococcus faecium* had very high resistance to amikacin (72.73%). The AMR profile of *Enterococcus species* is not well described in neonatal sepsis in South Africa, however, comparable data from Malawi reported high resistance to ampicillin (69.7%) and gentamicin (100%) [65]. The high resistance to individual agents indicates that the expected synergy from combination therapy in empirical treatment cannot be achieved in AMR sepsis caused by *Enterococcus species*. More research is required to understand the AMR profiles of Enterococcus species to the recommended better empirical therapy options.

5. Chapter 5: Conclusions

5.1 Study conclusions

All the objectives of this study were achieved.

The gram-negative bacteria are the leading causes of AMR sepsis at CHBAH. Mortality due to AMR among neonatal sepsis cases at CHBAH which is 30% is high but consistent with the global estimates. The AMR in *Acinetobacter baumannii* causes almost half of the total neonatal deaths in sepsis cases.

The risk factors for infections by pathogens resistant to antimicrobial agents remain high at CHBAH. Effective responses to reduce AMR sepsis should involve addressing the risk factors such as preterm delivery, and lower birth weight.

The high levels of resistance among causative organisms of AMR sepsis have rendered empiric therapeutic options less effective. This is demonstrated by the low susceptibility to Ampicillin, Gentamicin, Amikacin, Piperacillin/Tazobactam, and third-generation Cephalosporins. The South African Department of Health's and the WHO's recommended empiric therapeutic options are therefore both ineffective to treat AMR sepsis among neonates at CHBAH. While the available antibiotics have become less effective, lack of discovery of new antibiotics and the very slow progress in development of vaccines restricts options for containing AMR. This could lead to an outbreak of AMR pathogens with epidemic or pandemic potential , which could cause severe social and financial disruptions.

A vaccine for *Acinetobacter baumannii, Klebsiella pneumoniae* and *Staphylococcus aureus* could potentially prevent 67% of neonatal deaths due to AMR sepsis. This would complement the existing strategies, as IPC or antimicrobial stewardship cannot prevent every infection. Furthermore, vaccines are effective regardless of the mechanism of AMR or prevalence of AMR, hence a valuable addition to contain AMR sepsis. Once such vaccines become available, maternal immunisation should be explored to determine the potential of level of protection amongst the neonates.

The prevalence of ESBL and CRE is very high among the *Klebsiella pneumoniae* as demonstrated by the low susceptibility to third-generation Cephalosporins and Carbapenems, respectively.

There is an urgent need for a joint global effort similar to that seen with Covid-19 to develop vaccines against the AMR pathogens before full-scale outbreaks start emerging anywhere in the world. This requires serious political commitment of the governments and corporations to pool financial resources to fund the research for development of vaccines against AMR pathogens. Therefore, dedicated funding, assured market and need to accelerate research into development of AMR vaccines requires an urgent global commitment.

5.2 Limitations

The very high prevalence of AMR could have been due to a selection bias resulting from many tests done per neonate which suggests that many specimens were taken after the patients had failed therapy, thus increasing the chances of isolating a resistant organism. However, we did not compare the microorganism yield between blood and CSF culture samples to ascertain this effect.

We present data showing hospital-treated sepsis cases, not population-based cases. Therefore, our findings are not a population-based estimate and may either underestimate or overestimate actual AMR prevalence.

Due to the secondary nature of the data, we were not able to observe blood culture inoculum and sampling technique to ascertain blood culture yields and contamination rates.

5.3 Recommendations for further research

Prospective survival analysis of neonatal sepsis cases following hospital admission is required to ascertain the actual burden of AMR sepsis at population level.

Patient care activities should be audited to identify areas for further improvement.

The trend in effectiveness of the most common antibiotics used to treat neonatal sepsis should be analysed and the data used to decide to either remove or keep the antibiotics on the essential medicines list and to advocate for vaccine development for the most prevalent AMR pathogens as a priority.

There is need for a nationwide strategy to collect AMR data in neonatal sepsis cases to establish total infections in a year, total hospitalisations and mortality stratified according to the leading AMR pathogens. This data can be used to establish social and financial costs incurred due to AMR infections in neonatal sepsis. This will accurately guide policy decisions, allow better forecasts, and assess the impact of the existing interventions.

