MICROBIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF NEONATAL BLOOD STREAM INFECTIONS IN DURBAN, SOUTH AFRICA

By

Dharshni Pillay

(206501841)

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PREFACE

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of other work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Microbiology, School of Laboratory Medicine and Health Sciences, University of KwaZulu-Natal, South Africa, under the supervision of Dr Y. Mahabeer.

_____	_____
Dr Dharshni Pillay	Dr Yesholata Mahabeer
(206501841)	(Supervisor)

DECLARATION

I, Dr Dharshni Pillay (student number: 206501841), declare as follows:

1. The research presented in this dissertation, unless otherwise states, represents original work.

2. The work described in this dissertation has not been submitted to UKZN or any other institution for the purposes of an academic qualification, whether by me or any other party.

3. This dissertation does not contain other persons' data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.

4. My contribution to the project was the role of the primary researcher. The role encompassed conceptualisation of the project, development of the methodology and protocol and liaison/applications for ethical clearance and relevant consents. Data curation and formal data analysis and completion of the final written dissertation was also the responsibility of the primary author.

5. The contributions of others to the project include those of Dr Yesholata Mahabeer (National Health Laboratory Service and Department of Medical Microbiology at the University of KwaZulu-Natal). This contribution encompassed the role of project supervisor, which included synthesis of the protocol and editing and review of the dissertation.

DEDICATION

To all the “unusual” suspects in my life.

Thank you for your love and support.

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To Dr Yesholata Mahabeer, my mentor, for assisting my progress as a researcher.

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A special thank you to the National Health Laboratory Service for allowing me permission to access their laboratory data and to the patients whose results have contributed to the data of the study.

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List of Abbreviations

A. baumannii	*Acinetobacter baumannii*
AK	Amikacin
AMP	Ampicillin
AMR	Antimicrobial resistance
BSI	Bloodstream infection
C. parapsilosis	*Candida parapsilosis*
CAZ	Ceftazidime
CD	Cluster of differentiation
CIP	Ciprofloxacin
COL	Colistin
CoNS	Coagulase negative *Staphylococcal* species
CRE	Carbapenem-resistant Enterobacterales
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CTX	Cefotaxime
CMX	Cefuroxime
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
EMA	European Medicines Agency
EOS	Early neonatal sepsis
ESBL	Extended spectrum beta-lactamase

FLU	Flucloxacillin
GBS	Group B streptococcus
GN	Gentamicin
HIC	High-income countries
IALCH	Inkosi Albert Luthuli Central Hospital
IC	Invasive candidiasis
IL	Interleukin
IMI	Imipenem
IRR	Incidence rate ratio
K. pneumoniae	*Klebsiella pneumoniae*
L	litre
LMIC	Low-and-middle-income countries
LOS	Late neonatal sepsis
LPS	Lipopolysaccharide
LZD	Linezolid
MALDI-TOF	Matrix-assisted laser desorption ionisation – time-of-flight
MBL	Mannose-binding lectin
MDR	Multi-drug resistance
MDRO	Multi-drug resistant organisms
mg	milligrams
mL	millilitre
MP	Meropenem
MRSA	Methicillin-resistance *Staphylococcus aureus*
N/S	Not specified

NACS	Non-albicans *Candida* species
ng	nanograms
NHLS	National Health Laboratory Service
NICU	Neonatal intensive care unit
NMR	Neonatal mortality rate
NPMM	Neonatal Perinatal Morbidity and Mortality Committee
NPV	Negative predictive value
OR	Odds ratio
OX	Oxacillin
P. aeruginosa	*Pseudomonas aeruginosa*
PCR	Polymerase chain reaction
PCT	Procalcitonin
PDR	Pan-drug resistance
PG	Penicillin
PLT	Platelet count
PPV	Positive predictive value
S. aureus	*Staphylococcus aureus*
S. maltophilia	*Stenotrophomonas maltophilia*
spp	species
SXT	Cotrimoxazole
TG	Tigecycline
TNF	Tumour necrosis factor
UNICEF	United Nations Children's Fund
US$	United States dollar

USA	United States of America
UTI	Urinary tract infection
VA	Vancomycin
VRE	Vancomycin resistant enterococci
vs	Versus
WCC	White cell count
WHO	World Health Organization
XDR	Extensive drug resistance

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CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION AND BACKGROUND

Antimicrobial resistance (AMR) has emerged as a global threat to healthcare resulting in an increase in morbidity and mortality [1]. It has been estimated that 31.0 % of deaths attributed to neonatal sepsis were associated with AMR [2]. The diagnosis of neonatal sepsis is often difficult, resulting in the widespread use of empiric regimens. Empiric regimens can drive AMR. Alternatively, AMR can be influenced by empiric antimicrobial choices. Therefore, understanding the microbial profile and antibiogram of a unit such as the neonatal intensive care unit (NICU), can positively impact outcomes of neonatal sepsis.

1.1. Trends in neonatal mortality (global and local perspectives)

The leading causes of neonatal mortality are preterm birth (15.9%), intrapartum-related events (10.7%) and neonatal sepsis (6.8%) [3]. Concomitantly, sepsis, meningitis and pneumonia accounts for approximately 10.0% of neonatal deaths [3]. Therefore, insights into neonatal sepsis require an understanding of trends in neonatal mortality. Worldwide, 45.0% of under-5 mortality occurred within the neonatal period [4]. Data from the United Nations Children's Fund (UNICEF) has revealed a decrease in the global neonatal mortality rate by 51.0% between 1990 and 2017 (Figure 1) [5]. This decline has been associated with the Millennium Development Goal number 4, which aimed to reduce under-5 mortality by two-thirds between 2000 and 2015 [6].

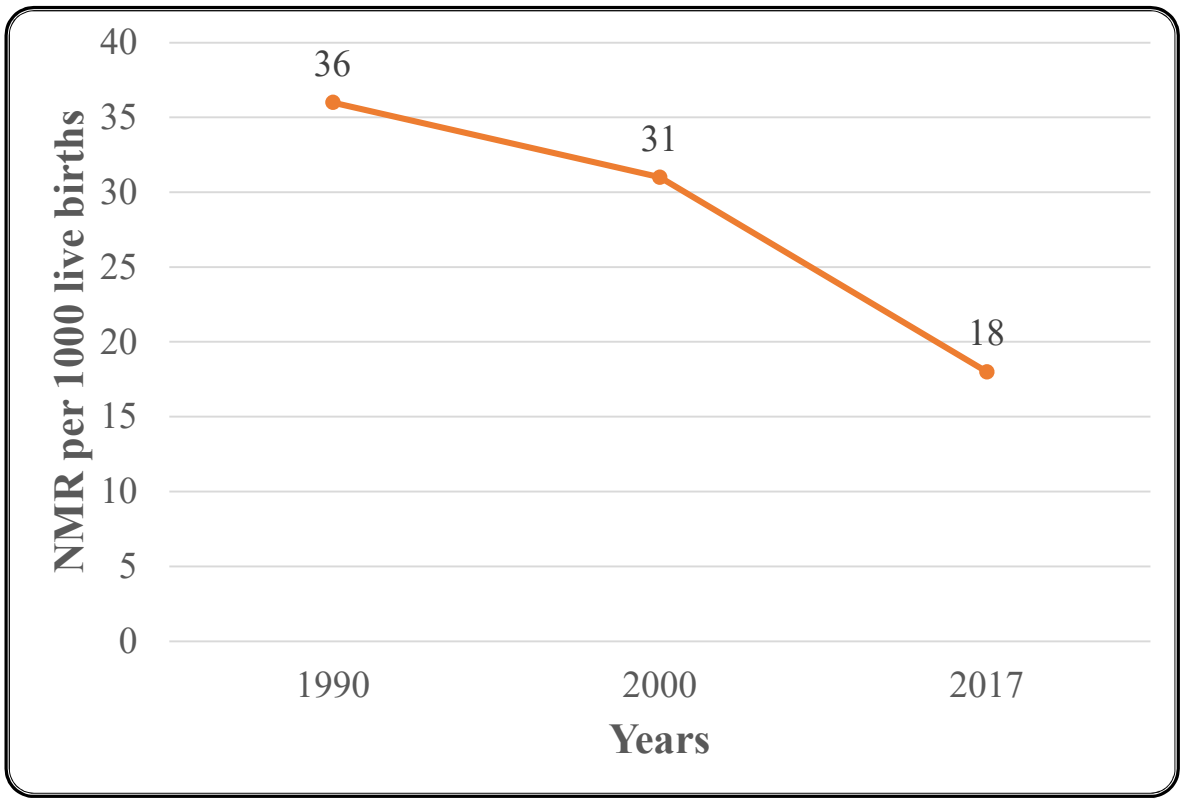

Figure 1: Global neonatal mortality rate (NMR) from 1990 to 2017 [5, 7]

Focussing on South Africa, it has been found that neonatal mortality constitutes approximately one third of under-5 mortality. However, conflicting reports on the South African neonatal mortality rate (NMR) exist [8]. The District Health Information System recorded an NMR of 12.6 deaths per 1000 live births in 2016 [9]. In contrast, the South African Demographic Health Survey documented the NMR to be 21 deaths per 1000 live births for the same year [9]. Also, UNICEF data has calculated the South African NMR to be 11.3 deaths per 1000 live births in 2016 (Table 1) [7].

Table 1: Neonatal mortality rate (NMR) in South Africa for 2016 from various sources

Source	**Neonatal mortality rate per 1000 live births**
District Health Information System	12.6
South African Demographic Health Survey	21.0
United Nation's Children Fund (UNICEF)	11.3

Despite conflicting reports, a downward trend in neonatal mortality in South Africa was observed using UNICEF data, including the period 2014 to 2018. (Figure 2). According to Rhoda et al. (2018) most childhood deaths occurred during the early neonatal period (10.2 deaths per 1000 live births) compared to the late neonatal period (2.4 deaths per 1000 live births) [9]. Consequently, the most common causes of mortality were prematurity (47.9%), intrapartum events (24.3%) and pneumonia (11.6%) [9, 10].

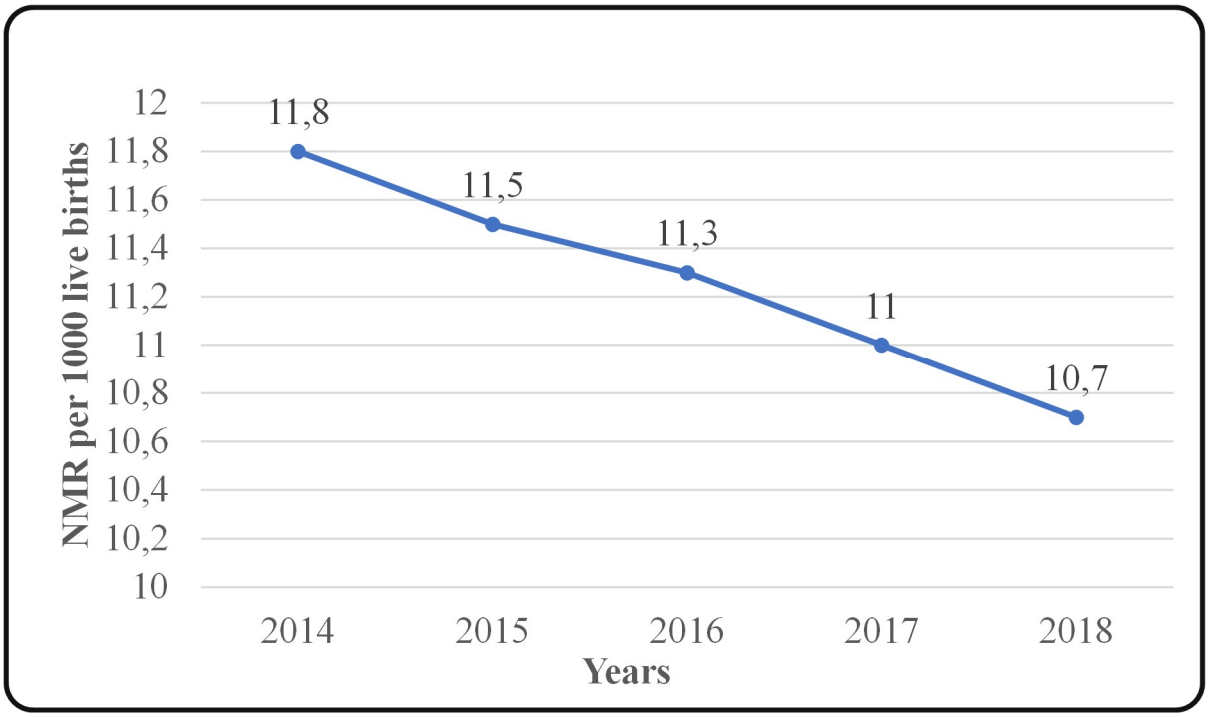

Figure 2: Trend in South African neonatal mortality rate (*Based on UNICEF data; accessed at*: https://data.unicef.org/)

Currently, the Sustainable Development Goals aim to reduce neonatal mortality to less than 12 deaths per 1000 live births by 2030 [11]. In order, to meet this target, interventions need to be accelerated globally with a commitment to address the burden of sepsis within the neonatal period.

1.2. Neonatal sepsis – burden, definitions, risk factors

Neonatal sepsis has traditionally been defined as the onset of sepsis within the first 28 days of life [12]. It entails a collection of nonspecific clinical features and positive microbiological cultures from a sterile sample which include blood, cerebrospinal fluid and urine [12].

1.2.1. Burden of neonatal sepsis

According to the World Health Organization (WHO), sepsis is a key priority condition. From estimates of neonatal mortality, it has been determined that 3 million cases of neonatal sepsis occur globally [13]. However, despite adequate resources, high-income countries report a paediatric and neonatal sepsis rate of 11.0% [13]. In Sub-Saharan Africa mortality rates from sepsis remain high at 26.0% which results in a loss of approximately 5.29 to 8.73 million disability-adjusted life years per annum and an estimated cost of US$10 billion to US$469 billion annually [14].

1.2.2. Definition of neonatal sepsis

According to literature a universal definition of neonatal sepsis is lacking [15]. Case definitions have been created by various neonatal networks worldwide, however, heterogeneity exists among these definitions of neonatal sepsis [16, 17]. This lack of a consensus definition may result in poor corroboration of statistics across various studies. Several attempts at a definition have been made:

1. The Young Infants Clinical Signs Group defined clinical criteria for the diagnosis of severe bacterial infection in neonates that informs WHO's *Integrated Management of Childhood Illness* guidelines [18, 19]. These guidelines displayed high sensitivity with low specificity, resulting in high numbers of referrals (including infants that were well). [18]
2. The European Medicines Agency (EMA) defined paediatric and neonatal sepsis for clinical trials. The panel reported the following definitions [20]:
    - Early neonatal sepsis (EOS) was defined as onset of sepsis within the first 72 hours of life.

- Late neonatal sepsis (LOS) was defined as onset of sepsis after, and including, 72 hours of life.
- Sepsis was defined as having as least two clinical symptoms **and** at least two laboratory findings in the presence of suspected or proven infection (Table 2).

Table 2: Clinical and laboratory features of neonatal sepsis

Clinical:	**Laboratory:**
• Modified body temperature • Cardiovascular instability such as bradycardia or tachycardia • Respiratory instability such as apnoea or tachypnoea • Gastro-intestinal complaints such as poor sucking or feed intolerance • Skin and subcutaneous lesions • Non-specific signs such as irritability, lethargy or hypotonia	• White blood cell count < 4000 x 10^9 cells/L or > 20 000 x 10^9 cells/L • Immature to total neutrophil ratio > 0.2 • Platelet count < 100 000 x 10^9 cells/L • C-reactive protein > 15mg/L • Procalcitonin > 2 ng/mL • Glucose intolerance confirmed at least twice • Metabolic acidosis
Microbiological tests: Microscopy, culture, polymerase chain reaction (PCR)	

Adapted from the EMA Report on the Expert Meeting on Neonatal and Paediatric, 2010 [20]

1.2.3. Risk factors of neonatal sepsis

Premature and low-birth weight infants are especially at risk for developing neonatal sepsis [21]. The timing of onset of sepsis is closely associated with certain risk factors (Table 3) [22]. Early-onset sepsis is strongly associated with maternal and obstetric factors such as maternal pyrexia or prolonged rupture of membranes beyond 18 hours. Hospital interventions predispose to late-onset sepsis which includes procedures such as central line placement and mechanical ventilation.

Table 3: Risk factors associated with early-onset sepsis and late-onset sepsis

Early-onset risk factors	Late-onset risk factors
Associated with maternal/obstetric factors: • Maternal intrapartum fever • Preterm rupture of membranes • Prolonged rupture of membranes (>18 hours) • Chorioamnionitis • Maternal Group B streptococcus genital colonisation	Associated with hospital interventions: • Invasive catheters and devices • Mechanical ventilation • Total parenteral nutrition • Prolonged hospitalisation • Underlying diseases (such as cardio-pulmonary diseases)

Adapted from Murthy et al. (2019)[22]

1.3. Neonatal Units

Due to economic differences between settings, two distinct profiles of neonatal units have emerged [23]. These include:

1. Facilities caring for term infants in poorly equipped, high dependency units with associated understaffing and overcrowding. This profile is seen in many African countries.
2. Tertiary neonatal facilities with developed supportive care. In these units most babies are born prematurely or are of low birthweight.

