THE INCIDENCE, CLINICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ACINETOBACTER BAUMANNII SEPSIS IN PREMATURE NEONATES AT UNIVERSITAS ACADEMIC HOSPITAL FOR THE PERIOD 1 JANUARY 2016 TO 31 DECEMBER 2018, A RETROSPECTIVE DESCRIPTIVE STUDY

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DECLARATION OF AUTHORSHIP

I, Dr Mark Moodley, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification in Paediatrics at the University of the Free State is my independent work and that I have not previously submitted it for a qualification at another institution of higher education.

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The contribution of each author of the article is stipulated below:

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All researchers declare that they have no conflict of interest and that they know no other situation of real, potential, or apparent conflict of interest. They undertake to inform the University of any change in these circumstances.

ABSTRACT

BACKGROUND: *Acinetobacter baumannii* is an emerging and challenging pathogen in neonatal units, due to its ability to develop resistance against antibiotics. Studies on *Acinetobacter baumannii* infection in premature neonates are lacking in developing countries.

OBJECTIVES: To determine the incidence, clinical presentation, antibiotic susceptibility profiles and the morbidity and mortality rate of premature neonates with *Acinetobacter baumannii* infection.

METHODS: We conducted a retrospective descriptive study at a tertiary hospital in Bloemfontein, South Africa. Medical records were reviewed over 36 months. Premature neonates admitted to the neonatal intensive care unit with positive blood, urine or cerebrospinal culture for *Acinetobacter baumannii* were identified through the National Health Laboratory Services.

RESULTS: Fifty premature neonates were enrolled. There was an incidence of 103 cases per 1000 admissions/ year. The median age at the onset of sepsis was 17 days (IQR 9-26). Known risks such as central venous catheters and parental nutrition were found in 78% (39/50), and 68% (34/50) respectively. Respiratory distress (56% [28/50]) and abdominal distension (50% [25/50]) were the most common clinical signs. Of all the isolates, 86% (43/50) were susceptible to colistin only. Supportive therapy was frequently required, 48% (24/50) received vasoactive drugs, and 70% (35/50) were mechanically ventilated. Mortality related to infection was 36% (18/50).

CONCLUSION: There was a high incidence of *Acinetobacter baumannii* sepsis in premature neonates at Universitas Academic Hospital. A significant number of neonates had a central catheter and received parental nutrition. Most isolates were resistant to the carbapenems but susceptible to colistin. Vasoactive drugs and mechanical ventilation were frequently required. The mortality rate was high from *Acinetobacter* infection which is in keeping with most studies. Strict infection control and antibiotic stewardship are imperative.

KEYWORDS: Neonate, neonatal sepsis, prematurity, *Acinetobacter baumannii,* multi-drug resistance, vasoactive drugs, ventilation, antibiotic stewardship, infection control, colistin

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ACRONYMS AND ABBREVIATIONS

AMS	Antimicrobial stewardship
CRP	C-reactive protein
CDC	Centre for Disease Control and Prevention
CSF	Cerebrospinal fluid
DOH	Department of Health
EOS	Early-onset sepsis
ICU	Intensive care unit
LOS	Late-onset sepsis
MDR	Multi-drug resistant
NHLS	National Health Laboratory Service
NICU	Neonatal Intensive Care Unit
PCT	Procalcitonin
TPN	Total Parental Nutrition
VLBW	Very low birth weight
WHO	World Health Organisation
WCC	White cell count

DEFINITIONS

Early-onset sepsis	Sepsis in the first 72 hours of life [1]
Late-onset sepsis	Sepsis after the first 72 hours of life [1]
Neonate	First 28 days of life[1]
Prematurity	Birth prior to 37 completed weeks gestation [2]
Mechanical ventilation	The technique through which gas is moved toward and from the lungs through an external device connected directly to the patient [3]
Thrombocytopenia	A platelet count of <150 x10/L[4]
Neutropenia	A neutrophil count of <1500/mm [5]
Multi-drug resistant	Resistance to >3 antibiotics [6]
Meditech	Medical Information Technology, Incorporated is a Massachusetts based software and service company selling information systems for health care organisations, Head Office situated in Midrand, South Africa

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CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION TO THE STUDY

In developing countries, neonatal sepsis is a significant health issue. Premature and low birth weight neonates are at an increased risk for the development of neonatal sepsis.[1]

Acinetobacter baumannii is an opportunistic gram-negative organism and a nosocomial pathogen. The organism is mainly multi-drug resistant with varying mechanisms of resistance to antibiotics.[2]

Neonates with *A.baumannii* infection are at an increased risk for severe complications and have high morbidity and mortality rates.[3]

Anecdotal evidence in our setting is that we have seen a high number of *A.baumannii* infections amongst premature neonates. We have also observed that many of these neonates will require vasoactive drugs and escalation of respiratory support for survival. We obtained many of the results for a positive *A.baumannii* retrospectively after the death of the neonate.

This study investigates the incidence, clinical characteristics, frequency of risk factors described in the literature, antibiotic susceptibility and the clinical outcomes of premature neonates infected with *A.baumannii* in the neonatal unit.

This project is a retrospective descriptive study conducted at the neonatal unit at Universitas Academic Hospital, Bloemfontein over 36 months.

1.2 LITERATURE REVIEW

1.2.1 Neonatal sepsis

1.2.1.1 Epidemiology

According to the *Global Report on the Epidemiology and Burden of Sepsis*, Molloy *et al*. claims that the World Health Organisation has identified neonatal sepsis as a priority in the neonatal population. Neonatal sepsis contributes substantially to mortality worldwide.[4]

Due to the lack of unified data, it is challenging to standardise international

data.[4]

An estimated three million neonates are affected globally, with an incidence of 22 per 1000 live births. Mortality rates in neonatal sepsis vary between 11% and 19%.[5]

1.2.1.2 Why neonates are susceptible to infection

Exposure to infection while in utero and an altered immune function makes neonates substantially different to adults and young children. Immune privilege allows the fetus to eliminate toxins more effectively. When the neonate is born, there is an enhanced pro-inflammatory response, similar to an "adult" immune response, which alters the fetus's immune privilege in utero. This phenomenon is displayed more in premature neonates.[4]

1.2.1.3 Prematurity and sepsis

Ten per cent of neonates are born prematurely. The healthcare system is financially burdened by sepsis, especially in preterm neonates. Physical and chemical barriers such as the skin and mucosa provide the first line of defence against pathogens. Premature neonates have an immature stratum corneum, and there is an absence of vernix caseosa. The scarcity of goblet cells and impaired mucociliary clearance increases the risk of infection. An immature gut and limited exposure to enteral feeds and breastmilk are associated with an increased risk for infection. Adaptive immunity in the premature neonate is impaired, which increases the risk for microbial invasion.[6]

1.2.1.4 Prevention of preterm delivery

One of the primary preventative methods in neonatal sepsis is to prevent premature delivery. Early antenatal care and antenatal progesterone in pregnant mothers are associated with lower premature delivery rates. Cervical cerclage should be offered to women with a shortened cervix. Postdelivery neonatal outcomes improve significantly by the administration of a course of steroids to pregnant women.[7]

1.2.1.5 Early onset sepsis

Early-onset neonatal sepsis is defined as bacteraemia or bacterial meningitis occurring less than 72 hours. In premature neonates, early-onset sepsis is better described as sepsis in the first three days of life from pathogens transmitted from the mother during or before delivery of the neonate.[8]

The risk of death from early-onset sepsis is determined mainly by an earlier gestational age and its complications.[8]

In a retrospective study by Almudeer *et al*., early-onset sepsis incidence was 4.44 per 1000 live births. *E.Coli, Group B streptococcus* and *coagulase-negative Staphylococcus* were the most common pathogens identified.[9]