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Appendices

Appendix 1: Study variables

Study number M191071

Variable Number	Variable Name	Source of data
1	Age (days)	Neonatal discharge summary CDW-NHLS
2	Gestation age (weeks)	Neonatal discharge summary
3	Place of delivery (facility or home)	Neonatal discharge summary
4	Mode of delivery	Neonatal discharge summary
5	Birth weight (grams)	Neonatal discharge summary
6	Apgar's score (1-10)	Neonatal discharge summary
7	Gender (M / F)	Neonatal discharge summary
8	Unit and ward of admission	Neonatal discharge summary
9	Date of admission / diagnosis (DD/MM/YYY)	Neonatal discharge summary
10	Age at admission (days)	Neonatal discharge summary
11	Admission weight (grams)	Neonatal discharge summary
12	Date of sample (DD/MM/YYY)	CDW-NHLS
13	Sample type (blood or CSF)	CDW-NHLS
14	Cells (RBC, WBC)	CDW-NHLS
15	Pathogens isolated	CDW-NHLS
16	AMR profile	CDW-NHLS
17	Outcome of admission	Neonatal discharge summary
18	Date of outcome (DD/MM/YYY)	Neonatal discharge summary
19	Mother's HIV status	Neonatal discharge summary

Appendix 2: Approval for laboratory data access from NHLS

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6142
Fax: +27 (0)11 386 6296
Email: babatyi.kgokong@nhls.ac.za
Web: www.nhls.ac.za

23 June 2020

Applicant: Ronald Opiecabo
Institution: University of the Witwatersrand
Department: Vaccinology
Email: ropiecabo@gmail.com
Tel: 061 534 1834

CC: Jeannette Wadula
Senior Pathologist – Chris Hani Baragwanath Academic Hospital

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project **"Antimicrobial Resistance Patterns in Neonatal Sepsis Cases at Chris Hani Baragwanath Academic Hospital (CHBAH)"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application. Submissions should be made annually on the AARMS system – https://aarms.nhls.ac.za.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- All data requested should be in accordance with the research protocol submitted and approved by the relevant Ethics Committee.
- Request for the inclusion of the NHLS as a source of data in the original protocol to be approved by Ethics as NHLS does not have a Human Research Ethics Committee.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- Jeannette Wadula is noted as NHLS collaborator for this research.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix 3: Approval from the department of Paediatrics, CHBAH

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Department of Paediatrics and Child Health
Metabolic Unit
Chris Hani Baragwanath Academic Hospital
P. O. Bertsham
2013

27 February 2020

The Research Protocol Review Committee
Chris Hani Baragwanath Academic Hospital
Soweto
Johannesburg
South Africa

Dear Madam/ Sir

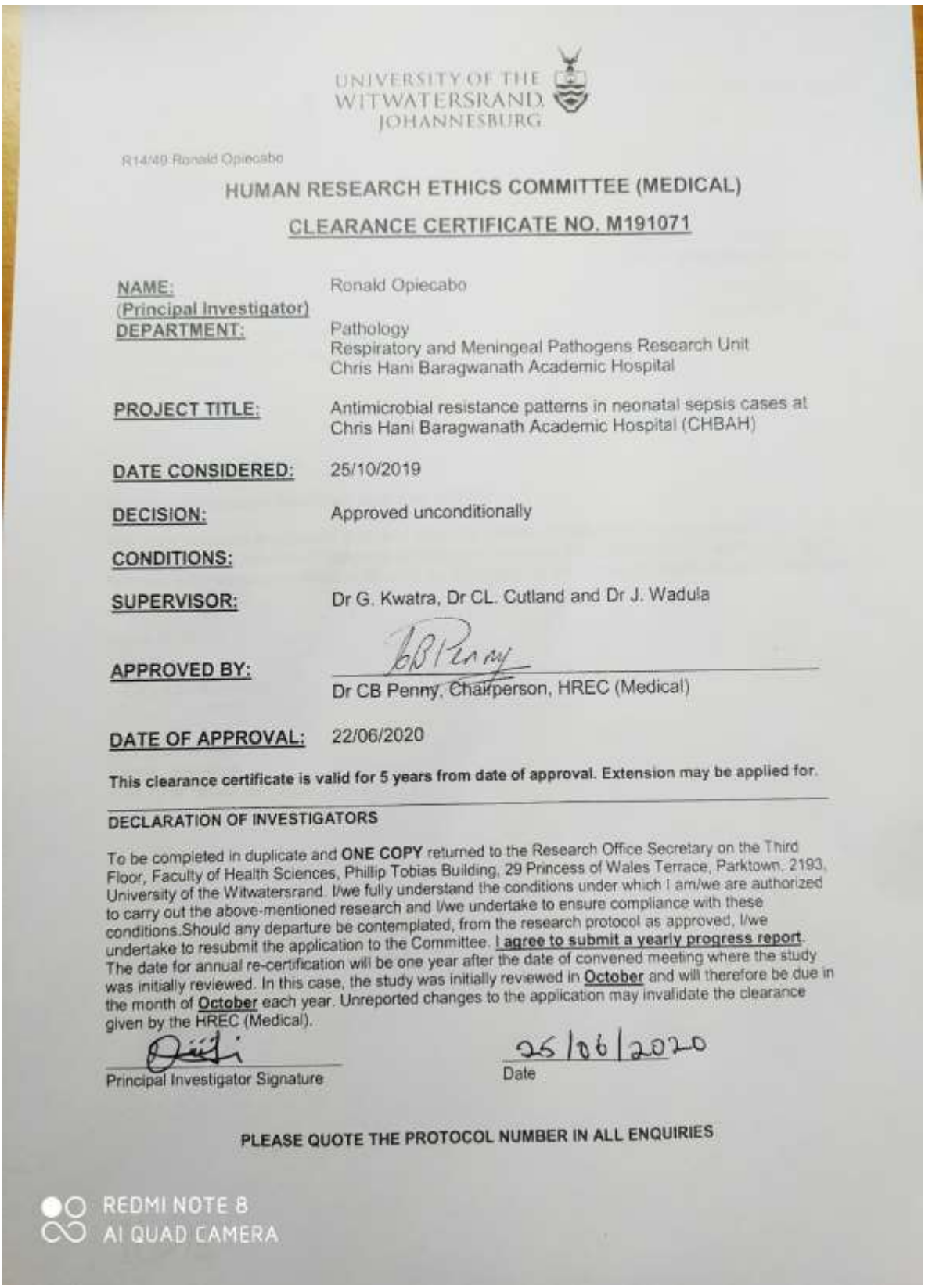
I would like to inform you that **Ronald Opiecabo** has been given a permission to conduct her research study in the Department of Paediatrics at Chris Hani Baragwanath Academic Hospital. The title of his study is: **"Antimicrobial resistance patterns in neonatal sepsis cases at Chris Hani Baragwanath Academic Hospital."**