It is probable that the stipulated differences will affect the microbial profile of sepsis within the neonatal units [23].

1.4. Bacteriological profiles of neonatal sepsis

The gold standard for diagnosis of neonatal sepsis is isolation of a positive blood culture [12]. Other sample sites include cerebrospinal fluid, respiratory samples, or urine. Many studies focus on a single sample types as an indicator of neonatal sepsis. A review of the current literature has indicated that there are differences in the microbial aetiology of sepsis between:

1. High-income (HIC) and low-and-middle income countries (LMIC).
2. Early-onset sepsis and late-onset sepsis.

1.4.1. Blood cultures

Early-onset sepsis (EOS): Group B streptococcus (GBS) is a major cause of early onset sepsis in HIC [24]. This contrasts with the bacteriological profile of resource-limited settings where GBS rates are lower [15].

On the Indian subcontinent, EOS had a variable gram-negative profile across studies. *Klebsiella pneumoniae* and *Enterobacter* species were predominant pathogens in both India and Nepal (Table 4) [25-27]. Bangladesh reported high rates of gram-negative sepsis, in which *K. pneumoniae* and *Serratia* species were the most common organisms [28].

In Africa, an Egyptian study implicated coagulase negative staphylococci (CoNS), *Acinetobacter baumannii,* and *Escherichia coli* in EOS [29]. When looking at the profile of EOS in Ghana, it was observed that gram-positive organisms predominated. These gram-positive organisms included CoNS and *Staphylococcus aureus* [30, 31]. However, a preponderance of GBS was noted from studies performed in Kenya and Malawi [32, 33].

A South African study recorded the incidence of neonatal GBS to be 2.72 cases per 1000 live births between 2004 and 2008 in Gauteng [34]. Therefore, GBS featured as a major causative organism of neonatal blood stream infections (BSI) [34-36]. Furthermore, findings have implicated viridans streptococci in association with EOS [34, 36]. Additionally, gram-negative organisms such as *K. pneumoniae* and *E. coli* were documented as causative agents [34, 37, 38].

Late onset sepsis (LOS): Studies from the Indian subcontinent have shown a predominance of gram-negative BSI over gram-positive BSI (Table 4) [26, 39, 40]. Furthermore, studies have indicated that *K. pneumoniae* and *Enterobacter* species were the leading pathogens among blood cultures, which are similar to findings, in EOS [25-27, 41]. Other important gram-negative organisms included *E. coli*, *Ps. aeruginosa* and *Acinetobacter* species [25, 27, 39, 42]. Leading gram-positive isolates included *S. aureus* and CoNS [25, 27, 43].

In an Egyptian study a greater number of cases were documented in LOS (55.8%) [29]. This study indicated that the most common organisms isolated were CoNS and *K. pneumoniae* which substantiate reports from Ghana where CoNS was the leading cause of LOS [30, 31]. Similar to Ghana, Botswana reported a predominating picture of CoNS, alongside enterococci, in LOS [44]. However, Enterobacterales, such as *Citrobacter* species, *Enterobacter* species and *E. coli,* were also detected [31].

From Malawi, Milledge et al. (2005) reported mostly gram-positive infections (54.0%) among blood and cerebrospinal fluid cultures. This study found that the most common cause of sepsis was GBS, which also compares to Kenyan findings [32, 33].

A South Africa study also associated GBS with LOS [34]. However, other gram-positives, such as CoNS, were also documented neonatal pathogens in this country [37]. Furthermore, *S. aureus,* enterococci, *K. pneumoniae* and *Acinetobacter* species are significant bacteria causing neonatal sepsis in South Africa [38, 40, 45, 46].

LOS occurs more often than EOS in many South African studies, although this finding may be dependent on the study setting utilised [35, 37, 38]. Most studies were conducted in neonatal units where hospital interventions may predispose to risk factors for LOS [22]. However, there is evidence to suggest that study settings may not influence the microbial profile of neonatal sepsis. As demonstrated by Crichton et al. (2018), GBS was isolated from both community and hospital settings [47].

Onset not-specified: A selection of studies did not specify the timing of sepsis onset (Table 4). However, *K. pneumoniae,* CoNS, *S. aureus* and enterococci were isolated most frequently, which is in keeping with abovementioned findings. [45, 46, 48, 49].

Table 4: Bacteriological profile of neonatal sepsis in low-and-middle income countries

Country	Author	Year published	No. cultures positive	Setting	Onset	Predominant pathogens
Southern Asia						
Bangladesh	Raha et al.[28]	2014	64	Hospital	Both	*K. pneumoniae, Serratia* spp
India	Roy et al.[25]	2002	346	Hospital	Early Late	*Klebsiella* spp, *Enterobacter* spp, *E. coli* *Enterobacter* spp, CoNS
	Rajendraprasad et al.[39]	2013	95	Hospital	N/S	*E. cloacae, K. pneumoniae, S. aureus*
	Muley et al. [26]	2015	48	Hospital	Both	*K. pneumoniae, S. aureus*
Nepal	Yadev et al.[133]	2015	37	Hospital	N/S	*S. aureus, K. pneumoniae, P. aeruginosa*
	Pokhrel et al.[27]	2018	69	Hospital	Both	*K. pneumoniae,* CoNS, *Enterobacter* spp
Africa						
Botswana	Mpinda-Joseph et al.[44]	2019	366	Hospital	Both	CoNS, enterococci, *K. pneumoniae*
Egypt	Shehab El-Din et al.[29]	2015	344	Hospital	Early Late	CoNS, *A. baumannii, E. coli* CoNS, *K. pneumoniae, Serratia* spp
Ghana	Labi et al.[30]	2016	8025	Hospital	Both	CoNS, *S. aureus*
	Aku et al.[31]	2018	26	Hospital	Both	*Staphylococcus epidermidis*
Kenya	Berkley et al.[32]	2005	1094	Community	Both	*E. coli*, Group B streptococcus
Malawi	Millege et al.[33]	2005	582	Hospital	Both	Group B streptococcus
Nigeria	Iregbu et al.[48]	2006	390	Hospital	N/S	*S. aureus, K. pneumoniae*
Zambia	Kabwe et al.[49]	2016	103	Hospital	N/S	*K. pneumoniae*, CoNS, *S. aureus*
CoNS – coagulase negative staphylococci; N/S – not specified; spp – species						
Country	**Author**	**Year published**	**No. culture episodes**	**Setting**	**Onset**	**Predominant pathogens**

South Africa						
South Africa	Motara et al.[37]	2005	140	Hospital	Both	*E. coli,* CoNS
	Ballot et al.[35]	2012	246	Hospital	Early Late	Group B streptococcus CoNS, *K. pneumoniae, A. baumannii,*
	Dramowski et al.[45]	2015	717	Hospital	N/S	*K. pneumoniae, S. aureus,* enterococci
	Cutland et al.[34]	2016	699	Hospital	Both	Group B streptococcus, *E.coli*
	Lebea et al.[38]	2017	236	Hospital	Both	*K. pneumoniae,* CoNS
	Crichton et al.[47]	2018	156	Hospital & community	N/S	Group B streptococcus, *S. aureus, E. coli*
	Velaphi et al.[36]	2019	858	Community	Early	Group B streptococcus, viridans streptococci
Multi-national (including South Africa)	Hamer et al.[46]	2015	947	Community	N/S	*S. aureus, K. pneumoniae,* enterococci

CoNS – coagulase negative staphylococci; N/S – not specified; spp – species

1.4.2. Cerebrospinal fluid

In both early-onset and late-onset neonatal meningitis developed nations demonstrate high rates of GBS and *E. coli* [50-53]. African countries also report *E. coli* and GBS in neonatal meningitis, along with a wider range of organisms, including *S. pneumoniae,* non-typhoidal *Salmonella* and aerobic gram-negatives (Table 5) [54-56]. *Listeria monocytogenes* is seldom isolated outside early-onset meningitis [53]

Table 5: Leading pathogens of neonatal meningitis in Africa

Country	Year	Predominant pathogens
Zimbabwe[54]	1991	Group B streptococcus, *S. pneumoniae, Streptococcus* species
Ethiopia[55]	1998	*K. pneumoniae, E. coli, Enterobacter* species
Kenya[57]	2003	*E. coli*, Group B Streptococcus, *K. pneumoniae*
Malawi[33]	2005	Group B streptococcus, *Streptococcus pneumoniae, Salmonella* species
Nigeria[56]	2008	*S. aureus, E. coli*

In keeping with studies from Africa, South African studies reported that common pathogens associated with neonatal meningitis included GBS, followed by *K. pneumoniae* and *E. coli.* Prior to the Listeriosis outbreak of 2017, *Listeria monocytogenes* was a rare cause of neonatal meningitis [58, 59]. However, during the outbreak, 43.0% of cases occurred in neonates [60].

Studies from Durban, South Africa, demonstrated a predominance of GBS and gram-negative organisms which mimic the national trends (Table 6). There were two major studies conducted within the same healthcare facility in different years, which may explain why the causative organisms reported were similar [61, 62]. Additional sporadic reports of neonatal meningococcal meningitis occur [63, 64]. However, meningococcal meningitis in the neonatal age group is a rare finding (2 – 9 cases per 100 000 live new-borns) [64].

Table 6: Predominant causes of neonatal meningitis in Durban, South Africa

Author	Year	Predominant pathogens
Coovadia et al.[61]	1989	*K. pneumoniae, E. coli,* Group B streptococcus
Haffejee et al.[65]	1991	Group B streptococcus, *E. faecalis*
Adhikari et al.[62]	1995	Group B streptococcus, *K. pneumoniae, E. coli*

1.4.3. Respiratory samples

Regarding respiratory infections, neonatal pneumonia may be divided into early- and late-onset [66]. Early-onset pneumonia is associated with aspiration of amniotic fluid during labour or early rupture of membranes, or initial low-grade intra-uterine infection. Common pathogens include *E. coli,* Group B streptococcus, *K. pneumoniae, S. aureus* and *S. pneumoniae.* As suggested by Duke et al. (2005), within neonatal units, late-onset pneumonia is precipitated by endotracheal tube colonisation and a breach in local immunity. According to this review a predominance of gram-positive infections such as *S. pneumoniae* and *S. aureus* was reported [66].

It has been shown that ventilator-associated pneumonia and nosocomial pneumonia are of major concern in neonatal intensive care units as gram-negative bacteria, notably *K. pneumoniae,* predominate the bacteriological profile [67].

The most common cause of community-acquired atypical neonatal pneumonia is *Chlamydia trachomatis* [66]. Tuberculosis should also be considered as a cause of neonatal pneumonia in South Africa [68].

1.4.4. Urine

According to Tan et al. (2016) the definition of urinary tract infections (UTI) in adults is a collective term for infections involving any part of the urinary tract [69]. However, a concise definition in neonates has not been established [70]. It has been found that neonatal UTIs are more common in male neonates and more frequently present as pyelonephritis [71]. However, the overall incidence remains low in developing nations [72].

E. coli and *K. pneumoniae* are leading causes of neonatal UTI [73-76]. However, the contribution of *E. coli* to UTIs is lower in neonates than in older infants and children [73].

According to Taheri et al. (2012) a high level of resistance to ampicillin (95.9%), gentamicin (52.6%) and cotrimoxazole (45.4%) among UTI isolates in neonates was found [75].

1.5. Fungal profile of neonatal sepsis

Fungal infections comprise a smaller proportion of neonatal pathogens compared to bacterial infections [77]. However, the incidence of candidemia is on an upward trend in South Africa [78]. In contrast, neonatal fungal sepsis in the United States of America (USA) was noted to be on the decline [79]. A strong association with LOS was detected, suggesting a causal relationship with hospital intervention [80]. These interventions included mechanical ventilation, central line insertion and abdominal surgery [77, 78, 81]. In addition, chorioamnionitis and vaginal deliveries were also associated with an increased risk of EOS with invasive candidiasis (IC) [82].

The spectrum of fungal species in the NICU may be divided into *Candida* and non-candida organisms. Under *Candida* infections, *C. albicans* and non-albicans *Candida* species (NACS) are of equal importance [83]. *Candida* colonisation may give rise to invasive candidiasis (IC), which may involve the brain (meningoencephalitis), eyes (endophthalmitis), heart (endocarditis), lung (pneumonia), kidney (abscesses) and urinary tract [84, 85].

Non-candida fungal aetiologies are rare in neonates, however, aspergillosis, zygomycosis and *Malassezia* sepsis have been reported. [86]

1.5.1. Blood cultures (candidaemia)

The incidence of candidaemia ranges from 2.3% to 24.0%, depending on geographic location, study setting and patient demographics [84, 87-90]. *Candida* species have been reported as the third leading cause of BSI amongst extremely-low-birthweight neonates [87]. Therefore, temporal trends of candidaemia vary across the globe, declining in the USA while increasing in South Africa [78, 91].

This may be related to the profile of candidaemia across neonatal units which is also variable. Studies conducted in United States of America, England and Europe established that *C. albicans* was the predominant pathogen causing candidaemia [79, 92-95]. However, *C. parapsilosis* is a significant cause of candidaemia in some European settings [77].

Differing aetiological profiles of candidaemia also occur across Asia. Ariff et al. (2011) suggested that *C. albicans* is the predominant cause of neonatal candidaemia in Pakistan [96]. However, the emergence of NACS now outweighs *C. albicans* as a cause of candidaemia in many other Asian countries [97-99]. The main NACS isolated with increased frequency include *C. parapsilosis, C. glabrata* and *C. tropicalis.*

Data on neonatal candidaemia within Africa remains limited. *C. albicans* has been implicated as a major pathogen in limited studies from Egypt and Nigeria [89, 100, 101]. However, in South Africa, studies have reported *C. parapsilosis* as the leading causative organism of candidaemia in neonates [78, 88, 102]. Of concern is the emergence of the multi-drug resistant yeast, *C. auris*, as a cause of neonatal sepsis [103].

1.5.2. Cerebrospinal fluid (CSF)

The central nervous system is the primary site of *Candida* infections in approximately 8,0% of cases, however, concomitant candidaemia may be absent [86, 104]. It has been demonstrated that CSF parameters may be normal in a percent of neonates with culture-positive *Candida* meningitis [105, 106]. *C. albicans* is predominantly implicated in fungal CNS infections. Other organisms include *C. parapsilosis, C. tropicalis* and *C. glabrata* [85, 105]. *Malassezia pachydermatis* may cause meningitis in preterm neonates [107].

1.5.3. Respiratory samples

Several factors affect the development of fungal pneumonia in neonates, including prematurity, low-birth weight, prolonged hospital stay and use of combination broad-spectrum antibiotic therapy [108]. Also, *Candida* pneumonia was strongly associated with early-onset IC in low-birth weight infants with *C. albicans* acting as a causative organism of congenital pneumonia [82, 109].

1.5.4. Urine

Candiduria may reflect colonisation, isolated urinary tract infection or IC [110]. This may predispose to the development of fungal bezoars and subsequent urinary tract obstruction in neonates [104]. *C. albicans* has been isolated most often compared to other fungi [110].

1.6. Diagnosis of neonatal sepsis

As a consequence of various definitions in use for neonatal sepsis, the diagnosis of neonatal sepsis is a challenge. The gold standard for diagnosis is culture from a sterile site [111]. Many factors may result in negative results such as intrapartum antibiotics, low or intermittent bacteraemia and suboptimal blood sampling practices [111]. However, positive cultures from a sterile site are not always present in neonatal sepsis but a significant isolate from a blood culture is the gold standard.

A variety of haematological indices, biochemical markers and microbiological tests may assist in diagnosing neonatal sepsis. Each test has unique performance indices that vary according to the timing of infection onset. Conventional tests include the white cell count, platelet count, C-reactive protein (CRP) and procalcitonin (PCT) (Table 7). These tests demonstrate suboptimal sensitivities with high specificities. Physiological, maternal, perinatal and neonatal factors, such as maternal pyrexia and mechanical ventilation, may affect the test results [111]. Novel markers, for examples interleukin 1β, interleukin 6 and lipopolysaccharide-binding protein, display improved performance characteristics when compared to conventional markers (Table 8A and Table 8B). Mannose-binding lectin correlates well with the presence of infection [111]. However, these novel tests are not routinely utilised for the diagnosis of neonatal sepsis. Polymerase chain reaction (PCR) and matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) may assist in diagnosing infections after antibiotic therapy administration and in culture negative infection. (Table 8B) [111]. Traditionally, microbiological diagnosis of neonatal sepsis has relied on culture. The limitations of culture and the complexities of diagnosis of this disease may be circumvented by use of combined testing modalities. However, careful consideration to test performance characteristics is advisable.