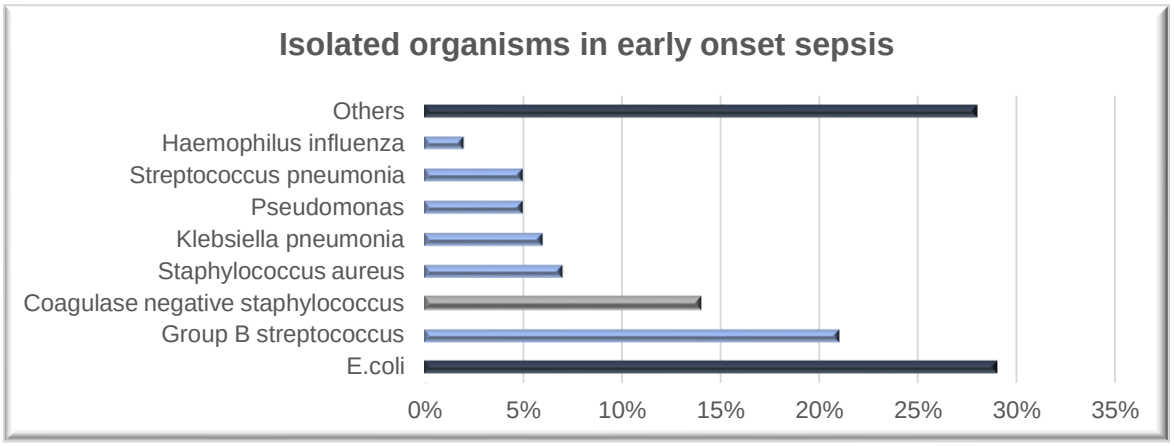

Figure 1: Graph showing organisms cultured in early-onset sepsis (Almudeer *et al*.)

1.2.1.6 Late-onset neonatal sepsis

Late-onset sepsis is defined as sepsis occurring after 72 hours of life.[10]

Neonates can have more than one episode of late-onset sepsis with the subsequent episodes associated with more severe presentations and poorer outcomes.[11]

Tsai *et al.* showed that one-fifth of neonates with an initial episode of late-onset sepsis would often have recurring episodes requiring a more extended stay in the neonatal unit. These neonates had a higher mortality rate (30.7%) than a single episode of sepsis (7.8%).[11]

Overcrowding, lack of neonatal staff, limited resources and inappropriate antibiotic use contribute to late-onset sepsis.[12]

Late-onset sepsis is inversely proportional to the gestational age of the neonate.[13]

Incidence peaks between days 10-22 of life and is attributed mainly to nosocomial infections.[13]

Coagulase-negative staphylococci, gram-negative bacilli and fungi are the common organisms isolated.[13]

Due to the increased risk of death and long-term neurodevelopmental complications, empirical antibiotics are often commenced.[13]

Multi-resistant Gram-negative infections are increasing in late-onset sepsis cases and pose a significant challenge on neonatal units worldwide.[13]

1.2.2 The emergence of multi-drug resistant organisms in NICUs

Multi-drug resistant (MDR) infections have become a global health issue. Clinicians are faced with significant challenges in treating multi-drug resistant infections. Progress in medicine and health is compromised.[14]

In a Taiwanese study, MDR infections were found in 18.6 % of all neonatal bacteraemia. Most of these neonates received broad-spectrum antibiotics. There was a high rate of carbapenem-resistant bacteria in the neonatal ICU.[11]

The incidence of MDR gram-negative bacilli is on an upward trend, and occasionally pan-resistant pathogens are cultured. Enzymatic action on bacterial morphology, mutations that inhibit the binding of antibiotics from targeting sites and modifications in uptake through drug efflux pumps are all factors that contribute to antibiotic resistance.[15]

A delay in initiating antimicrobials, limited resources and increased virulence of pathogens may be associated with the high mortality and morbidity rates from antimicrobial-resistant pathogens.[15]

Clinical outcomes in multi-drug resistant infections can be improved by trials of both old and new antibiotics.[16]

In an Egyptian study conducted by Awad *et al.* in 2016, 77% of isolated organisms cultured in the neonatal unit were multi-drug resistant. More than half the organisms were Gram-negative bacteria.[17]

Screening for multi-drug resistant organisms in the neonatal unit is costly, and the role of screening in low-income countries is controversial. Screening may assist in developing rational antibiotic use in neonatal units where microbiology services

are lacking.[18]

Labi *et al.* showed that multi-drug resistant infections were common in neonates with prolonged hospital stays and neonates who received excessive antibiotics.[18]

Agarwal *et al.* demonstrated significant numbers of multi-drug resistance in *Acinetobacter species* (22%), *Klebsiella spp* (17%) and *Escherichia coli* (14%) in the neonates.[19]

1.2.3 Antibiotic stewardship in the neonatal intensive care unit

Antibiotic stewardship is an integrated programme for the rational use of antimicrobials to sustain favourable patient outcomes, reduce microbial resistance, and lower the transmission of infections caused by multi-drug resistant pathogens.[20]

The World Health Organisation advocates for the judicious use of antibiotics in neonatal intensive care units. The irrational use of antibiotics and the resultant emergence of multi-drug resistance has posed a significant problem globally.[20]

The use of antimicrobials in the neonatal intensive unit is different from other settings for a few reasons.[21]

The neonate with sepsis can present with non-specific or subtle signs. The neonate may not have any signs of sepsis. In the early stages of infection, there are no laboratory investigations that can confidently exclude infection. These factors, therefore, contribute to the empirical use of antibiotics in neonates. A premature neonate may have complications due to prematurity, such as apnoeas or hypotension, but these signs are misinterpreted for sepsis and antibiotics are continued.[21]

Neonates born to mothers with chorioamnionitis and neonates undergoing surgery frequently receive prolonged courses of antibiotics. Studies are lacking regarding peri-operative antibiotics.[21]

Neonates have a lower glomerular filtration rate; therefore, there are significant differences in pharmacodynamics and pharmacokinetics. These differences make ideal dosages and drug-level monitoring challenging in the neonate.[21]

The ideal situation would be to administer the narrowest spectrum antibiotic only to neonates with proven infection.[22]

Ting *et al.* showed an association between prolonged antibiotic use and adverse

outcomes. Neonates who received prolonged antibiotics were at increased risk for intraventricular haemorrhage, periventricular leukomalacia, bronchopulmonary dysplasia, retinopathy of prematurity, necrotising enterocolitis and late-onset sepsis. There were > 20000 neonates enrolled in this study, and it is the most extensive study to date, showing the association between early antibiotic exposure and composite outcomes.[23]

The main aim of antibiotic stewardship in premature neonates is to reduce empirical prescribing after birth and reduce the duration of antibiotics in low-risk neonates.[24]

Lu *et al.* showed a significant decrease in late-onset sepsis (p=0.03) in the number of neonates whose antibiotics were discontinued after 48 hours (p=0.0001).[25]

Bhat *et al.* demonstrated a 10.6 % reduction of antibiotic use after implementation of an antibiotic stewardship programme.[26]

In a study conducted by Cantey *et al.*, antibiotic usage was reduced by 27 % after initiating antibiotic stewardship.[27]

1.2.4 Infection control in the neonatal intensive care unit

Infection prevention in the neonatal unit is imperative. The literature is limited on infection control in neonatal intensive care units.[28]

A telephonic survey was conducted amongst 87% of the 198 neonatal units in the United Kingdom in 2012. 12.2 % of neonatal units had closed due to issues with infection control. 22.1% of units employed the use of protective clothing for all staff working in the department. The use of gloves and aprons were observed in 5.8% and 7.6% respectively. Alarmingly, 54% of units were observed to practice hand sanitisation before entry into the neonatal unit.[28]

Hand sanitisation is considered the best way to prevent health-worker associated infections. The mechanism of proper handwashing and decontamination is poorly performed in most neonatal units. There are limited studies to evaluate the technique of hand decontamination in the neonatal intensive care unit.[29]

Risso *et al.* showed that healthcare team members, both nursing staff and physicians, displayed poor hygiene practices in a neonatal unit.[30]

In an article published in a South African journal in 2020, neonatal units in South

Africa have minimal staff, inadequate infrastructure and limited resources like gloves and alcohol hand sanitisers.[31] At a national level, quality improvement strategies aim to promote the establishment of breastfeeding, availability of donor milk banks, stringent cord care, and appropriate use of antimicrobials. Outbreaks of sepsis in neonatal units in South Africa are infrequently reported. Anecdotal evidence from South African clinicians suggests that sepsis outbreaks in the neonatal unit occur frequently.[31]