While he has been given permission to conduct this study, he cannot start with data collection until he has provided the Department with Ethics Committee Clearance Certificate.

Yours Sincerely

Professor SC Velaphi
Head of Department of Paediatrics
Chris Hani Baragwanath Academic Hospital

Appendix 4: Ethics clearance certificate



UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Ronald Opiecabo

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M191071

NAME: (Principal Investigator)	Ronald Opiecabo
DEPARTMENT:	Pathology Respiratory and Meningeal Pathogens Research Unit Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Antimicrobial resistance patterns in neonatal sepsis cases at Chris Hani Baragwanath Academic Hospital (CHBAH)
DATE CONSIDERED:	25/10/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr G. Kwatra, Dr CL. Cutland and Dr J. Wadula
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	22/06/2020

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 Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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**. 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 **October** and will therefore be due in the month of **October** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

25/06/2020
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

REDMI NOTE 8
AI QUAD CAMERA

Appendix 5: Clearance certificate from Medical Advisory Committee, CHBAH

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 11th March 2020

TITLE OF PROJECT:

Antimicrobial Resistance Patterns in Neonatal Sepsis at Chris Hani Baragwanath Academic Hospital (CHBAH).

UNIVERSITY: Witwatersrand

Principal Investigator: R Opiecabo

Department: Paediatrics / Pathology

Supervisor : Dr G Kwatra

Permission Head Department (where research conducted): Yes

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- **Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.**
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.

Recommended
(On behalf of the MAC)
Date: 11/3/2020

Approved/~~Not Approved~~
Hospital Management
Date: 17/03/2020

Appendix 6: Approval of title by Postgraduate Research Office of Faculty of Health Sciences



UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

Private Bag 3 Wits, 2050
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

07 September 2020
Person No: 2293192
PAG

Mr R Opiecabo
Plot 16 Aputi Road, Lira municipality
Plot 2 Ekwang Road, Lira municipality
Uganda

Dear Mr Ronald Opiecabo

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Antimicrobial resistance patterns in neonatal sepsis cases at Chris Hani Baragwanath Academic Hospital (CHBAH)* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 7: Clearence letter from RMPRU



DST/NRF:Vaccine Preventable Diseases
Respiratory and Meningeal Pathogens Research Unit
11th Floor, Central West wing, Nurses Residence
Chris Hani Baragwanath Hospital
Tel (011) 983 4283
Fax 086 646 4208

VPD
Vaccine Preventable Diseases
RMPRU
Respiratory & Meningeal Pathogens Research Unit

Tuesday, 24 March 2020

The Human Research Ethics Committee (HREC),
Faculty of Health sciences,
University of the Witwatersrand.
Re: Permission to conduct research at the Respiratory and Meningeal Pathogens Research Unit (RMPRU)

Dear Sir/ Madam,
This serves to confirm that permission is here by granted to Mr Ronald Opiecabo, student number 2293192 to conduct his research study at RMPRU. He is a student of MSc (Med) in the field of Vaccinology. His study tittle is Antimicrobial Resistance Patterns in Neonatal Sepsis cases at Chris Hani Baragwanath Academic Hospital (CHBAH).