Table 7: Conventional laboratory tests for diagnosis of neonatal sepsis

Test	Timing	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	Confounding factors
WCC	EOS	79 – 99	0.3 – 18	36	94 – 99.8	Bacteraemia may present with normal indices during the first hours of sepsis. Maternal and intrapartum factors influence the WCC.
	LOS	80 – 99	0.1 – 23	13 – 100	74 – 96	
PLT	EOS	97-99	0.8 - 4	13 – 14	-	Affected by several factors including viral infections and ventilation.
	LOS	89 – 98	8 – 48	9	94	
PCT		30 – 99	7 – 100	33-90	91-100	Increases can be noted in perinatal asphyxia, respiratory distress syndrome and foetal distress.
CRP		59 – 87	9 – 89	33 -96	50 – 94	Physiological increases at day 3 of life may occur.

Adapted from Tam et al. (2017)[111] - CRP – C-reactive protein; EOS – early onset sepsis; LOS – late onset sepsis; PCT – procalcitonin; PLT – platelet count; PPV – positive predictive value; NPV – negative predictive value; WCC- white cell coun

Table 8A: Novel tests for diagnosis of neonatal sepsis

Biomarkers						
Marker	**Timing**	**Specificity** (%)	**Sensitivity** (%)	**PPV** (%)	**NPV** (%)	**Confounding factors**
TNF-α	EOS	88	75	67	51	Associated with systemic inflammation.
	LOS	79 – 86	60 – 100	54 – 82	780 – 100	
LPS-binding protein		70 – 94	94 – 100	37 – 80	92 – 100	Cannot differentiate sepsis from SIRS.
IL-1β	EOS	70- 86	74 – 83	71	94	Elevated in cord blood following emergency caesarean sections or induced vaginal deliveries.
	LOS	59	95	35	97	
IL-6	EOS	70 – 100	54 – 84	38 – 100	59 – 97	May be elevated (intubation) or depressed (maternal hypertension) due to non-infectious causes.
	LOS	74 – 93	44 -100	40 – 86	74 – 100	

Table 8B: Novel tests for diagnosis of neonatal sepsis (continued)

Marker	Timing	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	Confounding factors
MBL		66	62	-	-	Correlates with risk of infection.
CD6 **CD14**		67 – 98	67 – 96	-	-	CD14 - Affected by the duration of labour.
Molecular Test						
Marker	**Timing**	**Specificity (%)**	**Sensitivity (%)**	**PPV (%)**	**NPV (%)**	**Confounding factors**
PCR		53 – 100	59 – 100	-	-	Potential to detect post-antibiotic, culture-negative samples.
MALDI-TOF		96 – 100	76 – 80	99.2	-	Similarities in ribosomal protein spectra may result in poor differentiation of species.

Adapted from Tam et al. (2017)[111]. CD – cluster of differentiation; EOS – early onset sepsis; IL – interleukin; LOS – late onset sepsis; LPS – lipopolysaccharide; MALDI-TOF- matrix-assisted laser desorption ionisation-time of flight; MBL – mannose-binding lectin; PCR – polymerase chain reaction; PPV – positive predictive value; NPV – negative predictive value; SIRS – systemic inflammatory response syndrome; TNF – tumour necrosis factor.

1.7. Management of neonatal sepsis

Treatment of neonatal sepsis utilises a multifaceted approach: support during organ dysfunction and antimicrobial administration [112]. The initial antibiotic therapy is empiric and based on the age at onset of sepsis, the likely underlying pathogens and the local antimicrobial susceptibility profile [112].

However, unreliable case definitions combined with the diminished sensitivity of culture methods, results in poor antimicrobial stewardship practices and excessive use of antimicrobials. Broad-spectrum antibiotics are necessary prior to the availability of culture results. However, keeping patients on long-term broad-spectrum therapy can have deleterious effects including disturbances to the normal flora and selection for resistant organisms [113].

1.7.1 Empiric antimicrobial regimens

Common antibiotic choices include beta-lactam antibiotics (such as penicillins, third generation cephalosporins or carbapenems), glycopeptides (such as vancomycin) and aminoglycosides (such as gentamicin or amikacin) [112]. The current WHO guidelines advocate the use of penicillin or ampicillin, in combination with gentamicin, for neonates with suspected sepsis [15]. Antibiotics, such as cloxacillin and gentamicin, are deemed necessary in patients at risk for *Staphylococcal* infections [15].

The prescription of recommended first-line antibiotics occurs in HIC with a greater frequency than other regimens, despite the emerging presence of multi-drug resistant organisms (MDRO) [114].

Labi et al. (2016) compared empiric antibiotic regimens in Ghana to antimicrobial susceptibility profiles and found that in both EOS and LOS, the most susceptible regimen was cloxacillin and gentamicin (71.6% and 63.6%). Less effective regimens included the first-line regimens ampicillin plus gentamicin (32.3% and 36.2%) and ampicillin plus cefotaxime (20.7% and 24.6%) [30]. It was suggested by Pokhrel et al. (2018) that to improve antimicrobial coverage a change in first-line antibiotic regimen, to piperacillin/tazobactam plus ofloxacin, and second-line regimen, to vancomycin plus meropenem, would be necessary [27].

There are studies from South Africa which suggests that treatment regimens containing a penicillin and an aminoglycoside may still be effective in treating EOS [35, 47]. However, in

settings of LOS, where organisms are predominantly hospital-acquired, broader spectrum antibiotics are required to treat the increased prevalence of MDROs [35].

1.8. Antimicrobial resistance in neonatal sepsis

The problem of antimicrobial resistance is common to both HICs and LMICs. In addition, multidrug resistance is an emerging issue that threatens empiric antimicrobial regimens.

1.8.1. Antimicrobial resistance in high-income countries

Resistance to first-line empiric antibiotic therapy has been recorded across Europe and North America. Bryan et al. (1985) reported the emergence of cefotaxime-resistant gram-negative bacilli after inclusion of cefotaxime into an empiric neonatal sepsis regimen [115]. It has been postulated that the greater use of intra-partum antibiotics has driven the emergence of ampicillin-resistant *E. coli* [116]. A 10-year study, by Cole et al. (2019), determined that *E. coli* isolates from neonatal BSI in the USA showed increased resistance to ampicillin (67.0%), gentamicin (14.0%) and ceftriaxone (2.0%) [117]. Other reports from the USA confirmed emerging ampicillin resistance, notably from *E. coli* [118, 119]. Single-centre studies have revealed multi-drug resistance in *Serratia marcesens*, *K. pneumoniae* and *E. cloacae* [120, 121]. Also, methicillin-resistant *S.aureus* (MRSA) has been associated with multiple outbreaks within NICU settings [122-125]. In Spain, neonatal *E. coli* isolates examined over 10 years harboured resistance to both ampicillin (92,8%) and gentamicin (22,6%) [126]. The neonIN infection surveillance network (United Kingdom) calculated a higher level of antimicrobial resistance among LOS isolates than EOS isolates [127].

1.8.2. Antimicrobial resistance in low-and-middle-income countries

In LMICs, antimicrobial resistance is increasing, especially in NICU settings [23]. Elevated levels of resistance have been demonstrated towards the common beta-lactam antibiotics (Table 9). Increased resistance towards amoxicillin and oxacillin in gram-positives has been reported [27, 31, 46]. Resistance to ampicillin and the third generation cephalosporins was demonstrated among gram-negative isolates [26, 27, 31, 46]. Similar findings have been corroborated by studies performed in Southern Asia and Africa [25, 49, 128]. An alternate

first-line antibiotic is gentamicin. However, resistance to gentamicin was observed in *Staphylococcus epidermidis* (57.0%), *P. aeruginosa* (25.0%), *Enterobacter* species (50.0%) and *Proteus mirabilis* (100.0%) [31].

Antibiotics that maintain high levels of susceptibility in gram-negative bacilli include the carbapenems, tigecycline and colistin, however, resistance to these antibiotics is increasing [27]. Gram-positive organisms display susceptibility to vancomycin and linezolid (Table 9) [27].

In South Africa, antimicrobial resistance was noted from both community-acquired neonatal sepsis and hospital acquired neonatal sepsis [47]. Literature suggests that first-line antibiotics are still effective against community-acquired neonatal infections (ampicillin and gentamicin). However, hospital-acquired isolates were reported to be more resistant while maintaining susceptibility to second-line, broader-spectrum agents (amikacin, piperacillin and meropenem) [35, 47]. Several South African studies documented the emergence of drug resistance to multiple antibiotics (Table 10) [35, 38, 40, 45, 47].

Table 9: Overview of the antimicrobial susceptibility patterns in BSI in LMIC

Country	Author	Year	Organisms	Susceptibility patterns
India	Pokhrel et al.[27]	2018	Gram-negatives	↑Resistance to CTX CIP, GN Susceptible to carbapenems, TG and COL
			Gram-positives (mostly CoNS)	↑Resistance to OX, CTX, MP; Susceptible to VA, LZD
	Muley et al.[26]	2015	Gram-negatives *S. aureus*	↑Resistance to CTX/CAZ Methicillin resistance
	Roy et al.[25]	2002	Enterobacterales	↑Resistance to PG and extended spectrum cephalosporins
	Kaistha et al.[128]	2009	Gram-negatives	Resistance to PG and third-generation cephalosporins Sensitive to IMI, AK
			Gram-positives	All sensitive to VA
	Aku et al.[31]	2018	Gram-negatives	↑Resistance to AMP, CXM, SXT and GN
			CoNS, *S. aureus*	↑Resistance to PG, FLU and SXT
Nigeria	Iregbu et al.[48]	2006	*K. pneumoniae*	ESBL production
Zambia	Kabwe et al.[29]	2016	*K. pneumoniae*	Resistance to third-generation cephalosporins
Multi-national	Hamer et al.[46]	2015	Gram-negatives	Resistance to PG, third-generation cephalosporins and GN
			S, aureus	Methicillin resistance

AK – amikacin; AMP – ampicillin; CAZ – ceftazidime; CIP – ciprofloxacin; CTX – cefotaxime; COL – colistin; CXM – cefuroxime; FLU – flucloxacillin; ESBL – extended-spectrum beta-lactamase; GN – gentamicin; IMI – imipenem; LZD – linezolid; MP – meropenem; OX – oxacillin, PG – penicillin; SXT – cotrimoxazole; TG – tigecycline; VA - vancomycin

1.8.3 Multi-drug resistance organisms

Multi-drug resistant (MDR) organisms can be defined as organisms that display non-susceptibility to one or more agents in three or more antimicrobial classes. Following on that definition, extensively-drug resistant (XDR) organisms demonstrate non-susceptibility to one or more agents in two or less categories of drugs. Pan-drug resistant (PDR) organisms are non-susceptible to all classes of antibiotics [129].

MDR organisms have been observed across the globe, affecting both gram-positive and gram-negative isolates [27, 29-31, 117]. These resistance patterns appear to increase during late-onset sepsis. It has been found that certain first-line antimicrobial regimens, such as ampicillin plus cefotaxime, have decreasing susceptibility [30]. For example, isolates of *K. pneumoniae* that often produce extended spectrum beta-lactamases (ESBL) remain susceptible to imipenem [48, 49]. Neonatal BSI *E. coli* isolates also produced ESBL [117].

Within South Africa certain multidrug resistant phenotypes have occurred frequently (Table 10). Extended spectrum beta-lactamase-producing organisms were identified amongst both community-acquired and hospital-acquired neonatal BSI [38, 45, 47]. There are variable reports on the incidence of carbapenem-resistant Enterobacterales (CRE) in neonatal infection throughout South Africa. Ballot et al. (2019) reported the emergence of CRE within a tertiary NICU in Johannesburg, South Africa [130]. However, CRE isolates did not occur within a neonatal population in Khayelitsha [47]. Among other gram negatives, the MDR phenotype was detected in association with *A. baumannii* [45, 131]. From the gram-positive spectrum, high levels of MRSA occur within neonatal units [35, 38, 40, 45].

Table 10: Patterns of multi-drug resistance in neonatal BSI (South Africa)

Study	Year	Organisms	Susceptibility pattern
Ballot et al.[35]	2012	*K. pneumoniae* CoNS, *S. aureus*	ESBL production Methicillin resistance
Morkel et al[40].	2014	*K. pneumoniae* *Acinetobacter* spp *S. aureus*	ESBL production MDR phenotype Methicillin resistance
Dramowski et al.[45]	2015	*K. pneumoniae* *A. baumannii* *S. aureus*	ESBL production MDR phenotype Methicillin resistance
Lebea et al.[38]	2017	*K. pneumoniae* *S. aureus*	ESBL production Methicillin resistance
Crichton et al.[47]	2018	*E. coli, K. pneumoniae*	ESBL production
Thomas et al.[59]	2018	*A. baumannii*	MDR phenotype
Ballot et al.[130]	2019	Enterobacterales	Carbapenem resistance

CoNS – coagulase negative staphylococci; ESBL – extended spectrum beta-lactamase; MDR – multi-drug resistance

1.9. Significance of the study

South Africa's National Perinatal Morbidity and Mortality (NPMM) Committee's HHAPI-NeSS strategy highlights key areas needed to improve neonatal survival [9]. Reducing deaths due to infection is advocated by the NPMM Committee to attain this goal. Suggested activities include:

- Ensuring presumptive antibiotic therapy for the at-risk neonate is available
- Management of neonatal sepsis, meningitis and pneumonia

Inappropriate or incorrect antibiotic therapy predisposes to longer hospital stays and prolonged antibiotic exposure with the consequent side effects [31]. In the era of multidrug resistance, deciding on an appropriate antibiotic is problematic due to increasing resistance to first-line antibiotics. Furthermore, microbiological culture results take on average 48 to 72 hours, delaying definitive therapy [12].

Due to difficulties diagnosing neonatal sepsis, antibiotic stewardship within an NICU remains a challenge for the neonatologist. In addition, antimicrobial susceptibility patterns may vary over time [132]. These changing patterns of resistance require that regular microbial surveys be conducted. Hence, understanding the microbial profile of a neonatal unit can contribute to appropriate early management of sepsis, thereby, improving therapeutic outcomes.

The microbial profile of neonatal units in KwaZulu-Natal remains under-explored and investigations into causes of neonatal sepsis are limited in our setting and require more attention. This study endeavours to contribute to the knowledge of trends in microbes and antimicrobial susceptibility patterns to guide empiric management and facilitate antimicrobial stewardship activities with the unit.

1.10. Aim and objectives

1.10.1. Aim

To establish the microbiological and antimicrobial susceptibility profiles and trends of neonatal bacteraemia in the Neonatal Intensive Care Unit (NICU) at Inkosi Albert Luthuli Central Hospital (IALCH), Durban at three biennial intervals (2014, 2016, 2018).

1.10.2. Objectives

1. To determine the common microbial pathogens isolated from blood cultures within the unit.
2. To establish temporal trends for common organisms and antimicrobial susceptibility profiles over three biennial intervals (2014, 2016, 2018).
3. To investigate the incidence and trend of multidrug resistant organisms in the unit.
4. To advise an empiric antimicrobial strategy based on current susceptibility data.

CHAPTER TWO

Neonatal sepsis in a tertiary unit in South Africa: Big drugs for baby bugs

Prepared according to the Instructions for Authors of **Journal of Infection**

Neonatal sepsis in a tertiary unit in South Africa: Big drugs for baby bugs

Running Title: Neonatal sepsis in South Africa

By

Dharshni Pillay[a,b] (dharshnipillay@gmail.com)

Yesholata Mahabeer[a,b] (MAHAB@ukzn.ac.za)

[a] Department of Medical Microbiology, National Health Laboratory Service, Inkosi Albert Luthuli Central Hospital, 800 Vusi Mzimela Road, Durban, 4091, KwaZulu-Natal, South Africa

[b] School of Laboratory Medicine and Medical Sciences, Nelson R. Mandela School of Medicine, University of KwaZulu Natal, 716 Umbilo Road, Berea, 4001, KwaZulu-Natal, South Africa

Corresponding author: Dharshni Pillay

Affiliation address: Inkosi Albert Luthuli Central Hospital, 800 Vusi Mzimela Road, Durban, 4091, KwaZulu-Natal, South Africa

Permanent address: 35 Seatides Drive, Seatides, 4399, KwaZulu-Natal, South Africa

Email: dharshnipillay@gmail.com

Contact number: +27-83-647-2317

HIGHLIGHTS

- **Antimicrobial resistance is a growing threat in neonatal intensive care units.**
- **Coagulase-negative staphylococci were predominant in early and late-onset sepsis.**
- **ESBL *Klebsiella pneumoniae* and MDR *Acinetobacter baumannii* were common gram-negatives isolated.**
- **High levels of resistance were noted among first line and second line antimicrobials.**
- **An empiric regimen of meropenem is advised with the addition of vancomycin depending on the clinical setting.**

ABSTRACT

Objectives

Antimicrobial resistance (AMR) has emerged as a global threat to healthcare resulting in an increase in morbidity and mortality. Neonatal sepsis is ranked as the third highest cause of neonatal demise globally, in which AMR accounted for 31.0% of deaths. This study analysed the aetiology and antimicrobial resistance patterns of bloodstream infections within the neonatal intensive care unit (NICU) at a tertiary hospital in Durban, South Africa.

Methods

A retrospective data review was conducted on all positive blood cultures at three time periods: 2014, 2016 and 2018. The organisms and antimicrobial susceptibilities were analysed for significant trends using Poisson and logistic regression.