1.2.5 Current challenges in neonatal sepsis

One of the Millenium Development Goals programme's most significant achievements was to reduce maternal and child mortality by 50%. The neonatal mortality rate, however, remains high with an estimated 2.9 million deaths per year. The top three causes of neonatal death were notably prematurity, intrapartum complications and sepsis.[32]

Infections cause nearly 23% of neonatal deaths globally. Early identification of neonatal sepsis and prompt treatment may lower the mortality rate, but the emergence of antimicrobial resistance remains a significant challenge. Because of limited therapeutic options, gram-negative infections are of most significant concern.[32]

There is currently a global shortage of antibiotics to treat neonatal infections. Neonates are not prioritised highly enough in research and development, especially in developing countries. Data to support optimal management of multi-drug resistant infections is significantly lacking.[32]

The Global Antibiotic Research and Development Partnership has initiated a programme to deal with antibiotic resistance issues in neonates. The NeoAMR Project identifies pitfalls in antibiotic regimens and institutes new regimens, especially in the setting of multi-drug resistant pathogens.[32]

1.3 ACINETOBACTER BAUMANNII

1.3.1 History

In 1911, the genus Acinetobacter was first identified and described as a nosocomial pathogen in the 1960s when isolated in an intensive care unit. Molecular tests were

used to determine the species. The persistence of *A.baumannii* in the hospital setting allowed it to develop resistance over the years. Due to the favourable environmental conditions, successful clones of *Acinetobacter baumannii* with a strong propensity for antibiotic resistance emerged. Resistance to the carbapenems was reported as early as the 1980s.[33]

1.3.2 Microbiology

Acinetobacter baumannii is an aerobic Gram-negative coccobacillus. The pathogen was first described as *Micrococcus* with the term *Acinetobacter* allocated in 1950. Over 50 variant species of *Acinetobacter species* have been identified to date with *Acinetobacter baumannii* being the most common species. *Acinetobacter baumannii* is an opportunistic pathogen with a limited number of virulence factors.[34]

1.3.3 Pathogenesis

The production of biofilms allows for *A.baumannii* to colonise environmental surfaces. Biofilm associated protein promotes adherence to cells and therefore facilitates colonisation.[34]

Outer membrane protein A is essential for manufacturing biofilm that is intact. It is involved in cell apoptosis and helps bind factor H. About a third of *Acinetobacter baumannii* strains produce the K1 capsule which is mainly constituted of polysaccharides. The K1 capsule inhibits activation of the complement pathway and alters phagocytosis of cells.[34]

Acinetobacter baumannii can thrive in patients with iron-deficiency due to the siderophore-mediated iron-acquisition system. Fimbriae help the pathogen to attach itself to environmental and biotic surfaces.[34]

1.3.4 Clinical significance

The prevalence of *Acinetobacter baumannii* infections has significantly increased in hospitals worldwide. The World Health Organisation identifies the pathogen as a critical priority owing to its ability to cause multi-drug resistant infections. Urgent treatment interventions are warranted, and it is anticipated that *Acinetobacter*

baumannii will soon be an untreatable pathogen.[35]

The *Infectious Diseases of Society of America* has identified *Acinetobacter baumannii* as one of the six top-priority lethal microorganisms.[36]

1.3.5 South African literature on *A.baumannii* in neonatal units

Thomas *et al.* undertook the most extensive study in South Africa on *A.baumannii* and the outcome in neonates. Three hundred ninety-nine neonates were enrolled in this study. 91% of neonates were born before 37 completed weeks of gestation. The mean birth weight was 1401 grams and 76% of neonates presented with late-onset sepsis. Respiratory distress (22%) was the most common clinical sign at the time of presentation. 33% of neonates received parental nutrition, and 36% had a central catheter inserted before the onset of infection with *A.baumannii*. Vasoactive drugs were administered to 29% of neonates, and 43% required escalating respiratory support due to sepsis. The study showed a high rate of susceptibility to the cephalosporins with few isolates susceptible to the carbapenems. The case fatality rate was 32%.[37]

Chromosomal studies have demonstrated that *A.baumannii* possess OXA beta-lactamases, OXA-48 has been shown to spare the cephalosporins but may have an antagonistic effect on the carbapenems.[38]

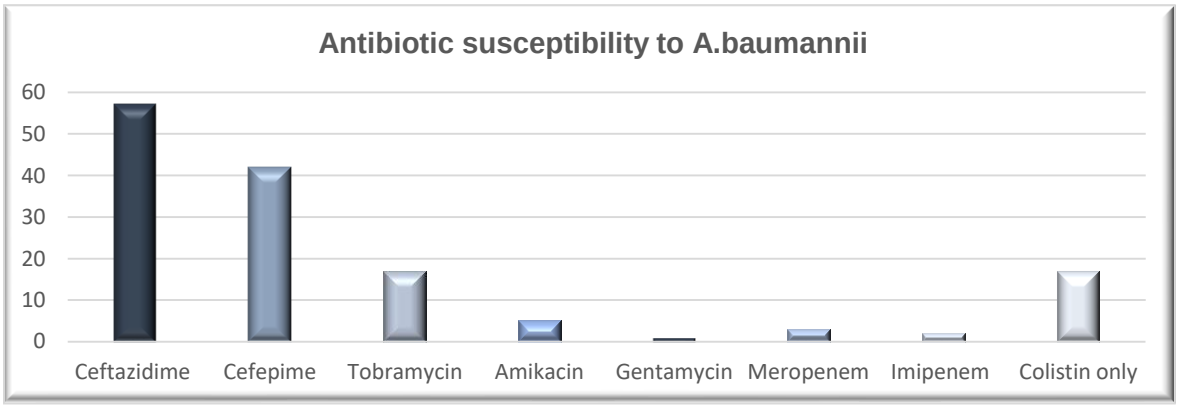

Figure 2: Graph showing antibiotic susceptibility profiles to A.baumannii (Thomas *et al.*)

In a study conducted by Reddy *et al.* over ten years at Tygerberg Hospital, Gram-negative organisms were the neonatal unit's predominant pathogens. *Klebsiella species, Serratia marcescens, E.coli* and *A.baumannii,* were the common pathogens isolated. *A.baumannii* contributed to many neonatal deaths in the unit

with a significant outbreak of carbapenem-resistant *A.baumannii* in 2017. Of the *A.baumannnii* pathogens isolated, 83.9 % were extensively drug-resistant.[39] Lebea *et al.* conducted a study on neonatal sepsis at Charlotte Maxexe Johannesburg Academic Hospital. The study showed that gram-negative pathogens (49%) were commonly isolated. Klebsiella Pneumonia, *A.baumannii* and E.coli were prevalent in the neonatal unit. *A.baumannii* contributed to 17.9% of early-onset infections and 6.6% of late-onset infections. Most strains of *A.baumannii* were sensitive to Amikacin (65%) and Ceftazidime (75%) with high resistance to both Gentamycin (85%) and Meropenem (80%).[40]

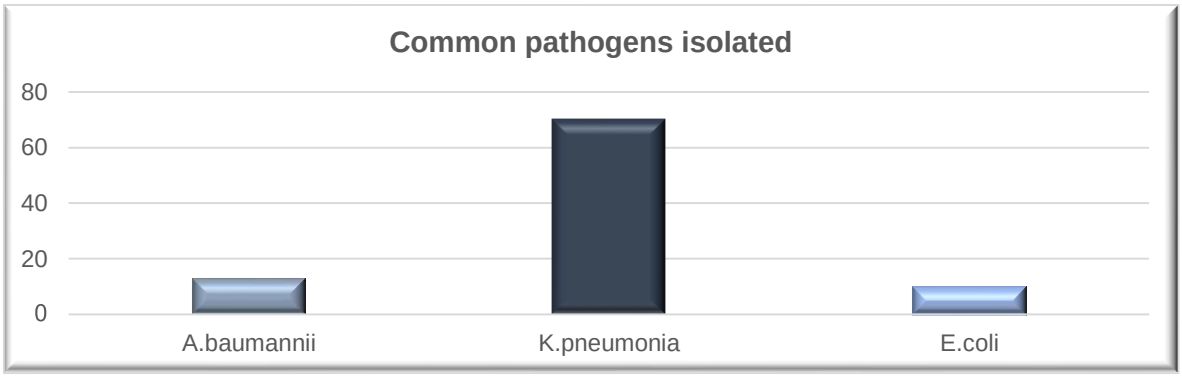

Figure 3: Graph showing common pathogens isolated in a South African neonatal unit (Lebea *et al*.)