He cannot start data collection until he has provided full Ethics committee clearance certificate to the research unit.

Yours faithfully,

.....
Shabir A Madhi
Professor of Vaccinology: University of Witwatersrand
Director: MRC Respiratory and Meningeal Pathogens Research Unit
DST/NRF South African Research Chair: Vaccine Preventable Diseases

REDMI NOTE 8
AI QUAD CAMERA

Appendix 8: Budget

Item	Quantity	Rate (Rands)	Amount (Rands)
Data analysis consultations	1	7000	7,000
Proofreading	1	5000	5000
Internet and airtime			2,500
Printing, scanning and photocopying	1000	1	1,000
Research trainings attended	6	300	1,800
Miscellaneous			3,000
Total			**21300**

Appendix 9: Plagiarism report

Antimicrobial Resistance Patterns in Neonatal Sepsis Cases

ORIGINALITY REPORT

%8 SIMILARITY INDEX %6 INTERNET SOURCES %5 PUBLICATIONS %2 STUDENT PAPERS

PRIMARY SOURCES

nccid.ca
Internet Source

wiredspace.wits.ac.za <%1
Internet Source

"13th European Congress of Clinical Microbiology and Infectious Diseases", Clinical Microbiology and Infection, 2003 <%1
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"Poster Abstracts", Open Forum Infectious Diseases, 2020 <%1
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Daniel F Monte, Nilton Lincopan, Paula J Fedorka-Cray, Mariza Landgraf. "Current insights on high priority antibiotic-resistant Salmonella enterica in food and foodstuffs: a review", Current Opinion in Food Science, 2019 <%1
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hdl.handle.net

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Mohanlal, Reena D., and Sugeshnee Pather. "Variability of HHV8 LNA-1 Immunohistochemical Staining Across the 3 Histologic Stages of HIV-Associated Mucocutaneous Kaposi Sarcoma : Is There a Relationship to Patients' CD4 Counts?",

American Journal of Dermatopathology, 2015. <%1

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Gemechu Ameya, Temesgen Weldemedhin, Tsegaye Tsalla, Feleke Gebremeskel. " Antimicrobial Susceptibility Pattern and Associated Factors of Pediatric Septicemia in Southern Ethiopia

", Infection and Drug Resistance, 2020 <%1

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"Abstracts cont.", Clinical Microbiology and

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M Coetzee, N Mbowane, T De Witt. "Neonatal sepsis: Highlighting the principles of diagnosis and management", South African Journal of

Child Health, 2017 <%1

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"Posters", Clinical Microbiology and Infection, 4/2007 <%1

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Brancart, F.. "Quantitative TaqMan PCR for detection of Pneumocystis jiroveci", Journal of Microbiological Methods, 200506 <%1

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Md. Abdur Rafi, M. Morsed Zaman Miah, Md.

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Nadire Seval Gündem, Nuriye Tarakçi Emiroglu, Erkan Atas. "Causative Agents, Antibiotic

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Érique José Peixoto De Miranda, Gerson Sobrinho Salvador De Oliveira, Felício Lopes Roque, Sílvia Regina Dos Santos et al. "SUSCEPTIBILITY TO ANTIBIOTICS IN URINARY TRACT INFECTIONS IN A SECONDARY CARE SETTING FROM 2005-2006 AND 2010-2011, IN SÃO PAULO, BRAZIL: DATA FROM 11,943 URINE CULTURES", Revista do Instituto de Medicina Tropical de São Paulo, 2014 <%1

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Zeinab Mahmoudi, Iraj Rasooli, Abolfazl Jahangiri, Shakiba Darvish Alipour Astaneh. " Prevention of nosocomial infections with a conserved immunogenic fimbrial protein ", APMIS, 2020

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Joe J. Harrison, Maryam Rabiei, Raymond J. Turner, Erin A. Badry, Kimberley M. Sproule, Howard Ceri. "Metal resistance in Candida

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Internet Source

Jing Liu, Zengyu Fang, Yonghui Yu, Yanjei Ding et al. "Pathogens Distribution and Antimicrobial Resistance of Bloodstream Infections at Twenty-Five NICUs in Eastern China, 2016-2019",

Research Square, 2020 <%1

Publication

EXCLUDE QUOTES ON
EXCLUDE
BIBLIOGRAPHY
ON
EXCLUDE MATCHES < 10
WORDS