Results

A preponderance of gram-positive organisms (68.7%) over gram-negatives (26.8%) and fungi (4.5%) was detected. Common pathogens included coagulase-negative staphylococci (53.5%), *Klebsiella pneumoniae* (11.6%), enterococci (9.3%), and *Acinetobacter baumannii* (7.7%). Late-onset sepsis (86.8%) predominated over early-onset sepsis (13.2%). High rates of resistance to first- and second-line antibiotics were noted among gram-positive and gram-negative organisms. Multidrug resistant organisms included extended-spectrum beta-lactamase (ESBL) *K. pneumoniae* (7.6%) and multi-drug resistant *A. baumannii* (7.0%). A statistically significant decrease in ESBL-producing organisms was documented between 2014 and 2018 ($p = 0.005$).

Conclusion

High resistance rates were seen for first- and second-line antibiotics used for the treatment of neonatal sepsis. Ongoing microbial surveillance is essential to tailor empiric antimicrobial choices in individual units.

Keywords: Neonatal sepsis, microbial profiles, antimicrobial resistance, multi-drug resistant organisms

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a global threat to healthcare resulting in an increase in morbidity and mortality [1]. An estimated 31.0% of deaths from neonatal sepsis are attributed to AMR [2]. Sepsis accounts for 6.8% of neonatal deaths, ranking it as the third highest cause of neonatal demise following preterm births and intrapartum-related events [3]. In Sub-Saharan Africa, sepsis-related neonatal mortality rates are high and range between 17.0% to 29.0%. [4].

Neonatal sepsis presents unique diagnostic challenges largely due to the absence of a universal definition [5]. Traditionally defined as sepsis with the first 28 days of life, neonatal sepsis can be further stratified into early-onset (< 3 days) and late-onset (≥ 3 days) [6, 7]. It entails a collection of non-specific clinical features or laboratory signs of sepsis with positive microbiological cultures from a sterile sample (although cultures may not always be positive). A significant isolate from a blood culture is the gold standard for diagnosis [7].

Based on blood culture data, early-onset sepsis (EOS) and late-onset sepsis (LOS) differ in microbial profiles. Group B streptococcus (GBS) is a major cause of early onset sepsis in high-income countries (HIC) [8]. This contrasts with the bacteriological profile of resource-limited settings where GBS rates are lower and *Klebsiella pneumoniae*, *Staphylococcus aureus* and coagulase-negative Staphylococci (CoNS) are the predominant pathogens of EOS [5, 9-15]. In South Africa, EOS is mainly caused by GBS, *K. pneumoniae* and *Escherichia coli* [16-20].

A wider spectrum of gram-negatives, including *K. pneumoniae*, *Citrobacter* species, *E. coli, Enterobacter* species, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are observed during LOS [13, 15]. Furthermore, CoNS cause significant cases of gram-positive LOS in many countries [19, 21]. Other important bacterial causes of LOS in South Africa are enterococci, *K. pneumoniae* and *Acinetobacter* species [20, 22-24].

A strong association between fungal infections and LOS suggests a causal relationship with hospital intervention [25]. *Candida* species have been reported as the third leading cause of blood-stream infection (BSI) amongst extremely-low-birthweight neonates [26]. In South Africa, studies have reported *C. parapsilosis* as the leading causative organism of candidaemia in neonates [27-29].

Global resistance to first-line empiric treatment regimens is on the increase [11, 15, 24, 30]. This pattern was observed in South Africa, as antimicrobial resistance was noted from both community-acquired and hospital-acquired neonatal sepsis [31]. Multi-drug resistance adds a

further complication to antimicrobial choices. As indicated, in several South African studies, the emergence of drug resistance to multiple antibiotics, including extended-spectrum β-lactamase production (ESBL) and methicillin-resistant *S. aureus* (MRSA), has been documented [16, 20, 22, 23, 31]. These changing patterns of resistance require that regular microbial surveys be conducted. Consequently, understanding the microbial profile of a neonatal unit contributes significantly to early appropriate empiric antimicrobial choices in the management of sepsis. This improves therapeutic outcomes and reduces mortality.

In South Africa, and notably in KwaZulu-Natal, a limited knowledge base surrounding local microbial profiles and AMR in neonatal sepsis exists, compared to other countries. Therefore, this study aims to establish the microbiological and antimicrobial susceptibility profiles and trends of neonatal bloodstream infections in the Neonatal Intensive Care Unit (NICU) of Inkosi Albert Luthuli Central Hospital (IALCH).

METHODS

Study design, location, and period

This study is a retrospective review of positive blood cultures from the NICU at IALCH, which is a tertiary and the only quaternary referral unit for KwaZulu-Natal, South Africa. The unit consists of 12 intensive care and 8 high-care beds, with approximately 700 to 800 admissions per year and a bed occupancy rate of 100%. The patients are usually from surgical, neurosurgical and cardiology disciplines.

Data was collected from 2014 to 2018 at three biennial periods: 2014, 2016 and 2018. The data was accessed from the National Health Laboratory Service (NHLS) Central Data Warehouse.

Study population

The study samples consisted of all positive blood cultures from NICU for the period January to December in years 2014, 2016 and 2018. Samples were included from patients aged 0 to 30 days of life. Repeat blood cultures taken within 14 days of the index culture, where the same organism was isolated again, were excluded from the study.

Laboratory methods

The techniques utilised by the laboratory to generate the microbial identifications and antimicrobial susceptibility test is included in Appendix F.

Measurements

The onset of sepsis was classified as early-onset (< 3 days old) and late-onset sepsis (≥ 3 days old) measured from the date of birth until collection of the index culture.

Primary outcomes: The prevalence of common organisms and antimicrobial susceptibility patterns were evaluated as a proportion of the total number of positive cultures. Antimicrobial susceptibility patterns were stratified and analysed according to gram-positive, gram-negative and fungal antimicrobial panels. These panels consisted of antimicrobials routinely tested within the IALCH microbiology laboratory.

Secondary outcomes: Rates of change in prevalence of organisms and antimicrobial susceptibilities per year were calculated and prevalence of specific multi-drug resistant organisms (MDRO) during the study period was sought. These MDROs included [32]:

- MDR gram-negatives – resistant to at least one agent from two or more classes of all tested antimicrobial agents.
- XDR - non-susceptibility to one or more agents in two or less categories of drugs.
- Carbapenem-resistant Enterobacterales (CRE) – resistant to at least one carbapenem (imipenem, meropenem, ertapenem).
- ESBL – resistant to a 3rd or 4th generation cephalosporins or detected as an ESBL through automated methods (Vitek® 2 Advanced Expert System, bioMerieux)
- MRSA – *S. aureus* resistant to cloxacillin.
- VRE – enterococci resistant to vancomycin.

Statistical analysis

Descriptive statistics were used to summarise the data. Categorical data were summarised by frequencies and percentage. The frequency of selected organisms was reported by year. Susceptibility of each drug was reported as the percentage susceptible. Prevalence was

calculated as a measure of the total number of samples in the data series. The number of each organism seen per year is a count variable. Comparisons of pathogens by subgroup, such as early-onset and late-onset neonatal sepsis, was done using Chi Square or Fisher's exact test. Temporal trends in the number of organisms and antimicrobial susceptibility patterns over time were analysed using Poisson and logistic regression. Stata V13.1 was used for the data analysis and *p*-value of 0.05 was considered statistically significant.

Ethical considerations

This study has received approval from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BE019/19), IALCH, NHLS and the KwaZulu-Natal Department of Health.

RESULTS

Six hundred and eighty-eight isolates were obtained during the three study periods. These were divided into eight organism types: *Staphylococcus aureus,* coagulase negative staphylococci (CoNS), *Streptococcus* species, *Enterococcus* species, Enterobacterales, non-fermenting gram-negative organisms, fungi, and "others". The "others" category comprised seven which include possible contaminant (*Micrococcus* species and *Rothia* species). Possible significant neonatal pathogens, such as *Listeria monocytogenes, Eikenella corrodens*, *Routella* species and M*oraxella catarrhalis,* were also included in the "others" category. However, the number of "other" organisms were low (one isolate in each group), therefore, the group was excluded to prevent distortion of the statistical and regression analyses. Thus, the final analysis included 681 isolates from three years: 2014 (207 isolates), 2016 (222 isolates) and 2018 (252 isolates).

Microbial profile

Coagulase negative staphylococci (363/681; 53.3%) were the most common isolates in this study. This was followed by Enterobacterales (118/681; 17.3%), enterococci (64/681; 9.4%), non-fermenting gram-negative organisms (64/681; 9.3%), *Candida* species (31/681; 4.6%), *Staphylococcus aureus* (24/681; 3.5%), and *Streptococcus* species (17/681; 2.5%) (Figure 1).

Gram-positive organisms were predominantly isolated (468/681; 68.7%) (Figure 2). Within the gram-positive category, the main organisms were CoNS (363/468; 77.6%), *Enterococcus* species (64/468; 13.7%), *S. aureus* (24/468; 5.1%) and *Streptococcus* species (17/468; 3.6%). Often, CoNS are common blood culture contaminants and in the absence of clinical correlation, the significance of these organisms is unclear. If CoNS were excluded from the analysis, enterococci emerge as the leading cause of gram-positive sepsis (64/105; 61.0%), comprising *Enterococcus faecium* (40/64, 62.5%); *Enterococcus faecalis* (22/64; 34.4%) and *Enterococcus* species (2/64; 3.1%). Only one *Streptococcus agalactiae* isolate was found during the study period.

Gram-negatives accounted for 26.8% of the total study population (182/681) and consisted of Enterobacterales (118/182; 64.8%) and non-fermenters (64/182; 35.1%). (Figure 2). Within the Enterobacterales family, the most common organisms were *K. pneumoniae* (79/118; 66.9%), *E. coli* (13/118; 11.0%) and *Serratia marcesans* (10/118; 8.5%). Non-fermenters consisted almost exclusively of *Acinetobacter baumannii* (53/64; 82.8%), followed by *Stenotrophomonas maltophilia* (6/64; 9.4%) and *Pseudomonas aeruginosa* (5/64; 7.8%).

Fungal isolates were less commonly isolated than bacterial isolates (4.5% vs. 95.5%) and consisted of *C. parapsilosis* (14/31; 45.2%), *C. albicans* (9/31; 29.0%) and other *Candida* species that were not speciated further (8/31; 25.8%). Non-albicans *Candida* species (22/31; 71.0%) predominated over *C. albicans* (9/31; 29.0%).

Early-onset sepsis versus late-onset sepsis

In this study, the majority of organisms were isolated during late-onset sepsis (591/681, 86.8%), with early-onset sepsis (EOS) accounting for only 13.2% (90/681) of cases ($p = 0.02$). There were no significant differences in the predominant organisms between EOS and LOS when analysed within the specified groups: CoNS (56.7% vs 52.8%, $p = 0.5$), Enterobacterales (11.1% vs 18.3%, $p = 0.09$), non-fermenters (7.8% vs 9.6%, $p = 0.6$) and enterococci (10.0% vs 9.3%, $p = 0.8$) (Figure 3). However, sub-analysis revealed that species-level differences between the two groups existed (Table 1). Notably, *S. aureus* and *E. faecalis* were more significant in EOS ($p = 0.006$ and $p = 0.048$, respectively). *E. faecium* emerged as an important gram-positive organism of LOS ($p = 0.2$). In addition, although *A. baumannii* is a predominant gram-negative organism in EOS, gram-negative sepsis was preponderated by *K. pneumoniae* isolates in LOS. However, this difference did not demonstrate statistical significance.

Trends in incidence of organisms

Coagulase negative staphylococci, *K. pneumoniae, A. baumannii* and enterococci were leading organisms over the three study periods (Table 2).

A comparison across the study period revealed similar organisms predominating each year (Figure 4). However, an increase in the number of enterococci was observed over time, and although not statistically significant, by 2018, it was the commonest organism in the unit after CoNS. Analysis of the trends of the other organisms revealed no significant patterns, except for *Streptococcus species* (Table 3). *Streptococcus* species, specifically viridans streptoccoci, demonstrated a significant increase in the number of isolates between 2014 and 2018 (IRR 9.04; CI 1.17 – 69.99; $p = 0.04$). Their overall contribution to the sample pool was low, 16/681 (1/16 in 2014, 4/16 in 2016 and 11/16 in 2018), therefore, there was no impact on the leading pathogens over the three study years.

Antimicrobial susceptibility patterns

Overall antimicrobial susceptibility patterns for the study period

Following the exclusion of absent data (n = 449 specific bug-drug combinations), group-specific antimicrobial susceptibilities were analysed for the total study period. (Table 3)

Among the gram-positive organisms, the susceptibility of *S. aureus* and CoNS to cloxacillin was 20.8% and 8.8%, respectively. Ampicillin susceptibility was only 37.5% for enterococci which may be attributed to the high number of *E. faecium* in the study. All gram-positive isolates tested during the study period were susceptible to vancomycin (100.0%).

The Enterobacterales revealed overall low susceptibilities to third generation cephalosporins, such as cefotaxime (34.2%) and ceftazidime (27.7%). Susceptibility to piperacillin/tazobactam was 55.5%. Amikacin displayed higher susceptibility than gentamicin (70.3% vs 39.7%, respectively). Susceptibilities of the carbapenems were 91.8% for imipenem, 92.3% for meropenem and 90.3% for ertapenem. Ciprofloxacin susceptibility was 62.4%.

Analysis of the non-fermenters, demonstrated low levels of susceptibility towards all tested antibiotics: piperacillin/tazobactam (15.5%), ceftazidime (15.3%), gentamicin (11.5%), amikacin (49.1%), meropenem (17.2%), imipenem (13.5%) and ciprofloxacin (19.7%).

Approximately half (54.8%) of the fungal isolates tested were susceptible to fluconazole.

Trends in susceptibility patterns over the study period

A rise in susceptibility to cloxacillin among *S. aureus* and CoNS between 2014 (4.4%) and 2016 (13.7%) was observed (p = 0.02; OR 3.46; 95% CI 1.23 – 9.72), which did not continue into 2018.

Among Enterobacterales, a statically significant increase in cefotaxime susceptibility was noted between 2014 (24.4%) and 2018 (55.1%) (p = 0.02; OR 3.29; 95% CI 1.15 – 7.09).

Combined analysis of all the gram-negatives demonstrated a significant decrease in susceptibility of amikacin between 2014 (85.8%) and 2018 (53.8%) (p = 0.002; OR 0.24; 95% CI 0.10 – 0.59). During the same period, there was an increase in gentamicin susceptibility (19.7% to 43.4%; p = 0.01; OR 3.14; 95% CI 1.33 – 7.42).

Fluconazole susceptibility initially increased between 2014 (22.2%) and 2016 (70.0%) (p = 0.047; OR 8.17; 95% CI 1.03 – 64.94), and then plateaued by 2018.

Susceptibility trends between 2014, 2016 and 2018, for the other antibiotics (ciprofloxacin, ceftazidime, imipenem, meropenem, penicillin, ampicillin, piperacillin/tazobactam, vancomycin) did not reveal statistical significance (Table 4).

Multidrug resistant organisms

MDROs constituted 20.0% of the total sample population (138/681) (Figure 5). The ESBL Enterobacterales isolates totaled 60/681 (8.0%) of which *K. pneumoniae* formed the majority (52/60; 86.7%). A statistically significant decrease in ESBL organisms was noted between 2014 (70.0%) and 2016 (45. 5%) (OR 0.36; CI 0.15 – 0, 88; p = 0.03). The downward trend continued further in 2018 (36.4%) (OR 0.24; CI 0.09 – 0.65; p = 0.005).

An MDR phenotype was observed among 76.6% (49/64) gram-negative non-fermenters and consisted solely of extensively-drug resistant (XDR) *A. baumannii*. There was upward trend in MDR isolates between 2014 (75.0%) and 2018 (88.9%) however this was not of statistical

significance (p = 0.3; OR 2.67; CI 0.47-15.14). The MDR phenotype equated to 7.0% of the total resistance observed in the study.

MRSA comprised 24/681 (3.0%) of the total study population and 19/24 (79.0%) of the *S. aureus* population. Completion of a regression analysis was challenging due to the small sample size of *S. aureus* and was therefore omitted.

The cohort of CREs constituted 1.4% (10/681) of MDROs with no appreciable differences in occurrence of CRE samples across the study period. When comparing the number of CRE samples from 2014 (2.5%) to 2018 (15.2%), the odds ratio equated to 6.96 (p 0.08; CI 0.77 – 62.93), which demonstrates a non-significant increase in the number of cases.

No VREs were detected during the entire study period.

DISCUSSION

According to our knowledge, this study represents the first published microbial profile of neonatal sepsis at a tertiary/quaternary unit in Kwazulu-Natal, South Africa. The leading organisms were CoNS, followed by enterococci, Enterobacterales and *A. baumannii*. Data has indicted that there was a preponderance of LOS versus EOS. A decrease in susceptibility to first- and second-line antibiotics, in both gram-positives and gram-negatives, occurred. Broader spectrum antibiotics remained susceptible, such as vancomycin for gram positives and carbapenems for Enterobacterales. Candida *species* demonstrated high fluconazole resistance. *K. pneumoniae* (ESBL) and XDR *Acinetobacter baumannii* represented the other predominant MDRO types.