Pillay *et al.* investigated an outbreak of multi-drug resistant *A.baumannii* from contaminated suction bottles and catheters at King Edward VIII Hospital in Durban, South Africa. Preterm neonates were the most at risk with a median gestational age of 33 weeks. The outbreak contributed more to early-onset sepsis than late-onset sepsis, with a median postnatal age of three days. The mortality rate of neonates infected with *A.baumannii* was 22%. Ciprofloxacin and amikacin were effective in treating the infection in 55% of cases.[41]

In meningitis cases, *Acinetobacter baumannii* is often regarded as a contaminant unless there is a neurosurgical predisposition. In a case study by Howell *et al.* at Inkosi Albert Luthuli Hospital, it was concluded that the pathogenicity of *A.baumannii* in neonatal meningitis might be underestimated and therefore warrants more studies.[42]

Snyman *et al.* demonstrated significant numbers of colistin-resistant *A.baumannii* in a hospital in the Western Cape. 77% of strains studied were not susceptible to colistin by broth microdilution testing.[43]

1.3.6 International studies on *A.baumannii*

1.3.6.1 Risk factors

Dalili *et al.* (2019) showed that *A.baumannii* was common in preterm neonates (84%) and frequently cultured in blood specimens (66.7%). The use of antifungals such as fluconazole and amphotericin and the number of prescribed antimicrobials may play a role in increasing the risk for *A.baumannii* infection.[44]

In a case-control study by Hsu *et al.* (2014), insertion of a central venous line (P=0.009) and total parental nutrition (P=0.010) were more common in the group of neonates infected with *Acinetobacter baumannii* as compared to the control group.[45]

Lee *et al.* (2017) demonstrated prematurity (95%) as a significant risk factor with most neonates (70%) being very low birth weight (<1500 grams). Central venous catheters were inserted in 65% of neonates before the onset of sepsis. 95% of neonates received parental nutrition.[46]

Sultan et al. (2018) showed that prematurity (P<0.01) and the previous use of carbapenems were significant risk factors for *A.baumannii* infection in neonates. 76.9 % of neonates enrolled in the study had an umbilical venous catheter inserted before the onset of sepsis. There was no statistical difference between early (49.5%) and late-onset sepsis (50.5%).[47]

Central catheters, ventilation exceeding seven days, prolonged hospital admission and lack of early breastfeeding were significant risk factors according to a study by Kumar *et al.* (2014).[48]

Colonisation with *A.baumannii* may be a significant risk factor to develop an infection. Arhoune *et al.* (2019), showed that 9.8 % of neonates were colonised with *A.baumannii* on admission to the neonatal unit. More than 66% of the colonised isolates were multi-drug resistant. The study concluded that the lack of infection control during labour and postnatal care might contribute to colonisation of *A.baumannii* during the first week of a neonates life.[49]

1.3.6.2 Site of culture

Saleem *et al.* (2010), showed that blood (44%) was the most common site for a positive culture. *A.baumannii* was rarely cultured in urine (1%) and cerebrospinal fluid (1%).[50]

1.3.6.3 Clinical presentation and complications

In a recent study by Mahich *et al.* (2020), respiratory symptoms such as tachypnoea (76%) and chest recessions (14%) were common initial findings in neonates with *A.baumannii* sepsis. Neurological signs such as apnoea (9.3%) and lethargy (9.3%) were not common findings. Only 4/43 (9.3%) of neonates studied had temperature disturbances. The onset of sepsis differed from most other studies in that most neonates (58.1%) had early-onset sepsis.[51]

More than half (58.1%) of neonates developed circulatory shock requiring the use of vasoactive drugs. 72.1% of neonates developed severe respiratory distress requiring mechanical ventilation. Many of these neonates had poor outcomes.[51]

A case-control study in Thailand by Thatrimontrichai *et al.* (2016) showed that septic shock was more common in neonates with *A.baumannii*.[52]

Thrombocytopenia is frequently seen in neonates with a gram-negative infection such as *A.baumannii*. The low platelet count may be present before pathogens are cultured on specimens and therefore may be considered an essential parameter in the early diagnosis of sepsis in the neonate.[53]

1.3.6.4 Antibiotic susceptibility

Studies show that most isolates of *A.baumanni* are sensitive to Colistin and resistant to carbapenems and many other drugs classes.

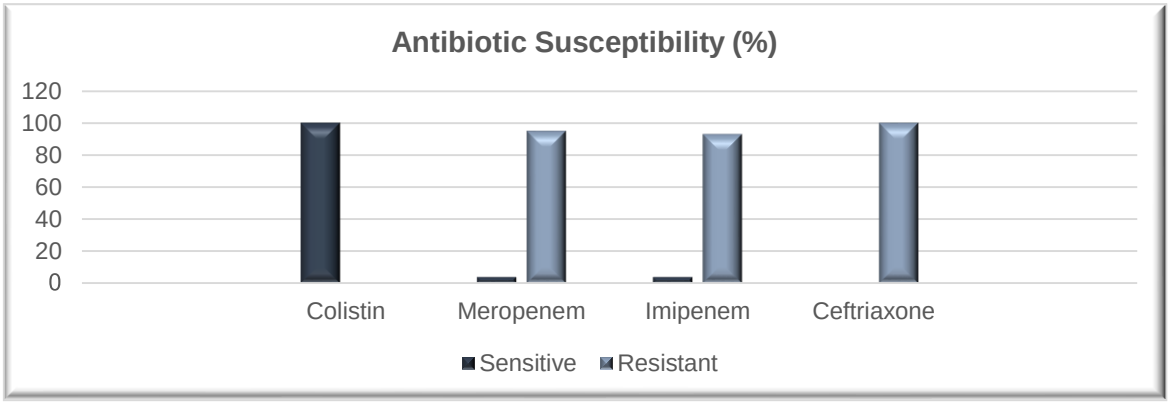

Figure 4: Graph showing antibiotic susceptibility to A.baumanni (Nazir *et al*., 2019)[54]

In a study conducted by Touati et al. (2009), *A.baumannii* was resistant to all beta-lactams, 94.9 % of isolates were resistant to Gentamycin and 87.2% for cotrimoxazole. All strains showed susceptibility to Colistin.[55]

Agarwal *et al. (2016),* showed an overall multi-drug resistant rate of 82% in

Acinetobacter species, 38% of isolates were resistant to the cephalosporins, and more than half (78%) of isolates displayed resistance to the carbapenems.[56]
Alsubaie *et al. (2019),* demonstrated in a Saudi Arabian study, 100 % resistance to carbapenems and beta-lactams and 100% sensitivity to Colistin.[57]

Shehab *et al. (2015)* showed that A.baumannii isolates were resistant to most antimicrobials with a small number of isolates demonstrating sensitivity to imipenem and amikacin.[58]

1.3.6.5 Mortality

Many studies have shown a high mortality rate from *Acinetobacter baumannii* related sepsis in neonates. Case fatality rates may be as high as 38%-59% according to a paper by Jajoo *et al. (2018).*[59]

Table 1: Showing mortality rates from A.baumannii sepsis in neonatal units

Researcher	**Mortality Rate**
Mishra *et al.* (1998)[60]	13.9%
Jarousha *et al.* (2009)[61]	37.5%
Nakwan *et al.* (2012)[62]	54.0%
De A *et al*. (2013)[63]	20.0%
Wei *et al.* (2015)[64]	20.3%

1.3.6.6 Colistin: the antibiotic of choice for multi-drug resistant infections in neonates

A few studies have demonstrated that overall Colistin is a safe and efficient antibiotic in treating multi-drug infections in neonates.