Tertiary NICU settings, such as the NICU at IALCH, treat neonates that are mostly premature and of a low birth weight [33]. Since the combination of healthcare setting and patient profile is likely to influence the occurrence of LOS versus EOS, the microbial aetiology of sepsis within the neonatal unit is likely to be influenced [33, 34]. According to Giannoni et al. (2018) hospital-acquired LOS was higher in preterm infants when compared to EOS [35]. A significant majority of late-onset neonatal sepsis (86,8%) was observed in our study which corroborated with other studies from tertiary level neonatal units in South African (LOS approximated 83.0% to 93.0% of cases) [16, 19, 20].

According to literature, almost 70.0% of first-onset infections in LOS were caused by gram-positive organisms, followed by gram-negatives (18.0%) and fungi (12.0%) [36].

Subsequently, this study established that gram-positive organisms outweighed gram-negative and fungal organisms. Apart from CoNS, the leading organisms were enterococci, *K. pneumoniae* and *A. baumannii*. These findings are consistent with reports from other African countries and India [13-15]. Coagulase negative staphylococci have been implicated as a significant pathogen in LOS of premature and very-low-birth-weight infants resulting from NICU interventions such as the use of invasive devices, and the presence of immune immaturity [37]. Despite the evidence surrounding CoNS as pathogens of neonatal sepsis, isolates may still represent blood culture contamination as skin colonisers [38]. Becker et al. (2014) defined clinically significant CoNS as culturing the same isolate from blood cultures taken within 5 days, or a single positive blood culture with clinical signs of infection [39]. However, the absence of clinical data in this study rendered determining clinical significance challenging. Furthermore, the majority of CoNS, in this study were not identified to species level, which is a common practice in many microbiology laboratories [40]. Of note, studies from other LMICs have shown that CoNS were leading pathogens of neonatal sepsis (19.1% - 59.1%) after adjusting for contamination [11, 14-16, 19, 21]. If CoNS were removed from the data set, the microbial profile shifts towards a predominance of gram-negative organisms. These results then correlated with other South Africa studies [20, 23].

Studies from Botswana and South Africa have documented enterococci as a leading cause of gram-positive sepsis (12.2% to 18.0%) [21, 23]. Frequently enterococcal infections have been reported in LOS [36, 41, 42]. Risk factors in these infections include prematurity, the use of non-umbilical central lines or prolonged placement of a central line, and bowel resection [41, 43, 44]. In the current study, enterococci emerged as significant pathogens of gram-positive sepsis with a predominance of *E. faecium* in LOS and *E. faecalis* in EOS. The possibility remains that antibiotic selective pressure may drive the shift from ampicillin-susceptible *E. faecalis* in EOS to ampicillin-resistant *E. faecium* in LOS. This species-specific differentiation requires confirmation with larger studies.

South African studies determined *K. pneumoniae* and *S. aureus* are leading pathogens of neonatal sepsis [19, 20]. However, in this study, *S. aureus* was not a common cause of sepsis. From the Enterobacterales order, *K. pneumoniae* was isolated most frequently followed by *E. coli.* Studies confirm that *E. coli* and *K. pneumoniae* are well-recognized pathogens of neonatal sepsis [9-11, 17, 45]. Additionally, our study found that non-fermenters such as *A. baumannii*, *S. maltophilia* and *P. aeruginosa* were also causes of sepsis. Thomas et al. (2018) reported that *A. baumannii* is a significant pathogen in neonatal sepsis, associated with central venous

catheter placement, mechanical ventilation, and inotropic support [46]. A study undertaken by Viswanathan et al. (2011) described the emergence of gram-negative non-fermenters, particularly *A. baumannii,* in neonatal sepsis [47]. Recently, *Pseudomonas* species was identified as an important neonatal pathogen [48].

Though overall rates of candidaemia remained low when compared to bacteraemia, *C. parapsilosis* was a notable cause of neonatal candidaemia. This observation is in keeping with findings from another South African study [23]. Subsequently, Govender et al. (2016) reported an association between neonates and *C. parapsilosis* BSI [49]. There is also evidence to suggest that undetected outbreaks and intra-hospital transmission of *C. parapsilosis* occur [28].

The analysis of trends in organisms over the three intervals showed a statically significant increase in viridans streptococci (p = 0.04). This was the only significant trend in incidence observed between 2014 and 2018. Evidence from other studies have indicated the importance of viridans streptococci as a pathogen in EOS [50-54]. It is advised that careful attention be paid to these organisms, especially in the setting of a clinically ill child with serial positive cultures of this isolate from a normally sterile sample site [51].

Currently, ampicillin, cloxacillin and vancomycin are advocated in the treatment regimen of gram-positive organisms. *Staphylococcus aureus* and CoNS showed high resistance to cloxacillin, which has been demonstrated both globally and in South Africa [14, 16, 19, 23, 55]. In addition, due to the prevalence of *E. faecium* within the NICU, ampicillin susceptibility was low, which was also described in another South African setting [16]. Labi et al. (2016) reported high resistance levels in enterococci to a regimen of ampicillin and gentamicin [14]. Vancomycin susceptibility was preserved (100.0%) for the entire study period, which has been confirmed in another South Africa study [16].

It has been observed that resistance amongst the gram-negative population is on the rise within NICUs [40]. This study corroborates these findings, as Enterobacterales in this unit demonstrated high levels of resistance to first-line antibiotics (i.e. cefotaxime and gentamicin). However, susceptibility to broader-spectrum agents which included meropenem, imipenem and amikacin was evident. Patel et al. (2010) noted the loss of susceptibility to certain antibiotics, such as piperacillin/tazobactam, ceftazidime and gentamicin, and attributed this loss to the rise in ESBL Enterobacterales [40]. Amongst the non-fermenter gram-negative organisms, low levels of susceptibility were noted across several antibiotics including ceftazidime, piperacillin/tazobactam, carbapenems, gentamicin and ciprofloxacin. Many other

studies reported high levels of resistance in antimicrobials used to treat gram-negatives, including ampicillin, ceftazidime, cefotaxime, imipenem, gentamicin, ciprofloxacin while maintaining susceptibility to meropenem as confirmed by this study. [56-60]. The use of colistin for the treatment of XDR *A. baumannii* should be considered.

Neonatal units have demonstrated variable fluconazole susceptibility patterns that are dependent upon the species of their predominating fungal pathogens [61-63]. This study demonstrated that approximately half of all candida isolates were susceptible to fluconazole. However, the administration of fluconazole prophylaxis to high-risk patients may lead to selection of fluconazole resistant species [40]. Resistance was especially high among *C. parapsilosis*, which is supported by another South African study that reported fluconazole resistance amongst *C. parapsilosis* of 54.0% [28].

When susceptibility trends over the three study periods were compared for all antibiotics, susceptibility of cefotaxime amongst Enterobacterales increased significantly during the study period, however, it remained below 50.0%. This observation may be attributed to the decreasing levels of ESBL organisms noted. There were no statistically significant downward trends in susceptibility apart from amikacin. Literature includes mixed reports regarding the susceptibility of aminoglycosides, such as amikacin and gentamicin. Roy et al. (2002) reported increased amikacin resistance in an Indian NICU over a 15-year period [64]. In contrast, low amikacin resistance has been documented in other studies; therefore, amikacin is frequently included in empiric regimens [57, 58]. The temporal increase in susceptibility of gentamicin found in this study, may be attributed to the preferential use of amikacin, instead of gentamicin, in the unit. This occurrence requires further observation over time.

Although other antibiotics did not demonstrate significant trends across the duration of the study, a general shift of decreasing antimicrobial susceptibility has been seen for antibiotics used to treat gram-positive and gram-negative organisms from low-and-middle-income countries [47, 65-69].

This study documented a 20.0% MDRO incidence. However, this figure may be higher than calculated due to the presence of potential CoNS within the denominator. The highest occurring MDRO were ESBL-producing Enterobacterales. High levels of ESBL-producing Enterobacterales were noted in a systematic review of neonatal sepsis which was subsequently confirmed in a South African study [20, 69]. This study lends support to those findings. However, despite the global trend towards increasing antibiotic resistance, the rates of ESBLs

in our study have undergone a statistically significant decrease from 2014 to 2018, which is in keeping with the increased cefotaxime and gentamicin susceptibilities observed. Changes in ESBL rates may also be attributed to the increase of other MDROs within the unit. XDR *A. baumannii* were important isolates during the study period. Evidence suggests that these organisms are associated with neonatal sepsis in South Africa [46]. This study demonstrated the emergence of carbapenem-resistant Enterobacterales (CRE), which has been implicated in neonatal sepsis in a recent South African study [59].

MRSA has been found to cause neonatal sepsis in other South African studies [16, 19, 20, 23]. The overall contribution of MRSA to the study population was lower than described in other South Africa neonatal settings. However, a high percentage of MRSA amongst *S. aureus* was identified in this study. Methicillin-susceptible *S. aureus* accounted for only 20.8% of the total *S. aureus* cohort. An absence of VRE was noted, although VRE has been observed in other neonatal populations in association with prematurity and prolonged antibiotic administration [70, 71]. Two neonatal VRE outbreaks occurred in 2013 and 2014, which were limited to the Western Cape, South Africa [72].

According to the WHO, first line antibiotic therapy for neonatal sepsis consists of benzylpenicillin/ampicillin and gentamicin [5]. However, changes to empiric first- and second-line antibiotic therapy regimens have been undertaken globally. Marzban et al. (2010) advised that cephalothin and amikacin were no longer effective as empiric management of LOS in an NICU in Tehran. Therefore, a change to vancomycin plus amikacin was recommended [65]. In Nepal, Pokhrel et al. (2018) reported that changing the first-line regimen to piperacillin/tazobactam and ofloxacin improved antimicrobial cover for resistant organisms [11]. In Ghana, Labi et al. (2016) demonstrated that cloxacillin and gentamicin was more effective than ampicillin plus gentamicin or ampicillin plus cefotaxime. [14].

Choosing an appropriate empiric antimicrobial regimen in the IALCH NICU remains a challenge. In view of the high levels of antimicrobial resistance observed in this setting, meropenem with or without vancomycin provides optimal empiric cover. The addition of vancomycin would depend on the presence of risk factors for staphylococcal infection or the correlation to the clinical condition (such as the presence of intra-abdominal sepsis). This regimen would be effective due to the high prevalence of ESBL organisms and resistant gram-positive organisms in the neonatal unit. However, this regimen would not be effective against XDR *A.baumannii.* Therefore, the addition of amikacin may be considered in the critically ill

neonate, while awaiting microbiological results. Newer antimicrobials are available for the management of sepsis. However, further investigation into use in the neonatal population is required.

The Infectious Diseases Society of America guidelines recommends amphotericin B for treatment of patients with disseminated candidiasis [73]. Due to the emergence of *C. parapsilosis* in the neonatal unit, this recommendation is supported.

When assessing the appropriateness of the above recommendations, the study's limitations need to be considered. This study is based on laboratory surveillance and clinical data was not collected. However, clinical correlation is required to assist decisions regarding clinical relevance of potential pathogens in blood cultures, especially for CoNS. Additional parameters, such as patient-days, could not calculated due to the absence of in-patient clinical data. Results cannot be generalised to other settings, hospitals or patient profiles as a single centre was utilised for the analysis. Premature neonates were not stratified within the study population and may present a different bacteriological profile than other groups of neonates. This study may be underpowered to determine temporal fluctuations amongst less frequently occurring organisms. Lastly, some antimicrobial data was absent.

In conclusion, first-line antimicrobials, advocated by the WHO for treatment of neonatal sepsis, have proven ineffective in this unit due to high levels of AMR. Gram-positive sepsis, caused by CoNS and enterococci, were leading causes of sepsis in this study. Gram-negative sepsis occurs to a lesser extent and is mainly comprised of MDR *A. baumannii* and ESBL *K. pneumoniae*. These MDROs create a therapeutic challenge and require broad-spectrum agents or combination therapy. The resistance noted in fungal isolates also calls for broad-spectrum antifungals. Therefore, the way forward is surveillance of the microbial profile of neonatal sepsis which can provide evidence to assist in development of empiric regimen and antimicrobial stewardship activities. With the advent of AMR, antibiograms are needed to provide better empiric cover which, ultimately, improves sepsis outcomes.

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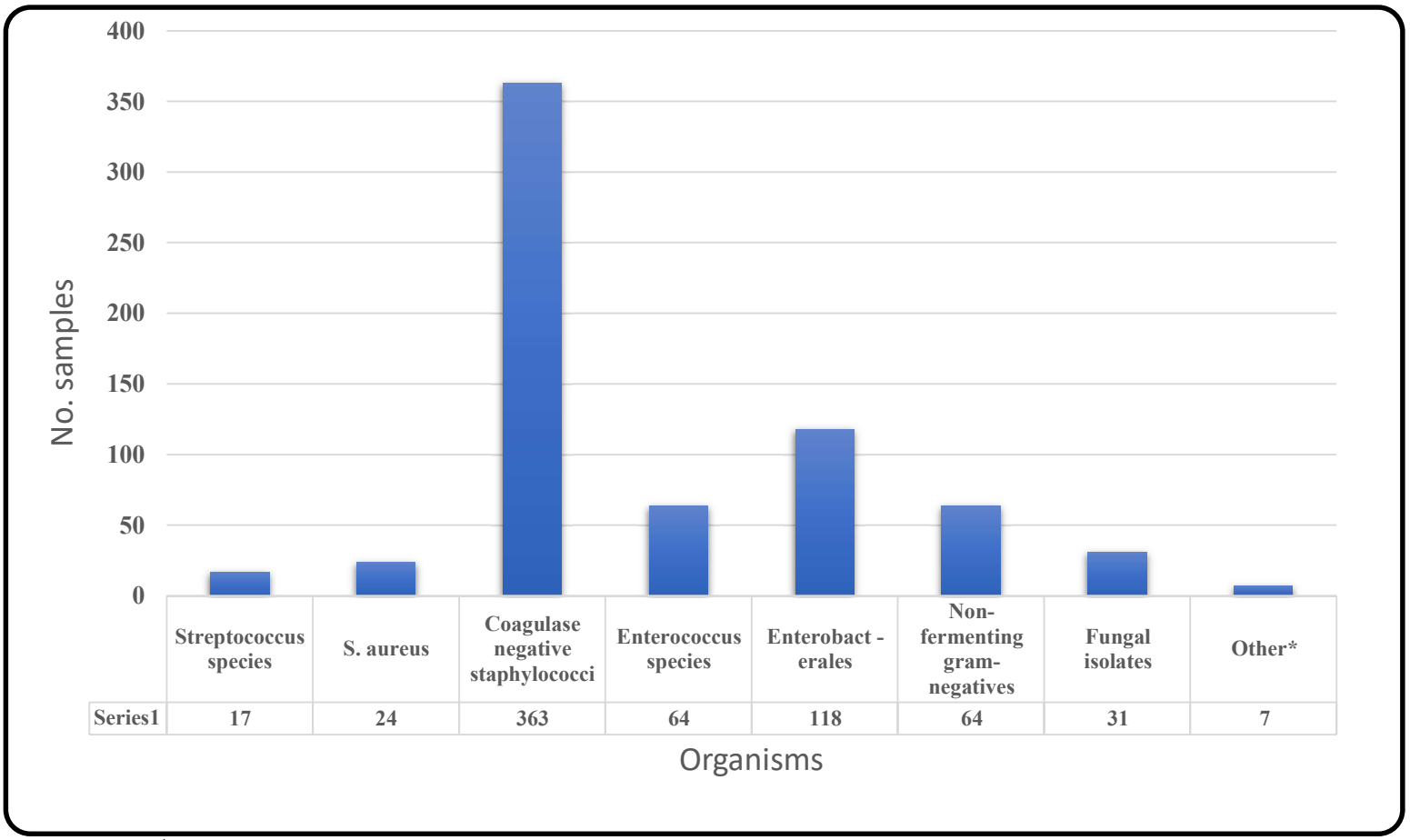


* *Listeria monocytogenes, Eikenella corrodens*, *Routella* species, *Moraxella catarrhalis, Micrococcus* species, *Rothia* species.

Figure 1: Overall distribution of organisms over the three study periods (2014, 2016 and 2018); n = 688.

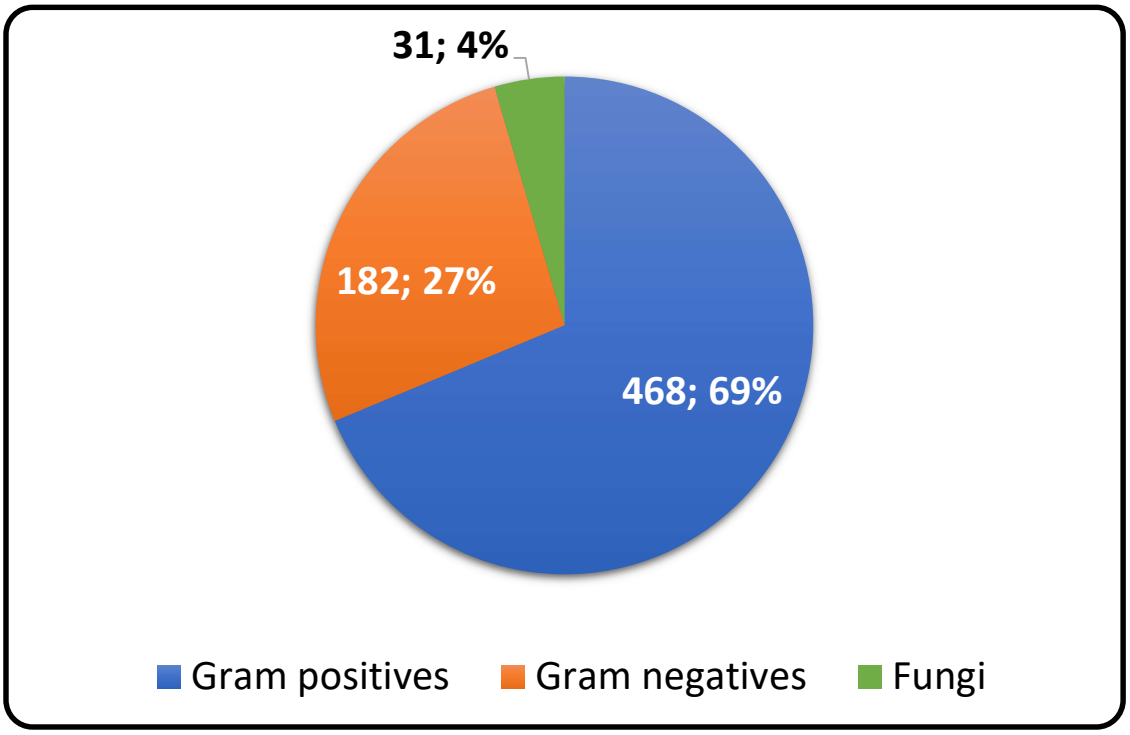
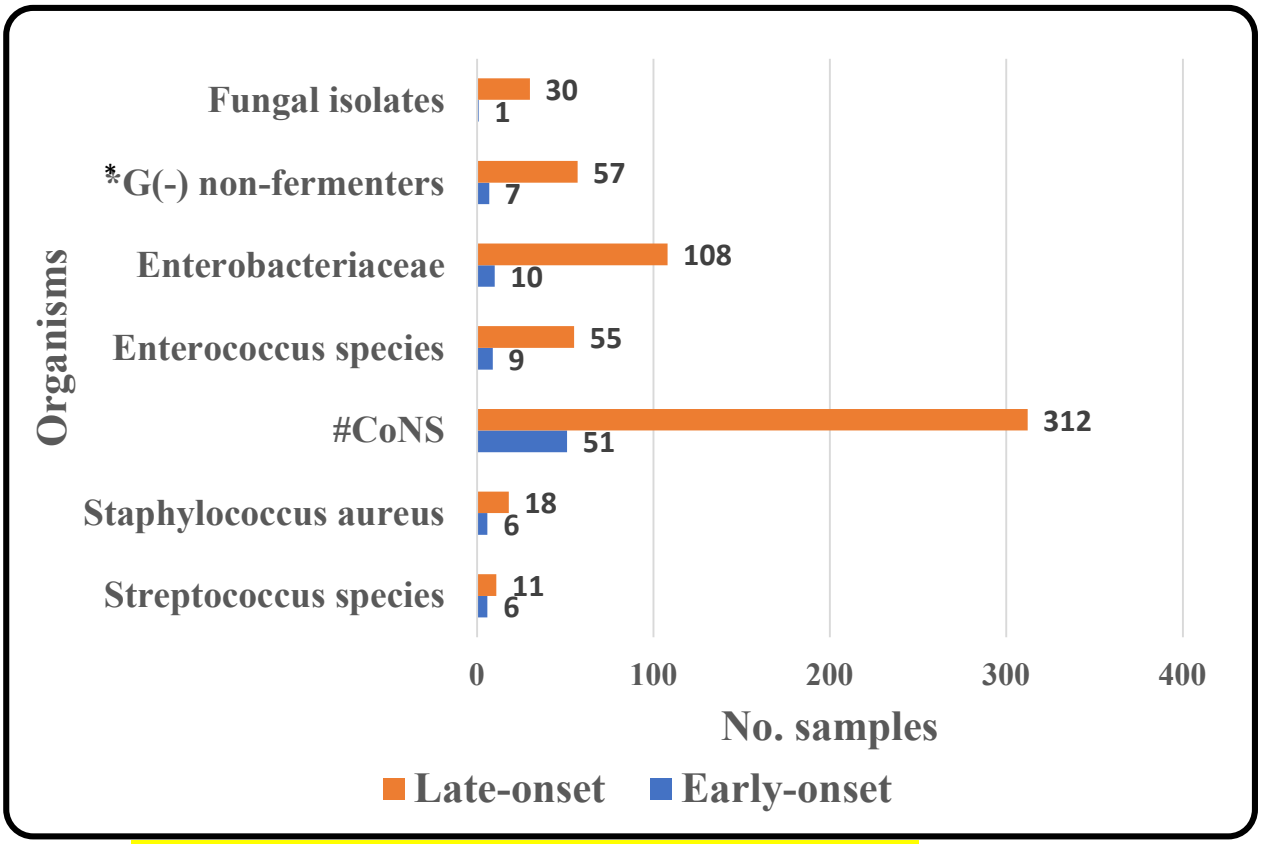


Figure 2: Distribution of isolates according to organism type over the three study periods (2014, 2016 and 2018); n = 681.



*G(-) – gram-negative/ #CoNS – coagulase negative staphylococci

Figure 3: Aetiology of early-onset sepsis (<3 days old) versus late-onset sepsis (≥ 3 days old); n = 681.

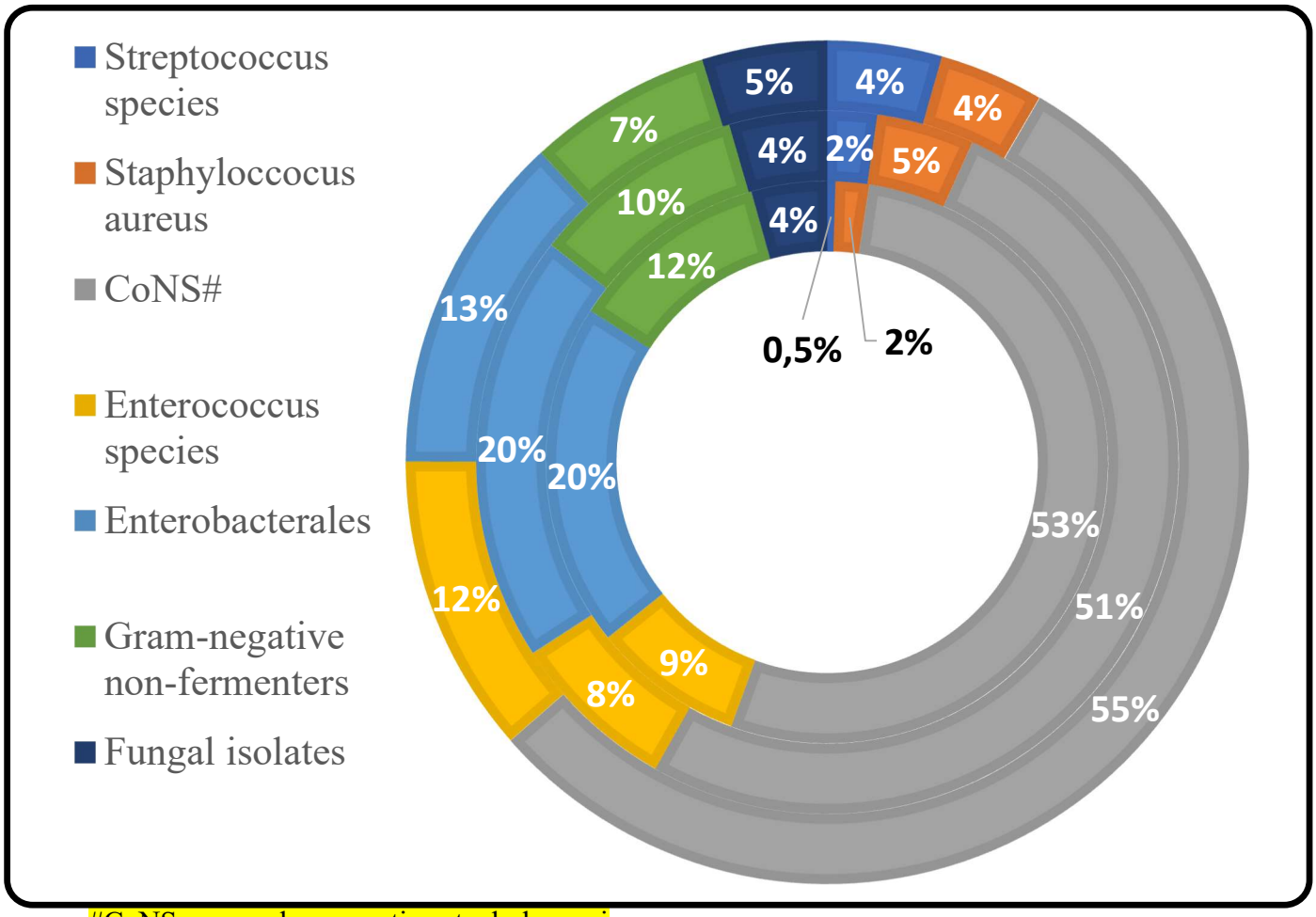
Table 1: Species-specific differences of leading pathogens in early-onset sepsis versus late-onset sepsis

	Early-onset sepsis (< 3 days)		**Late-onset sepsis (≥ 3 days)**	
Rank	**Organisms**	**Percent**	**Organisms**	**Percent**
1	CoNS	56,7	CoNS	52,8
2	*A. baumannii*	7,8	*K. pneumoniae*	12,4
3	*Staphylococcus aureus*	6,7	*A. baumannii*	7,8
4	*E. faecalis*	6,7	*E. faecium*	6,6
5	*K. pneumoniae*	6,7		

Table 2: Leading pathogens over the three study periods (2014, 2016, 2018)

Year	Rank	Organisms	Percentage
2014	1	CoNS	53,0
	2	*K. pneumoniae*	14,4
	3	*A. baumannii*	9,0
	4	*Enterococcus* species	9,0
2016	1	CoNS	51,4
	2	*K. pneumoniae*	16,7
	3	*Enterococcus* species	7,6
	4	*A. baumannii*	6,7
2018	1	CoNS	55,2
	2	*Enterococcus* species	11,5
	3	*K. pneumoniae*	9,9
	4	*A. baumannii*	5,1

*****CoNS – coagulase negative staphylococci



#CoNS – coagulase negative staphylococci

Figure 4: Comparison of organisms across the three study periods. The inner circle represents 2014, the middle circle represents 2016 and the outer circle represents 2018; n = 681.

Table 3: Overall antimicrobial susceptibility results for the study period

	Number isolates sensitive	Total isolates tested	% Susceptible	Number not tested*
Staphylococcus aureus				
Cloxacillin	5	24	20,8	0
Vancomycin	22	22	100,0	0
CoNS				
Cloxacillin	32	363	8,8	0
Vancomycin	46	46	100,0	317
***Enterococcus* species**				
Ampicillin	24	64	37,5	0
Vancomycin	64	64	100,0	0
Enterobacterales				
Amikacin	94	117	80,3	1
Cefotaxime	40	117	34,2	1
Ceftazidime	23	83	27,7	35
Ciprofloxacin	73	114	62,4	4
Ertapenem	93	103	90,3	25
Gentamicin	46	116	39,7	2
Imipenem	101	110	91,8	8
Meropenem	108	117	92,3	1
Piperacillin/Tazobactam	61	110	55,5	8
Gram-negative non-fermenters				
Amikacin	26	53	49,1	11
Ceftazidime	9	59	15,3	6
Ciprofloxacin	12	61	19,7	3
Gentamicin	6	52	11,5	12
Piperacillin/Tazobactam	9	58	15,5	6
Imipenem	7	52	13,5	12
Meropenem	10	58	17,2	6
Fungal isolates				
Fluconazole	17	31	54,8	0

* Antimicrobial data for some organisms was absent

Table 4 : Antimicrobial susceptibility trend for 2014, 2016 and 2018

Antimicrobials	2014 % susceptible	2016 % susceptible	2018 % susceptible
Staphylococcus aureus			
Cloxacillin	0.0	40.0	10.0
Vancomycin	100.0	100.0	100.0
CoNS			
Cloxacillin	4.5	10.4	11.1
Vancomycin	100.0	100.0	100.0
***Enterococcus* species**			
Ampicillin	33.3	41.2	37.9
Vancomycin	100.0	100.0	100.0
Enterobacterales			
Amikacin	90.2	38.6	57.6
Cefotaxime	24.4	30.2	51.5
Ceftazidime	25.0	30.2	0.0
Ciprofloxacin	53.7	70.5	62.5
Ertapenem	97.1	89.5	83.9
Gentamicin	25.6	38.6	57.6
Imipenem	100.0	90.7	84.8
Meropenem	97.6	90.7	84.8
Piperacillin/Tazobactam	56.1	53.8	56.7
Gram-negative non-fermenters			
Amikacin	76.2	52.9	6.7
Ceftazidime	4.8	33.3	6.3
Ciprofloxacin	22.7	27.3	5.9
Gentamicin	9.1	17.6	7.7
Piperacillin/Tazobactam	18.2	20.0	6.3
Imipenem	12.5	25	0.0
Meropenem	27.2	25.0	0.0
Fungal isolates			
Fluconazole	22.5	70.0	54.8

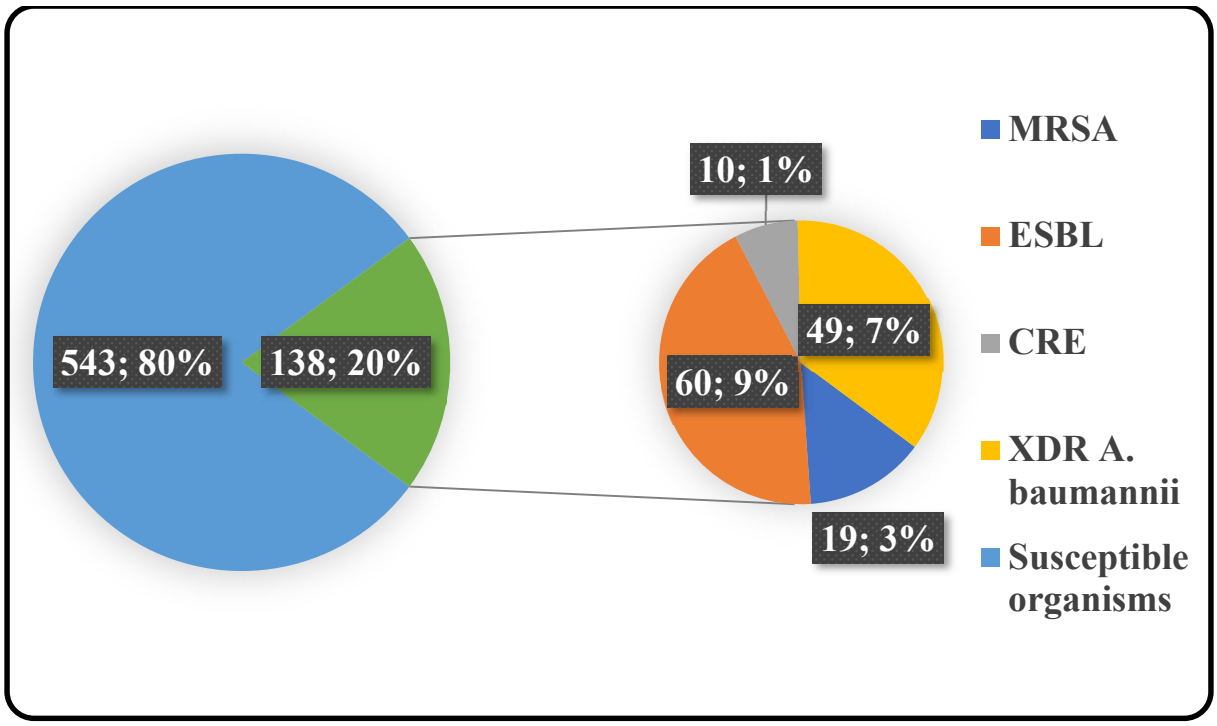


Figure 5: Distribution of antimicrobial resistance patterns among isolates (2014 – 2018); n = 681.

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APPENDICES

Appendix A

Study protocol

The study protocol was approved by UKZN Bioethics Research Committee, the Department of Health, Inkosi Albert Luthuli Central Hospital and the National Health Laboratory Service

University of KwaZulu-Natal

College of Health Sciences

School of Laboratory Medicine and Medical Sciences

(Microbiology)

Bacteriological profile and antimicrobial susceptibility patterns of neonatal bacteraemia in Durban, South Africa

Masters in Medicine (Pathology – Microbiology)

Principal investigator: Dr Dharshni Pillay

Student number: 206501841

Contact details:

Number: 0836472317

Email: dharshnipillay@gmail.com

Supervisor: Dr Yesholata Mahabeer

Email: MAHAB@ukzn.ac.za

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1.0 AIMS AND OBJECTIVE

1.1 Aim

To establish the microbiological and antimicrobial susceptibility profiles of neonatal bacteraemia in the Neonatal Intensive Care Unit (NICU) at Inkosi Albert Luthuli Central Hospital (IALCH) at 3 intervals over a 5 year period (2014, 2016, 2018).