Tekgunduz *et al. (2015)* showed that the common adverse effects associated with Colistin were low sodium and potassium levels. Hypomagnesaemia was demonstrated in most neonates, requiring magnesium supplementation. The study concluded that Colistin was a safe and efficacious antibiotic for treating multi-drug infections in premature neonates.[65]

In a study by Jasani *et al. (2016),* 62 neonates received Colistin for multi-drug resistant infections. None of the neonates developed neurotoxicity or nephrotoxicity.[66]

Ipek *et al. (2017)* showed that Colistin was not associated with severe adverse outcomes in neonates with multi-drug gram-negative infections. The study showed

that neonates might be susceptible to hypomagnesaemia and hypokalaemia while on Colistin, hence frequent monitoring of these electrolytes is warranted.[67]

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CHAPTER 2

ARTICLE MANUSCRIPT

The publishable article was prepared according to the journal submission guidelines for the South African Journal of Child Health (SAJCH) (cf. Appendix G).

PUBLISHABLE ARTICLE

Acinetobacter baumannii sepsis in premature neonates at a tertiary hospital in South Africa

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ABSTRACT

BACKGROUND: *Acinetobacter baumannii* is an emerging and challenging pathogen in neonatal units, due to its ability to develop resistance against antibiotics. Studies on *Acinetobacter baumannii* infection in premature neonates are lacking in developing countries.

OBJECTIVES: To determine the incidence, clinical presentation, antibiotic susceptibility profiles and the morbidity and mortality rate of premature neonates with *Acinetobacter baumannii*.

METHODS: We conducted a retrospective descriptive study at a South African tertiary hospital. Medical records were reviewed over 36 months. Premature neonates admitted to the neonatal intensive care unit with positive blood, urine or cerebrospinal culture for *Acinetobacter baumannii* were identified through the National Health Laboratory Services.

RESULTS: Fifty premature neonates were enrolled. There was an incidence of 103 cases per 1000 admissions/ year. The median age at the onset of sepsis was 17 days (IQR 9-26). Known risks such as central venous catheters and parental

nutrition were found in 78% (39/50), and 68% (34/50) respectively. Respiratory distress (56% [28/50]) and abdominal distension (50% [25/50]) were the most common clinical signs. Of all the isolates, 86% (43/50) were susceptible to colistin only. Supportive therapy was frequently required, 48% (24/50) received vasoactive drugs, and 70% (35/50) were mechanically ventilated. Mortality related to infection was 36% (18/50).

CONCLUSION: There is a high incidence of *Acinetobacter baumannii* sepsis in premature neonates at Universitas Academic Hospital. A significant number of neonates had a central catheter and received parental nutrition. Most isolates were resistant to the carbapenems but susceptible to colistin. Vasoactive drugs and mechanical ventilation were frequently required. The mortality rate is high from *Acinetobacter* infection which is in keeping with most studies. Strict infection control and antibiotic stewardship are imperative.

INTRODUCTION

An estimated 3 million neonates are affected with sepsis globally with an incidence of 22/1000 live births. Neonatal sepsis is the third common cause of death in neonates with mortality rates between 11% and 19%.[1]

Antimicrobial resistance in low and middle-income countries are significantly increasing. Multi-drug resistance patterns are seen mainly in Gram-negative pathogens. In an observational cohort study by Li *et al*. (2020), Bangladesh and South Africa had the highest resistance rates to cephalosporins and carbapenems. Choice of second-line antibiotics in these countries are restricted. Carbapenem-resistant rates may be as high as 84% in Gram-negative pathogens.[2]

Acinetobacter baumannii is an aerobic Gram-negative coccobacillus with a limited number of virulence factors. In the 1960s, *Acinetobacter baumannii* was first described as a nosocomial pathogen, and it has developed significant resistance over the years.[3,4].

Recent studies have shown that neonates with *Acinetobacter baumannii* have high rates of morbidity. In a paper by Mahich *et al*. (2020), most neonates developed septic shock requiring vasoactive drugs for survival. 72 % of neonates needed escalation of respiratory care and subsequently ventilated.[5] Studies from 1998 to 2015 have demonstrated high mortality rates from *Acinetobacter baumannii* (13-54%).[6–10]

The literature on the clinical presentation, antimicrobial susceptibility, morbidity, and case-fatality rates of *Acinetobacter baumannii* in South Africa is limited. Most South African studies have focused on neonatal sepsis as a whole and the incidence of common pathogens isolated. These studies have identified *Acinetobacter baumannii* as a common pathogen in South African neonatal units.[11,12] There have only been two recent studies that have focused on *Acinetobacter baumannii* as a unique pathogen in the neonatal intensive care unit. Pillay *et al*. (1999) demonstrated an outbreak with *Acinetobacter baumannii* in the neonatal unit, from contaminated suction catheters.[13] Thomas *et al*. (2018) conducted the most extensive South African study to date and demonstrated factors such as clinical presentation, antimicrobial susceptibility pattern and case fatality rate amongst 399 *A.baumannii* isolates.[14] Our research is the first in South Africa to show the outcomes of *Acinetobacter baumannii* in exclusively premature neonates. This

study aims to look at the incidence, clinical profiles, antimicrobial susceptibilities, and neonates' clinical outcomes, admitted to a tertiary hospital.

METHODOLOGY

Study design and population

A retrospective descriptive study of premature neonates admitted to the neonatal intensive care unit with culture-confirmed *A.baumannii* from either blood, urine or cerebrospinal fluid during the study period 1st January 2016 to 31st December 2018.

Study setting

Universitas Hospital is a tertiary level hospital located in Bloemfontein, Free State. The hospital receives referrals from the Free State, Northern Cape and Lesotho and has a 14-bed neonatal intensive care unit.

The microbiology laboratory uses an automated continuous monitoring blood culture system (BacT/Alert system, Biomerieux, Marcy I'Etoile, France). If bacterial growth is detected on a specimen, gram-stain is performed and the sample sub-cultured onto appropriate media and incubated overnight. Identification and antibiotic susceptibility are performed using either the Kirby-Bauer method or the automated system, Microscan, Siemens, USA.[15]

Study population and sampling strategy

The study population consisted of premature neonates (< 37 completed gestational weeks) with confirmed *A.baumannii* grown on cultures, admitted to the neonatal intensive care unit at Universitas Academic Hospital. Neonates, who acquired *A.baumannii* after 28 days of life were excluded from the study. Neonates who had a positive *A.baumannii* culture but not treated with antibiotics due to the neonate not having clinical signs of sepsis or normal infective markers were excluded. These isolates were regarded as contaminants. Neonates with a gestational age of >37 weeks were excluded. No other sampling strategy was employed. Patients were identified by logging all admissions from the admissions register for January 2016 to December 2018 on the NHLS database and the Meditech system. All blood, urine and cerebrospinal fluid specimens flagged positive for *A.baumannii* in premature neonates were identified and captured.

Data collection

Consent was obtained from the National Health Laboratory Services (NHLS) regarding the use of laboratory results. Further information was obtained from the Meditech system. Any outstanding information was obtained from patient statistic forms that are compulsory for every neonate admitted to the neonatal unit. Demographic data were collected, including patient age, sex and patient days at the onset of sepsis. Known risk factors from the literature such as neutropenia and thrombocytopenia, the use of a central venous catheter and parental nutrition were collected. Infection sites, infection markers such as C-reactive protein, white cell count, procalcitonin levels and antibiotic susceptibility profiles were obtained from the NHLS database. The use of vasoactive drugs and respiratory support, such as mechanical ventilation, were also obtained from patient summaries. Outcomes of the neonate, such as death or discharge, were collected.

Data capturing and analysis

Data was captured onto Microsoft Office Excel 2010. The data was stored on a password-protected computer. The data was captured daily, and only the principal researcher had access to the data. Completed information was sent to the Department of Biostatistics at the University of Free State for analysis. Medians and ranges were used to describe continuous variables that were not normally distributed. Frequencies and proportions were used to describe categorical variables.

Ethical considerations

Approval for this study was obtained externally from the Free State Department of Health and internally from the Health Sciences Research Ethics Committee of the University of Free State. ***(UFS-HSD2020/0667/2909)***. Data records were kept confidential and depersonalised; therefore, no further consent was sought. Appropriate measures were taken to ensure the security of all patient data.

Results

A total of 50 neonates were included in the study population. There was a total of 485 neonates admitted to the neonatal intensive unit over 36 months. The incidence was 103 cases per 1000 admissions/year.

Demographic characteristics, including gender, birth weight and age, are

presented for the study population. The majority of included patients were male (35/50 [70%]). The median age of included neonates at the onset of sepsis was 17 days (IQR 9-26). The median birth weight was 1295 grams (IQR 1010-1770).

Table 1 summarises the frequency of risk factors described in the literature. Most patients had a central venous catheter inserted (39/50 [78.0%]) and received total parental nutrition (34/50 [68.0%]) before the onset of infection with *A.baumannii*. The majority of neonates (31/50 [62%]) had thrombocytopenia while a small number of neonates (12/50 [24.0%]) had neutropenia before the onset of *A.baumannii* sepsis.

Table 1: Risk factors (n=50)

Risk Factors	n (%)
Central catheter	39 (78.0%)
Parental nutrition	34 (68.0%)
Thrombocytopenia	31 (62.0%)
Neutropenia	12 (24.0%)

Table 2 shows the frequency of clinical signs in neonates infected with *A.baumannii*.

Table 2: Clinical signs

Clinical signs	Yes
Respiratory distress	28 (56.0%)
Apnoea	13 (26.0%)
Abdominal distension	25 (50.0%)
Fever (Temp >37.5^0 C)	0 (0.0%)
Hypothermia (Temp <36.5^0 C)	6 (12.0%)

Respiratory distress (28/50 [56.0 %]) and abdominal distension (25/50 [50.0 %]) were the common clinical signs.

The laboratory results of included neonates are presented in Table 3.

Table 3: Laboratory results

Neonates	WCC x10^9/L	PLT x10^9/L	CRP mg/L	PCT ug/L
N	50	50	48	28
Median	12.1	56.5	75.0	3.6
IQR	7.8-18.4	18.0-120.0	41.5-110.0	0.6-11.3

WCC: white cell count, PLT: platelet count, CRP: C-reactive protein, PCT: procalcitonin

A summary of the site of positive *A.baumannii* culture of the study population is presented in Table 4.

Table 4: Site of positive culture for *A.baumannii* (n = 50)

Culture	n (%)
Blood	50 (100.0%)
CSF	1 (2.0%)
Urine	1 (2.0%)

CSF: cerebrospinal fluid

All patients (50/50 [100 %]) included in the study had a positive blood culture for *A.baumannii.* One neonate had both positive urine and CSF culture for *A.baumannii* (1/50 [2.0 %]).

A summary of antimicrobial sensitivity is presented in Table 5.

Table 5: Antimicrobial sensitivity profile for *A.baumannii* (n=50)

Antibiotic	n (%)
Amikacin	2 (4.0%)
Cefepime	1 (2.0%)
Ceftazadime	1 (2.0%)
Ciprofloxacin	0 (0.0%)
Imipenem	0 (0.0%)
Levofloxacin	0 (0.0%)
Meropenem	1 (2.0%)
Piperacillan/Tazobactam	1 (2.0%)
Tigecycline	1 (2.0%)
Tobramycin	0 (0.0%)
Co-trimoxazole	2 (4.0%)
Colistin	43 (86.0)

(43/50 [86%]) of isolates were sensitive to colistin, (2/50 [4%]) of isolates were sensitive to amikacin and (1/50 [2%]) of isolates were sensitive to Meropenem.

The resistance profiles of antimicrobials are presented in Table 6.

Table 6: Antimicrobial resistance profile for *A.baumannii* (n=50)

Antibiotic	n (%)
Amikacin	41 (82.0%)
Cefepime	44 (88.0%)
Ceftazidime	44 (88.0%)
Ciprofloxacin	45 (90.0%)
Imipenem	45 (90.0%)
Levofloxacin	12 (24.0%)
Meropenem	44 (88.0%)
Piperacillan/Tazobactam	44 (88.0%)

Tigecycline	8 (16.0%)
Tobramycin	41 (82.0%)
Co-trimoxazole	30 (60.0%)
Colistin	0 (0.0%)

The use of vasoactive drugs and respiratory support in neonates with *A.baumannii* sepsis is presented in Table 7.

Table 7: Use of vasoactive drugs and respiratory support in premature neonates with *A.baumannii* sepsis (n=50)

Modality	n (%)
Vasoactive drugs	24 (48.0%)
Mechanical ventilation	35 (70.0%)
High frequency oscillatory ventilation	10 (20.0%)

The outcomes of neonates with *A.baumannii* infection is presented in Table 8.

Table 8: Outcomes of neonates with *A.baumannii* infection (n=50)

Outcome	n (%)
Discharged	31 (62.0%)
Died	18 (36.0%)
Unknown	1 (2.0%)

Most patients (31/50 [62.0 %]) were discharged from the neonatal unit, but a large number of patients had died from *A.baumannii* sepsis (18/36) [36.0%]).

DISCUSSION

Incidence

The incidence of *Acinetobacter baumannii* in this study (103 cases/1000 admissions) was significantly higher than a previous South African study by Thomas *et al*. (22.8 patients/1000 admissions). The study by Thomas *et al*. included both term and preterm neonates, but 91% of neonates were born preterm.[14] It is difficult to compare the incidence to international studies. Most international studies have not commented on the incidence in premature neonates but have looked at neonates at all gestational ages.

Acinetobacter baumannii was found more in males than females ($p > .05$). This finding isin keeping with a few studies. Wei *et al*. showed a female to male ratio of

0.79:1 in neonates with multidrug-resistant infection. [10] In a recent study by Mahich *et al*. in2020, 2/3 of neonates were of the male sex.[5]

Our study showed a median age of 17 days which is considered late-onset sepsis.[16] A study has shown that overcrowding, lack of neonatal staff, limited resources and inappropriate antibiotic use contribute to late-onset sepsis.[17] The incidence of late-onset sepsis peaks between days 10-22 of life, and it is mainly attributed to nosocomial infections with gram-negative bacilli being one of the common pathogens isolated.[18]

Risk factors

A significant number of neonates in this study had a central venous catheter inserted and was administered parental nutrition before the onset of sepsis. Many studies show central catheters and parental nutrition to be significant risk factors for sepsis.[19–22]

A systematic review by Rosado *et al.* in 2018 showed that catheter-associated infections were more common in prematurity, low-birth-weight infants and prolonged use of central lines.[23]

Kung *et al.* demonstrated parental nutrition to increase the risk of sepsis by almost six-fold.[24]

Clinical presentation

Our study identified respiratory distress and abdominal distension as the most common clinical signs. Temperature disturbances were a rare finding. These results are in keeping with Mahich *et al*., who showed respiratory distress in 76% of neonates and temperature disturbances in less than 10% of neonates. Inadequate information on patient summaries and technical issues such as improper placement of the temperature probe on the skin of the neonate may have contributed to these low numbers.