1.2 Objectives

1. To determine the common microbial pathogens within the unit.
2. To correlate common organisms with known antimicrobial susceptibility profiles.
3. To establish temporal trends for common organisms and antimicrobial susceptibility profiles over a 5 year spread with 3 intervals (2014, 2016, 2018).
4. To advise an empiric antimicrobial strategy based on current susceptibility data.

2.0 BACKGROUND AND LITERATURE

Neonatal mortality and epidemiology

Neonatal sepsis is a significant cause of morbidity and mortality in developing countries, posing a major public health. The neonatal period represents the most vulnerable time in a child's life and childhood and neonatal mortality rates reflect a country's health status. Worldwide, forty-five percent of under-5 mortality occurred within the neonatal period. The leading causes of neonatal demise were preterm birth (15,9%), intrapartum-related events (10,7%) and neonatal sepsis (6,8%). Concomitantly, sepsis, meningitis and pneumonia account for approximately 10% of neonatal deaths. In Sub-Saharan Africa, neonatal mortality remains one of the highest in the world (29 deaths per 1000 live births compared to the global average of 19 deaths per 1000 live births), accounting for 35% of under-5 mortality.

Conflicting reports on the South African neonatal mortality rate (NMR) exist. The District Health Information System (DHIS) recorded an NMR of 12,6 deaths per 1000 live births in 2016. The South African Demographic Health Survey documented the NMR at 21 deaths per 1000 live births for the same year. The majority of childhood deaths occur during the early neonatal period. Sepsis was the fourth leading of early neonatal mortality as documented by

Stats SA in 2014. One study from South Africa ranked pneumonia as the third leading cause of neonatal death. The province of KwaZulu-Natal demonstrated an increase in NMR between 2013 and 2015 with a partial decline observed for 2016. Similar trends were noticed in the Western Cape and Gauteng.

Currently, the Sustainable Development Goals (SDG) aim to reduce neonatal mortality to less than 12 deaths per 1000 live births by 2030. To meet this goal, the burden of sepsis in the neonatal period needs to be addressed.

Definition of neonatal sepsis

Neonatal sepsis has traditionally been defined as the onset of sepsis within the first 28 days of life. It entails a collection of nonspecific clinical features and positive microbiological cultures from a sterile site. Sites include blood, cerebrospinal fluid and urine.

A universal definition of neonatal sepsis is lacking. Therefore, various organisations and publications have established case definitions in an attempt to define neonatal sepsis.

The Integrated Management of Childhood Illnesses (IMCI) handbook created by the World Health Organisation (WHO) defines clinical criteria for the diagnosis of severe bacterial infection in neonates. The Young Infants Clinical Signs Group defined criteria that would inform *WHO's Integrated Management of Childhood Illness* (IMCI) guidelines. An algorithm was generated stating that significant clinical signs include one of:

- history of difficulty feeding
- history of convulsions
- movement only when stimulated
- respiratory rate of ≥ 60 breaths per minutes
- severe chest retractions
- a temperature of ≥ 37.5°C or ≤ 35.5°C

These guidelines are highly sensitive with low specificity, resulting in high numbers of referrals (including well infants).

The European Medicines Agency (EMA) convened an expert meeting on neonatal and paediatric sepsis in 2010. The aim of this meeting was to define sepsis for clinical. The panel reported the following definitions:

- Early neonatal sepsis was defined as onset of sepsis within the first 72 hours of life.
- Late neonatal sepsis was defined as onset of sepsis after, and including, 72 hours of life.
- Sepsis was defined as having as least two clinical symptoms **and** at least two laboratory findings in the presence of suspected or proven infection (Table 1).

Table 1: Clinical and laboratory features of neonatal sepsis

Clinical	**Laboratory**
• Modified body temperature • Cardiovascular instability such as bradycardia or tachycardia • Respiratory instability such as apnoea or tachypnoea • Gastro-intestinal complaints such as poor sucking or feed intolerance • Skin and subcutaneous lesions • Non-specific signs such as irritability, lethargy or hypotonia	• White blood cell count < 4000 x 10^9 cells/L or > 20 000 x 10^9 cells/L • Immature to total neutrophil ratio > 0.2 • Platelet count < 100000 x 10^9 cells/L • C-reactive protein > 15mg/L • Procalcitonin > 2 ng/mL • Glucose intolerance confirmed at least twice • Metabolic acidosis
Microbiological tests: Microscopy, culture, polymerase chain reaction (PCR)	

Adapted from the EMA Report on the Expert Meeting on Neonatal and Paediatric, 2010.

Case definitions have been created by neonatal networks worldwide but heterogeneity exists among case definitions of neonatal sepsis and the lack of a consensus definition could impact the quality of data gathered on the subject.

Traditionally, neonatal sepsis has be stratified into early-onset sepsis (≤ 3 days of life) and late-onset sepsis (4-30 days of life)

Neonatal Units

The profile of neonatal units differs geographically which results in two distinct profiles Facilities caring for term infants in poorly equipped, high dependency units. There is associated understaffing and overcrowding. This profile is seen in many African countries.

1. Tertiary neonatal facilities with developed supportive care. Most babies are born prematurely or are of low-birth weight (LBW).

It is probable that the stipulated differences will affect the bacteriological profile of sepsis within the unit.

Bacteriological and antimicrobial susceptibility profiles

The gold standard for diagnosis of neonatal sepsis is isolation of a positive culture from a sterile site such as a blood culture.

The bacteriological profile of neonatal sepsis differs between high-income countries (HIC) and low-to-middle income countries (LMIC). Group B streptococcus is a frequent cause of early onset sepsis in HIC. This is in contrast to the bacteriological profile of resource-limited setting where group B streptococcus rates are much lower (Table 2).

Bloodstream infections (BSI) have shown a predominance of gram-negative infections over gram-positive sepsis. Studies have indicated that *Klebsiella pneumoniae* is a leading pathogen among blood cultures. Other important gram-negative organisms include *Enterobacter* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter* species. Leading gram positive isolates include *Staphylococcus aureus* and coagulase negative *Staphylococci*.

Elevated levels of resistance have been demonstrated towards the common beta-lactam antibiotics. Pohkrel et al. (2018) found increased resistance towards amoxicillin, oxacillin and ceftriaxone across both gram positives and gram negatives. A study by Muley et al. (2015) documented high levels of gram-negative resistance for ceftazidime and ceftriaxone. Similar findings have been corroborated by earlier studies. Multidrug resistance remains a problem. However, antibiotics that maintain high levels of susceptibility include the carbapenems, tigecycline and colistin (gram negative organisms) and vancomycin and linezolid (gram positive organisms) (Table3).

In keeping with other studies from Southern Asia, Bangladesh reported high rates of gram negative sepsis *K. pneumoniae* (sensitive to imipenem and ciprofloxacin) was the commonest organism. Other organisms included *E. coli* (sensitive to imipenem and amikacin) and *Serratia* species (sensitive to imipenem and ciprofloxacin.

In Ghana, late onset sepsis predominates over early onset The majority of blood stream pathogens are gram-positive organisms with *Staphylococcus epidermidis* as the leading cause. This finding was documented in both early and late neonatal sepsis. *Staphylococcus aureus* and *Streptococcus* specie are among the gram-positive organisms isolated Early onset sepsis had a variable gram negative profile across studies. Common organisms include *Citrobacter* species, *Enterobacter* species, *P. aeruginosa* and *E. coli* However, this bacteriological profile included a wider subset of *Enterobacteriaceae* during late-onset sepsis.

Aku et al. (2016), found a 100% rate of penicillin, flucloxacillin and cotrimoxazole resistance among *Staphylococcus epidermidis* and *S. aureus*. The same study reported total resistance to ampicillin (a first-line antibiotic) among the gram-negative isolates. An alternate first-line antibiotic is gentamicin. Resistance to gentamicin was observed in *S. epidermidis* (57%)*, P. aeruginosa* (25%), *Enterobacter* species (50%) and *P. mirabilis* (100%)

An equal preponderance between gram negative and gram positive organisms during neonatal septicaemia was established in Nigeria. *K. pneumoniae* and *S. aureus* were most frequently isolated. *K. pneumoniae* isolates often produced extended spectrum beta-lactamases (ESBLs) but remained sensitive to imipenem.

Milledge et al. (2001) reported a gram positive majority (54%) among blood and cerebrospinal fluid cultures from Malawi. The commonest causes of sepsis included Group B Streptococcus and non-typhoidal *Salmonella*. This pattern of sepsis compares to Kenyan findings but contrasts to other resource-limited settings.

An Egyptian study documented a greater number of late onset sepsis (55,8%). The commonest organisms were coagulase negative Staphylococci, followed by *K.*.

In Zambia, *K. pneumoniae,* coagulase negative Staphylococci and *S. aureus* are the three most likely organisms implicated in neonatal sepsis. Most *K. pneumoniae* demonstrated resistance to third-generation cephalosporins.

There is limited, current South African data on the bacteriological profile of neonatal sepsis. The incidence of group B streptococcus was 2,72 cases per 1000 live births between 2004 and 2008 in Soweto, Gauteng. In an analysis of neonatal blood cultures (2002-2003), gram negative organisms (EOS) and coagulase negative Staphylococci (LOS) were commonly isolated. Lebea et al. (2017) documented the common causative NICU pathogens to be *K. pneumoniae,* coagulase negative Staphylococci and Methicillin-resistant *S. aureus* (MRSA). *K. pneumoniae* isolates were predominantly ESBL producing. LOS occurs more often in the South African setting.

Table 2: Overview of the bacteriological profile of blood-stream infections

Country	**Author**	**Year**	**Top Pathogens**
Bangladesh	Jahan et al.	2017	*K. pneumoniae, E. coli*
Egypt	Shehab El-Din et al	2015	Coagulase negative staphylococci, *K. pneumoniae*
Ghana	Labi et al.	2016	Coagulase negative staphylococci, *S. aureus*
India	Roy et al.	2002	*Klebsiella* spp, *Enterobacter* spp, coagulase negative staphylococci
	Rajendraprasad et al.	2013	*E. cloacae, S. aureus*
	Marwah etal	2015	*S. aureus, K. pneumoniae, A. baumannii*
	Muley et al.	2015	*K. pneumoniae, S. aureus*
	Pokhrel et al.	2018	*K. pneumoniae,* coagulase negative staphylococci
	Aku et al.	2018	*Staphylococcus epidermidis*
Kenya	Berkley et al.	2005	Group B streptococcus, *E. coli*
Malawi	Millege et al.	2005	Group B streptococcus, non-typhoidal *Salmonella*
Nigeria	Iregbu et al.	2006	*S. aureus, K. pneumoniae*
South Africa	Motara et al.	2005	*E. coli,* coagulase negative staphylococci
	Lebea et al.	2017	*K. pneumoniae,* coagulase negative staphylococci, *S. aureus*
Zambia	Kabwe et al.	2016	*K. pneumoniae*, coagulase negative staphylococci, *S. aureus*

Table 3: Overview of the antimicrobial susceptibility patterns in blood-stream infections

Country	Author	Year	Organisms	Resistance/susceptibility patterns
India	Pokhrel et al.	2018	Gram negatives Gram positives (mostly coagulase-negative staphylococci)	Increased resistance to cefotaxime, ciprofloxacin, gentamicin Susceptible to carbapenems, tigecycline and colistin Increased resistance to oxacillin, cefotaxime, meropenem Susceptible to vancomycin, linezolid
	Muley et al.	2015	Gram negatives *S. aureus*	Increased resistance to ceftriaxone and ceftazidime Methicillin resistance
	Roy et al.	2002	Enterobacteriaceae	Increased resistance to penicillin and extended spectrum cephalosporins
	Kaistha et al.	2009	Gram negative Gram positives	Resistance to penicillin and third-generation cephalosporins Sensitive to imipenem, amikacin All sensitive to Vancomycin
	Aku et al.	2018	*S. epidermidis, S. aureus* Gram negatives	Increased resistance to penicillin, flucloxacillin and cotrimoxazole Increased resistance to ampicillin, cefuroxime, cotrimoxazole and gentamicin
Nigeria	Iregbu et al.	2006	*K. pneumoniae*	Extended spectrum beta-lactamase production
South Africa	Lebea et al.	2017	*K. pneumoniae* *S. aureus*	Extended spectrum beta-lactamase production Methicillin resistance

Multidrug resistance

Multi-drug resistance has been observed across the globe affecting both gram-positive and gram-negative organisms. Certain drug regimens, such as ampicillin plus cefotaxime, have proven decreasing susceptibility.

Empiric regimens

Broad-spectrum antibiotics are necessary prior to the availability of culture results. Keeping patients on long-term broad-spectrum therapy can have deleterious effects. These effects include disturbances to the normal flora and selection for resistant organisms.

Current World Health Organisation guidelines advocate the use of ampicillin plus gentamicin in neonates with suspected sepsis. Cloxacillin and gentamicin are deemed necessary in patients at risk for *Staphylococcal* infections.

Labi et al. (2016) contrasted empiric antibiotic regimens in Ghana to antimicrobial susceptibility profiles. In EOS and LOS the most susceptible regimen was cloxacillin and gentamicin (71,6% and 63.6%), followed by ampicillin plus gentamicin (32,3% and 36,2%) and ampicillin plus cefotaxime (20,7% and 24,6%).

Pokhrel et al. (2018) suggests substituting the first-line antibiotics (for Piperacillin/ Tazobactam and Ofloxacin) and second line antibiotics (for vancomycin plus meropenem) to reduce resistance in a Nepalese NICU by 22% and 46%, respectively.

Significance of Study

The South Africa's National Perinatal Morbidity and Mortality (NPMM) Committee's HHAPI-NeSS strategy highlights key areas needed to improve neonatal survival. Reducing deaths due to infection is advocated by NPMM. Suggested activities include:

- Ensuring presumptive antibiotic therapy for the at-risk neonate is available
- Management of neonatal sepsis, meningitis and pneumonia

Inappropriate or incorrect antibiotic therapy predisposes to longer hospital stays and prolonged antibiotic exposure (and the resultant side effects). In the era of multidrug resistance deciding on an appropriate antibiotic is challenging. Increasing resistance to first-

line antibiotics is emerging. Furthermore, microbiological culture results take on average 48 to 72 hours, delaying definitive.

A challenge for neonatologist within an NICU remains antibiotic usage. This influences antimicrobial susceptibility patterns, which tend to vary over. Changing patterns of resistance require that regular bacteriological surveys be conducted. Understanding the bacteriological profile of a neonatal unit can contribute to appropriate early management of sepsis, improving therapeutic outcomes.

There is a scarcity of data on epidemiology of neonatal sepsis in South Africa. The bacteriological profile of neonatal units in KwaZulu-Natal remains under-explored. Investigations into causes of neonatal sepsis are limited in our setting and require more attention.

3.0 METHODS

3.1 Study Design

This study is a quantitative, retrospective, descriptive data review.

3.2 Study Location

Data, pertaining to blood cultures, will be collected from a tertiary hospital, which is Inkosi Albert Luthuli Central Hospital (IALCH), in Durban, KwaZulu-Natal, South Africa. The study will focus on data from blood culture records within the neonatal intensive care unit (NICU).

3.3 Study period

The study years will encompass years 2014, 2016 and 2018 (months January to December).

3.4 Study population and sampling strategy

The study population will consist of neonates from the NICU at IALCH.

Subjects will be stratified into early-onset sepsis (birth – 3 days old) and late-onset sepsis (4 - 30 days old) or analysis of the primary outcomes. No randomisation of samples will be performed. The National Health Laboratory Service (NHLS) Central Data Warehouse (CDW) will be accessed for data.

Inclusion criteria:

- Positive blood cultures from NICU from January to December of years 2014, 2016 and 2018.
- Patients aged 0 – 30 days of life

Exclusion criteria:

- Blood cultures taken within 14 days of the index culture where the same organism is isolated again.

3.5 Sample size

A sample size of 300 is required to estimate the proportion of blood cultures resistant to a drug to within ± 8% with probability of 95% and a baseline estimate of 50%. Sample size was calculated using Stata Statistical Software V13.1.

3.6 Data collection

A structured spreadsheet consisting of standardised data fields shall be used (Appendix A)

Each patient shall be identified using the hospital number only. This number will only be recorded on the primary data sheet. Specimen identification will be recorded using NHLS assigned episode numbers (starting with "AA").

The general variables to be documented include:

- Age (and date of birth)
- Onset of sepsis (early-onset versus late onset)
- Gender

- Date of collection (to assist with identification of duplicate samples indicating the same infection).
- Sample type – blood cultures only
- Previous duplicate cultures
- Identification and classification of the organism as either a gram-positive organism, a gram negative organism or a fungal isolate.

Classification into the gram positive, gram negative or fungal categories informs the selection of the antimicrobial spreadsheet (Table 4). Data is recorded onto the antimicrobial spreadsheet as either susceptible, intermediately susceptible, sensitive dose dependent (fungal isolates only) or resistant to an antimicrobial. No available data for a particular antimicrobial will also be recorded.

Table 4: Antibiotic panels for analysis

Gram positive panel	Gram negative panel	Antifungal panel
• Penicillin • Ampicillin • Oxacillin/Cloxacillin • Vancomycin	• Cefotaxime • Ceftazidime • Imipenem • Meropenem • Ertapenem • Piperacillin/Tazobactam • Gentamycin • Amikacin • Ciprofloxacin • Tigecycline	• Fluconazole • Voriconazole

4.0 STATISTICAL PLANNING AND ANALYSIS

4.1 Measurements

The following outcome variables will be assessed:

Primary outcomes:

1. Prevalence of common organisms on blood culture within the unit during the total study period.
2. Antimicrobial susceptibility patterns for organisms isolated, based on the following testing categories:
    a. *Staphylococcus aureus* → Cloxacillin, Vancomycin
    b. *Staphylococcus* species → Cloxacillin, Vancomycin
    c. *Streptococcus* species → Penicillin
    d. *Enterococcus* species → Ampicillin, Vancomycin
    e. *Enterobacteriaceae* → Third-generation cephalosporins (ceftazidime, cefotaxime) or extended spectrum beta-lactamase test positive by automated testing, carbapenems (imipenem, meropenem, ertapenem), piperacillin/tazobactam, aminoglycoside (gentamycin, amikacin), quinolone (ciprofloxacin) and tigecycline.
    f. Gram-negative non-fermenter → piperacillin/tazobactam, carbapenems (imipenem, meropenem), extensively-drug resistance organisms (susceptible to ≤ 2 classes of antibiotics)
    g. Fungal isolates → fluconazole, voriconazole

Secondary outcomes:

1. Rates of change in prevalence of common organism per year (within the study period) – common organisms that are identified within the primary objective.
2. Rates of change in antimicrobial susceptibility patterns per year (within the study period) – based on susceptibility categories outlined above.
3. Prevalence of specific multidrug resistant organisms (MDROs) during the study period:
    a. XDR *A. baumannii* – if susceptible to ≤ 2 classes of all tested agents
    b. Carbapenem-resistance *Enterobacteriaceae* (CRE) – if resistant to at least 1 carbapenem.

c. Extended-spectrum beta-lactamase organisms (ESBL) – if resistant to a 3rd or 4th generation cephalosporins or detected as an ESBL through automated methods.
d. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus* species – if resistant to oxacillin
e. Vancomycin-resistant *Enterococci* (VRE) – if resistant to vancomycin

4.2 Statistical Analysis

Descriptive statistics will be used to summarise the data. Categorical data, like gender, will be summarised by frequencies and percentage. The frequency of selected organisms will be reported by year. Susceptibility of each drug will be reported as the percent susceptible or resistant.

Comparisons of pathogens by subgroup, such as early-onset and late-onset neonatal sepsis, will be done using Chi Square or Fisher's exact test. The number of each organism seen per year is a count variable and the temporal trend will be analysed using Poisson Regression. The change in susceptibility patterns over time will be analysed using linear regression.

Data will be analysed using Stata V13.1 and *p* value of 0.05 will be considered statistically significant.

5.0 STUDY LIMITATIONS

- Premature neonates will not be stratified within the study population. The premature neonate cohort may present a different bacteriological profile than other groups.
- Clinical data will not be collected as part of this analysis. Therefore, determining clinically relevant significance from contamination is challenging.
- This study may be underpowered to determine adequate significance of temporal fluctuations amongst less frequently occurring organisms.

6.0 ETHICAL CONSIDERATIONS

- This study will be submitted to the Biomedical Research Ethics Committee (BREC) at University of KwaZulu Natal for review and approval.
- Consent shall be obtained from Inkosi Albert Luthuli Central Hospital management and National Health Laboratory Service (NHLS) for use of patient data.
- Patient confidentiality shall be maintained by excluding names and surnames from the data collection process. Patients will be identified through hospital numbers. Samples will be identified using episode numbers.
- Work with clinical specimens and live culture do not form part of this study. Therefore, no biosafety hazards are posed.
- Collected data will be securely stored (on a single, password protected computer), handled only by the principal investigator and supervisor and destroyed upon completion of the project and/or publication.

APPENDIX B

Approval Letter:

Biomedical Research Ethics Council (BREC) at the University of KwaZulu-Natal,

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

27 August 2019

Dr D Pillay
School of Laboratory Medicine and Medical Sciences
College of Health Science
dharshnipillay@gmail.com

Dear Dr Pillay

Protocol: Bacteriology profile and antimicrobial susceptibility patterns of neonatal bacteraemia in Durban, South Africa
Degree: MMEd
BREC Ref No: BE019/19

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 09 January 2019.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 20 August 2019 to BREC letter dated 07 February 2019 has been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have been met and the study is given **full ethics approval** and may begin as from **27 August 2019. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.**

This approval is valid for one year from **27 August 2019.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

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

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

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

The sub-committee's decision will be **noted** by a full Committee at its next meeting taking place on **08 October 2019.**

Yours sincerely

[redacted]

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc: supervisor: mahab@ukzn.ac.za Postgrad Admin: dudhrajhp@ukzn.ac.za

APPENDIX C

Approval Letter:

Department of Health

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Physical Address: 330 Langalibalele Street, Pietermaritburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email:
www.kznhealth.gov.za

DIRECTORATE:
Health Research & Knowledge Management

NHRD Ref: KZ_201905_008

Dear Dr D. Pillay
UKZN

Approval of research

1. The research proposal titled **'Bacteriological profile and antimicrobial susceptibility patterns of neonatal bacteraemia in Durban, South Africa'** was reviewed by the KwaZulu-Natal Department of Health.

   The proposal is hereby **approved** for research to be undertaken at Inkosi Albert Luthuli Central Hospital.

2. You are requested to take note of the following:
   a. Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.
   b. Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.
   c. Provide an interim progress report and final report (electronic and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mr X. Xaba on 033-395 2805.

Yours Sincerely

[redacted]

Dr E Lutge

Chairperson, Health Research Committee

Date: 21/06/19

Appendix D

Approval Letter:

Inkosi Albert Luthuli Central Hospital

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

DIRECTORATE

Physical Address: 800 Bellair Road, Mayville, 4058
Postal Address: Private Bag X08, Mayville, 4058
Tel: 0312401059 Fax: 0312401050 Email: ursulanun@ ialch.co.za
www.kznhealth.gov.za

Office of The Medical Manager
IALCH

Reference: BE 019/19
Enquiries: Medical Managemer

15 March 2019

Dr D Pillay
School of Laboratory Medicine and Medical Sciences
College of Health

Dear Dr Pillay

RE: PERMISSION TO CONDUCT RESEARCH AT IALCH

I have pleasure in informing you that permission has been granted to you by the Medical Manager to conduct research on: **Bacteriology profile and antimicrobial susceptibility patterns of neonatal bacteraemia in Durban, South Africa**

Kindly take note of the following information before you continue:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regards to this research.
2. This research will only commence once this office has received confirmation from the Provincial Health Research Committee in the KZN Department of Health.
3. Kindly ensure that this office is informed before you commence your research.
4. The hospital will not provide any resources for this research.
5. You will be expected to provide feedback once your research is complete to the Medical Manager.

Yours faithfully

[redacted]

.....

Dr L P Mtshali pp/Acting
Medical Manager

Appendix E

Approval Letter:

National Health Laboratory Service

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

08 August 2019

Applicant: Dharshini Pillay
Institution: NHLS / IALCH
Department: Medical Microbiology
Email: Dharshni.pillay@nhls.ac.za
Cell: 083 642 2317

CC: Yesholata Mahabeer
Principal Pathologist
Inkosi Albert Luthuli Central Hospital

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

Your application to undertake a research project **"Bacteriological profile and antimicrobial susceptibility patterns of neonatal bacteraemia in Durban, South Africa"** using data from the NHLS database has been reviewed. This letter serves to advise that the application has been provisionally approved ***without patent names***.

Please note that the final approval will be 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.

- Ethics approval is obtained from a recognised SA Health Research Ethics Committee..
- 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.
- 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.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.
- Yesholata Mahabeer is noted as NHLS collaborator for this research.

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

[redacted]

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

Appendix F

Procedures for the processing and interpretation of blood cultures and antimicrobial susceptibility test results at the Inkosi Albert Luthuli Central Hospital's microbiology laboratory.

Procedure for processing of blood cultures at Inkosi Albert Luthuli Microbiology Laboratory

Adapted from National Health Laboratory Services Standard Operating Procedures (MIC1906v4, MICRO15v1) and Clinical Laboratory Standards Institute M100-S25 (2015).

1. Incubation of blood culture bottles

Upon arrival in the lab, all blood culture bottles are loaded and incubated in the BD BACTEC™ FX instrument (Becton Dickinson, USA). The total time of incubation is 7 days, If the bottle flags positive within the incubation period microscopy, culture and antimicrobial susceptibility testing is performed. However, if the bottle remains negative until the seventh day of incubation it is discarded. A longer incubation period may be utilised if fastidious organisms are suspected.

2. Microscopy, culture, and direct antimicrobial susceptibility tests set-up

The Gram stain is performed on all positive blood culture bottles. Organisms are classified into the categories and the result of the Gram stain directs further testing methods. Blood cultures are plated out onto agar plates for culture which are supplied by DMP (Sandringham) (Table 1 and 2). Kirby-Bauer disk diffusion is performed in conjunction with culture (direct senses).

Table 1: Culture algorithms as determined by Gram stain results

Gram result	Culture Method	Kirby-Bauer Disk Diffusion
Gram-negative bacilli Gram-negative cocci (resembling *Acinetobacter* species)	Chocolate Agar MacConkey Agar API20E	GN1 panel GN2 panel ESBL panel
Gram-negative cocci (resembling *Neisseria* species)	Blood Agar Chocolate Agar MacConkey Agar	Nil
Gram positive cocci in clusters	Blood Agar Mannitol Salt Agar DNAse Agar (with controls)	Mueller-Hinton Agar with cefoxitin disc
Gram positive cocci in chains	Blood Agar (Optochin + Bacitracin discs) MacConkey Agar Aesculin Bile Agar (with controls)	Mueller-Hinton Agar with 5% sheep blood (GP panel)
Gram positive in pairs (resembling possible *S. pneumoniae*)	Blood Agar (Optochin + Bacitracin discs) MacConkey Agar Aesculin Bile Agar (with controls)	Mueller-Hinton Agar with 5% sheep blood (GP panel + oxacillin)
Gram positive bacilli (large)	Blood Agar MYP Agar (with controls)	Nil
Gram positive bacilli (small)	Blood Agar Aesculin Bile Agar (with controls)	Nil
Yeast	Blood Agar Sabouraud Dextrose Agar	
Mixed organisms	Blood Agar Colistin Nalidixic Acid Chocolate Agar MacConkey Agar Sabouraud Dextrose Agar	Meuller-Hinton Agar (GN1 + GN2 + ESBL) panels Colistin Nalidixic Acid Agar (GP panel)
No organisms observed	Bloos Agar Chocolate Agar MacConkey	
No organisms + anaerobic bottle (to be reloaded within 3 hours).	10% Blood Aagar 10% Blood Agar + Amiakcin Anaerobic incubation	

Table 2: Control organisms for culture

Agar plate	Positive control	Negative control
DNAase	*S. aureus* ATCC 25923	*S. epidermidis* ATCC 12228
Aesculin Bile Agar	*E. faecalis* ATCC 29212	*S. pyogenes* ATCC 27893
MYP Agar	*B. cereus* ATCC 9592	*B. subtilis* ATCC 6051

3. Examination of Cultures

The agar plates may exhibit growth or no growth:

- Plates with visible growth:
  - Identify all organisms according to standard operation procedures (SOP) including the use of appropriate bench tests, manual identification, and automated identification methods (Vitek 2 Advanced Expert System, bioMeriuex).
  - If included in the work-up, read the API 20E (bioMerieux).
  - Record the results of the direct antimicrobial susceptibility test results.
  - Perform standardised antimicrobial susceptibility testing.
  - For *S. pneumoniae* set-up E-tests (bioMerieux) for penicillin and ceftriaxone.
  - Identify anaerobes according to their own SOP
- Plates without visible growth:
  - Re-incubate the plates for a further 24 hours.
  - If organisms were observed on the Gram stain, liaise with medical staff.
  - Set-up media for the isolation of fastidious organisms
  - For anaerobic bottles, a 10% Blood Agar plate and a 10% Blood Agar plare with amikacin should be inoculated and incubate anaerobically for 24-48 hours.

4. Standardised antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) is performed using a controlled inoculum (determined by the organism) using Kirby-Bauer disk diffusion or an automated method (Vitek 2, bioMerieux). Susceptibility testing of anaerobic isolates is not performed at this laboratory. Interpretation of AST results is performed using the Clinical Laboratory Standards Institute M100-S25(2015) criteria (Table 3, 4 and 5).

Table 3: Gram Negative Panels

Antimicrobial	Disc Content	Zone Diameter		
		Sensitive	Intermediate	Resistant
Enterobacterales				
Ampicillin	10 ug	≥ 17	14-16	≤ 13
Meropenem	10 ug	≥ 23	20-22	≤19
Imipenem	10 ug	≥ 23	20-22	≤ 19
Cefoxitin	30 ug	≥ 18	15-17	≤ 14
Cotrimoxazole	25 ug	≥ 16	11-15	≤ 10
Amikacin	30 ug	≥ 17	15-16	≤ 14
Ceftazidime	30 ug	≥ 21	18-20	≤ 17
Coamoxiclav	30 ug	≥ 18	14-17	≤ 13
Cefotaxime	30 ug	≥ 26	23-25	≤ 22
Piperacillin/Tazobactam	110 ug	≥ 21	18-20	≤ 17
Nitrofurantoin	300 ug	≥ 17	15-16	≤ 14
Ciprofloxacin	5 ug	≥ 21	16-20	≤ 15
***Acinetobacter* species**				
Meropenem	10 ug	≥ 18	15-17	≤ 14
Ceftazidime	30 ug	≥ 18	15-17	≤ 14
Amikacin	30 ug	≥ 17	15-16	≤ 14
Imipenem	10 ug	≥ 22	19-21	≤ 18
Piperacillin/Tazobactam	110 ug	≥ 21	18-20	≤ 17
Ciprofloxacin	5 ug	≥ 21	16-20	≤ 15
Pseudomonas aeruginosa				
Meropenem	10 ug	≥ 19	16-18	≤ 15
Ceftazidime	30 ug	≥ 18	15-17	≤ 14
Amikacin	30 ug	≥ 17	15-16	≤ 14
Imipenem	10 ug	≥ 19	16-18	≤ 15
Piperacillin/Tazobactam	110 ug	≥ 21	15-20	≤ 14
Ciprofloxacin	5 ug	≥ 21	16-20	≤ 15

Table 4: Gram positive panels

Antimicrobial	Disc Content	Zone Diameter		
		Sensitive	Intermediate	Resistant
***Staphylococcus aureus/Staphylococcus* species**				
Penicillin	10 units	≥ 29	-	≤ 28
Erythromycin	15 ug	≥ 23	14-22	≤13
Clindamycin	2 ug	≥ 21	15-20	≤ 4
Cefoxitin (*S. aureus/S. lugdunensis*	30 ug	≥ 22	-	≤ 21
Cefoxitin (*Staphylococcus* species except *S. lugdunensis*	30 ug	≥ 25	-	≤ 24
***Streptococcus* species (excluding enterococci)**				
Penicillin	10 units	≥ 24	-	-
Erythromycin	15 ug	≥ 21	16-20	≤ 15
Clindamycin	2 ug	≥ 19	16-18	≤ 15
Vancomycin	30 ug	≥ 17	-	-
Chloramphenicol	30 ug	≥ 21	18-20	≤ 17
Enterococci				
Penicillin	10 units	≥ 15	-	≤ 14
Ampicillin	10 ug	≥ 17	-	≤ 16
Erythromycin	30 ug	≥ 23	14-22	≤ 13
Vancomycin	15 ug	≥ 17	15-16	≤ 14
Streptococcus pneumoniae				
Erythromycin	15 ug	≥ 21	16-20	≤ 15
Clindamycin	2 ug	≥ 19	16-18	≤ 15
Tetracycline	30 ug	≥ 28	25-27	≤ 24
Cotrimoxazole	25 ug	≥ 19	16-18	≤ 15
Oxacillin	1 ug	≥ 20	-	-

Table 5: Antifungal panel

Antimicrobial	Disc Content	Zone Diameter		
		Sensitive	Intermediate	Resistant
***Candida albicans/Candida* species**				
Fluconazole	25 ug	≥ 19	15 - 18	≤ 14

Appendix G

Turnitin Report

MMed version 1

ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Posters", Clinical Microbiology and Infection, 5/2008 Publication	1%
2	"Posters", Clinical Microbiology and Infection, 4/2007 Publication	1%
3	Submitted to Liverpool School of Tropical Medicine Student Paper	<1%
4	www.ncbi.nlm.nih.gov Internet Source	<1%
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