A key finding in our study was a median platelet count of $56.5x10^9$/L (IQR 41.5-110.0). Ree *et al*. demonstrated thrombocytopenia (platelet count < $150x10^9$/L) was associated more with gram-negative than gram-positive infections (p=0.003). In their study, neonates with severe thrombocytopenia (platelet count <$50x10^9$/L), were more prone to intraventricular haemorrhage.[25] Our study showed 44% (22/50) of neonates who had platelet counts of less than $50x10^9$/L. Levit *et al.* showed

increased mortality rates in neonates who had thrombocytopenia.[26]

Site of culture

Most isolates were cultured from blood specimens in our study. One neonate had *Acinetobacter* cultured in both urine and cerebrospinal fluid. These findings are similar to a study by Saleem *et al*.[27] Studies on *Acinetobacter baumannii* as a cause of urinary tract infections in the neonate are scanty. Cases of meningitis and ventriculitis from *Acinetobacter baumannii* in the neonatal population are not common. Meningitis occurs predominantly in neonates, post-neuro-surgical procedures.[28]

Antimicrobial profiles

There was a 90% (45/50) and 88% (44/50) resistance rate to imipenem and meropenem respectively. A previous study in 2018 by Sultan *et al.* has shown prematurity to be a significant risk factor for carbapenem-resistant *Acinetobacter baumannii*.[29] Studies have identified *bla*$_{\text{OXA-23}}$ as the most common gene responsible for resistance to the carbapenems due to its carbapenemase action.[30] Since the introduction of imipenem, a new gene *bla*$_{\text{OXA58}}$ has evolved, and the current studies are now focusing on this gene.[31] In a South African study, Nogbou et al., the *bla*$_{\text{OXA-23}}$ gene was detected in 98% of *Acinetobacter baumannii* isolates, and the first cases of *bla*$_{\text{OXA-40}}$ and *bla*$_{\text{SIM-1}}$ isolates were reported. Most isolates showed susceptibility to colistin and tigecycline.[32]

In this study, only nine isolates were tested for susceptibility to tigecycline. Of the isolates tested, 2% were susceptible, and 18% of isolates showed resistance to tigecycline. Nazir *et al*.[33] and Wei *et al*.[10] demonstrated high susceptibility rates to tigecycline apart from colistin. It is difficult to comment on the antibiotic profile of tigecycline in our study due to the low number of isolates tested. In the largest study in South Africa by Thomas et al. tigecycline susceptibility was not reviewed.[14] The literature on the safety of tigecycline use in the neonatal population is limited, but in a study by Ipek *et al.,* tigecycline may be recommended in the neonate if no other drugs are available.[34]

Our study showed high susceptibility to colistin. This finding is similar to other studies that have shown high sensitivity patterns to colistin.[33,35,36] Nazir *et al.* postulated in a paper that the susceptibility to colistin might be high due to the

previously restricted use in fear of the nephrotoxic effects.[33] Ambreen *et al.* demonstrated that the early use of colistin in neonates with multi-drug resistant *Acinetobacter baumannii* has been associated with higher survival rates.[37]

Morbidity and mortality

Previous studies have shown that *Acinetobacter baumannii* is associated with a high morbidity and mortality rate.[7,27] Almost half of the neonates in this study required vasoactive drugs due to poor perfusion from sepsis and majority of neonates required escalation of respiratory support. One-fifth of the study population required progression to high-frequency oscillatory ventilation as they had coped poorly on conventional ventilation. These findings are similar to a study by Mahich *et al*.[5] Septic shock was a common finding in neonates with *Acinetobacter baumannii* according to a study by Thatrimontrichai *et al*.[38]

The study showed a high mortality rate of 36%, which is in keeping with most international studies.[7–9] This may be explained by the tendency of serious complications in neonates with *Acinetobacter baumannii* infection and the pathogen's multi-drug resistance that makes it a complicated infection treat. Anecdotally, many of the positive *Acinetobacter baumannii* culture results in our study were obtained retrospectively after neonates' death. The delay in starting colistin may have contributed to the high mortality rate.

Study limitations

This project is a retrospective, descriptive study. The incidence of *Acinetobacter baumannii* infection in the neonatal intensive care unit may not be a true reflection of the actual incidence as inadequate culture specimens may have been taken at the onset of sepsis leading to a lower yield. It is challenging to isolate contaminants from actual infection; therefore neonates who were not treated with antimicrobials or neonates who did not have any clinical signs of infection with normal infection markers and follow up cultures were excluded. Incomplete patient information may contribute to the overall accuracy of the results obtained.

CONCLUSION

There was a high incidence of *Acinetobacter baumannii* sepsis in premature neonates at Universitas Academic Hospital. Neonates prior to the onset of sepsis,

were more likely to have a had a central catheter and total parental nutrition which is in keeping with most studies. Carbapenem-resistance was demonstrated in most isolates. There was a high susceptibility rate to colistin. Many neonates required the use of vasoactive drugs and further respiratory support. There was a high mortality rate from *Acinetobacter baumannii* infection in premature neonates at Universitas Academic Hospital.

RECOMMENDATIONS

Maternal education on early antenatal care should be employed to reduce the rate of premature deliveries. This goal can be achieved at primary health care level. Local clinics and health care facilities should institute educative workshops on antenatal care.

Mothers in preterm labour should be administered antenatal steroids, which has been shown to lower the risk for infection in the neonate.

The use of central venous catheters and total parental nutrition should be used with caution and removed timeously.

Neonatal units should adopt an antibiotic stewardship programme. A multi-disciplinary approach involving pharmacists, doctors, and nursing staff would be useful.

Infection control policies and protocols should be maintained in the neonatal unit. Frequent surveillance is necessary.

An early index of suspicion for neonatal-sepsis is imperative. Septic screens should be done on neonates in whom sepsis is suspected without delays. Thrombocytopenia may be used as an early indicator for possible infection.

Neonatal units should promote breastfeeding and a donor milk bank should be available.

Further research is recommended in the following areas

i. A large cohort study has identified South Africa as one of the top two countries with a high incidence of multidrug-resistance in the neonatal unit; more studies are needed in South African neonatal units on multi-drug resistance such as the incidence and common pathogens associated with resistance.
ii. Case-control studies to identify if thrombocytopenia is an independent risk factor

for increased mortality in neonates with *Acinetobacter baumannii* .

iii. Studies on the safety of colistin are limited, more studies are warranted on the efficacy and safety of colistin in the neonate, and research should be dedicated to novel treatments for multi-drug resistance.

iv. Many studies have reported high susceptibility rates to tigecycline in multi-drug resistant *Acinetobacter baumannii*. Research on the efficacy and safety of tigecycline use in the neonate is needed.

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APPENDICES

Appendix A:
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Appendix A:

Letter of Approval from Research Ethics Committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

29-Jan-2021

Dear **Dr Mark Moodley**

Ethics Clearance: **The incidence, clinical profile and antimicrobial susceptibility pattern of Acinetobacter baumannii sepsis in premature neonates at Universitas Academic Hospital for the period 1 January 2016 to 31 December 2018, a retrospective descriptive study**

Principal Investigator: **Dr Mark Moodley**

Department: **Paediatrics and Child Health Department (Bloemfontein Campus)**

Submission Page

APPLICATION APPROVED

Please ensure that you read the whole document

With reference to your application for ethical clearance with the Faculty of Health Sciences, I am pleased to inform you on behalf of the Health Sciences Research Ethics Committee that you have been granted ethical clearance for your project.

Your ethical clearance number, to be used in all correspondence is:**UFS-HSD2020/0667/2909**

The ethical clearance number is valid for research conducted for one year from issuance. Should you require more time to complete this research, please apply for an extension.

We request that any changes that may take place during the course of your research project be submitted to the HSREC for approval to ensure we are kept up to date with your progress and any ethical implications that may arise. This includes any serious adverse events and/or termination of the study.

A progress report should be submitted within one year of approval, and annually for long term studies. A final report should be submitted at the completion of the study.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this proposal for ethical clearance and we wish you every success with your research.

Yours Sincerely

Prof. A. Sherriff
Chairperson: Health Sciences Research Ethics Committee

Appendix B:

Permission from FS DOH

health
Department of Health
FREE STATE PROVINCE

10 August 2020

Dr M Moodley
Dept. of Paediatrics and Child Health
UFS

Dear Dr M Moodley

Subject: A retrospective case-control study on the clinical profile of premature neonates with Acinetobacter baumannii sepsis in the Neonatal Unit at Universitas Academic Hospital for the period January 2016 to December 2018.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of **Universitas Hospital** nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of the Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** sebeelats@fshealth.gov.za / makenamr@fshealth.gov.za **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- **Please discuss your study with Institution Manager on commencement for logistical arrangements see 2nd page for contact details.**
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- **As part of feedback you will be required to present your study findings/results at the Free State Provincial health research day**

Trust you find the above in order.

Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 18/8/2020

Head : Health
PO Box 227, Bloemfotein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and, Harvey Road, Bloemfotein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail:khusemj@fshealth.gov.za@fshealth.gov.za/chikobvup@fshealth.gov.za

Appendix C:

Approval from NHLS

NATIONAL HEALTH LABORATORY SERVICE

Practice No. 5200296

Office of the Business Manager
UNIVERSITAS ACADEMIC LABORATORIES
PO BOX 339(G3)
C/O: CHEMICAL PATHOLOGY
1st FLOOR
BLOCK C
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF FREE STATE
BLOEMFONTEIN
9301

REQUEST FOR APPROVAL OF LABORATORY RESOURCES FOR ACADEMIC PURPOSES

Date: 18 October 2019

Requestor: Dr. M Moodley,

Project Name: "A RETROSPECTIVE STUDY ON THE RISK FACTORS, ANTIBIOTIC SUSCEPTIBILITY, CLINICAL PROFILE AND OUTCOMES OF PREMATURE NEONATES WITH ACINETOBACTER BAUMANNII SEPSIS IN THE NEONATAL UNIT AT UNIVERSITAS ACADEMIC HOSPITAL FROM THE PERIOD JANUARY 2016-DECEMBER 2018"

Dear Dr. Moodley,

Your request for use of laboratory facilities / data is hereby granted under following conditions:

1) That University Ethical Committee approval and approval from the Universitas Hospital management is obtained
2) All laboratory data remain confidential to the patient and doctor (anonymity is maintained)
3) This Office must be notified before any publication of any results / findings are made.
4) NHLS is recognised in all publications
5) That a successful K-Project application be made and relevant NHLS project cost centre be created to utilise testing at NHLS as per your protocol.

May your project be successful.

Regards,

NATIONAL HEALTH LABORATORY SERVICE
23 OCT 2019
Acting
BUSINESS MANAGER
UNIVERSITAS ACADEMIC LABORATORIES

Mr. Pakiso Letanta
Acting Business Manager

Appendix D:

Biostatistics Approval Letter

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

30 June 2020

For attention: Ethics Committee

Faculty of Health Sciences

Title of project:

The incidence, clinical profile and antimicrobial susceptibility pattern of Acinetobacter baumannii sepsis in premature neonates at Universitas Academic Hospital for the period 1 January 2016 to 31 December 2018, a retrospective descriptive study.

Researcher:

DR M MOODLEY

I have given input regarding the above mentioned project's protocol on the following aspects of the protocol, namely the study design, sample, measurement, and statistical analysis.

The input will be implemented under supervision of the study leader.

Yours faithfully

FC van Rooyen

Departement Biostatistiek
Department of Biostatistics
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205 Nelson Mandela Drive/Rylaan, Park West/Parkwes, Bloemfontein 9301, South Africa/Suid-Afrika
P.O. Box/Posbus 339 (G31), Bloemfontein 9300, South Africa/Suid-Afrika, www.ufs.ac.za

Appendix E:

Permission from HOD

UNIVERSITY OF THE FREE STATE
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HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

The Chair: Health Sciences Research Ethics Committee
Dr SM le Grange
For Attention: Mrs. M Marais
Block D, Room 104,
Francois Retief Building
Faculty of Health Sciences
University of the Free State
Bloemfontein
9300

Dear Dr. SM. Le Grange

Student: Dr Mark Moodley
Student Number: 2017556248.

Topic: The incidence, clinical profile and antimicrobial susceptibility pattern of Acinetobacter baumannii sepsis in premature neonates at Universitas Academic Hospital for the period 1 January 2016 to 31 December 2018, a retrospective descriptive study"

I Dr NOMAKHUWA ELIZABETH TABANE hereby grand MARK MOODLEY permission to conduct the abovementioned research project. The research will be complete in accordance with myself as Head of Department of DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH and DR MICHAEL PIERNAAR as supervisor of this study.

Kind Regards,

Date: 26 June 2020

Dr Elizabeth Tabane
HOCD: Paediatrics and Child Health
Universitas Academic Hospital
Faculty: Health Sciences
PO Box 339, Bloemfontein 9300, Republic of South Africa
051/401-9111
27834718015
TabaneNE@ufs.ac.za

M Moodley Digitally signed by M Moodley
Date: 2020.06.30 20:36:55 +02'00'

Appendix F:

Data collection form

QUESTIONNAIRE

SECTION 1 – BIOGRAPHICAL INFORMATION Office use only ☐☐☐ **1 – 3**

1.	**Gestation**	1-13 weeks	1	☐**4**
		14-26 weeks	2	
		27-40 weeks	3	
		40+ weeks	4	
2.	**Age (days)**	1-10 days	1	☐**5**
		11-20 days	2	
		21-30 days	3	
		30+ days	4	
3.	**Gender**	Boy	1	☐**6**
		Girl	2	
		Other	3	
4.	**Birth weight (grams)**	500g – 1000g	1	☐**7**
		1001g – 1999g	2	
		2000g – 2999g	3	
		3000g – 3999g	4	
		4000g+	5	

SECTION 2 – RISK FACTORS

Please mark the option you choose with an X and only mark one option.
Please add your comments where you feel that it is necessary.
Extra comments can be added on a loose piece of folio paper.

1 = Yes / 2 = No / 3 = Unknown

		1	2	3	Comments	
5.	Central line					☐**8**
6.	Total parental nutrition					☐**9**
7.	Thrombocytopenia					☐**10**
8.	Neutropenia					☐**11**

SECTION 3 – CLINICAL SIGNS

Please mark the option you choose with an X and only mark one option.
Please add your comments where you feel that it is necessary.
Extra comments can be added on a loose piece of folio paper.

1 = Yes / 2 = No / 3 = Unknown

		1	2	3	Comments	
9.	Respiratory distress					☐**12**
10.	Apnoea					☐**13**
11.	Abdominal distension					☐**14**
12.	Fever					☐**15**
13.	Hypothermia					☐**16**

SECTION 4 – LABORATORY RESULTS

14.	**White cell count**		1	
			2	☐**17**
			3	
			4	
15.	**Platelets**		1	
			2	☐**18**
			3	
			4	
16.	**Procalcitonin**		1	
			2	☐**19**
			3	
			4	
17.	**C-reactive protein**		1	
			2	☐**17**
			3	
			4	

SECTION 5 – SITE OF CULTURE

Please mark the option you choose with an X and only mark one option.
Please add your comments where you feel that it is necessary.
Extra comments can be added on a loose piece of folio paper.

1 = Yes / 2 = No / 3 = Unknown

		1	2	3	Comments	
18.	Blood					☐**18**
19.	CSF					☐**19**
20.	Urine					☐**20**

SECTION 6 – ANTIBIOTIC PROFILE

Please mark the option you choose with an X and only mark one option.
Please add your comments where you feel that it is necessary.
Extra comments can be added on a loose piece of folio paper.

1 = Resistant / 2 = Sensitive / 3 = Not tested

		1	2	3	Comments	
21.	Amikacin					☐**18**
22.	Cefepime					☐**19**
23.	Ceftazidime					☐**20**
24.	Ciprofloxacin					☐**21**
25.	Imipenam					☐**22**
26.	Levofloxacin					☐**23**
27.	Meropenam					☐**24**
28.	Piperacillan-tazobactam					☐**25**
29.	Tigecyline					☐**26**
30.	Tobramycin					☐**27**
31.	Bactrim					☐**28**
32.	Colistin					☐**29**

SECTION 7 – CLINICAL PROGRESSION

Please mark the option you choose with an X and only mark one option.
Please add your comments where you feel that it is necessary.
Extra comments can be added on a loose piece of folio paper.

1 = Yes / 2 = No / 3 = Unknown

		1	2	3	Comments	
33.	Inotropic support					☐**30**
34.	Mechanical ventilation					☐**31**
35.	High frequency oscillatory ventilation					☐**32**

SECTION 8 – OUTCOME OF PATIENT

Please mark the option you choose with an X and only mark one option.
Please add your comments where you feel that it is necessary.
Extra comments can be added on a loose piece of folio paper.

1 = Discharged / 2 = Died / 3 = Unknown

		1	2	3	Comments	
36.						☐**33**
37.						☐**34**
38.						☐**35**

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** NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'
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- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

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Guideline word limit: 3 000 words (excluding abstract and bibliography)

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

- May include up to 6 illustrations or tables.
- A max of 20 - 25references

Structured abstract

- This should be no more than 250words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
  - Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors. It should be able to be intelligible to the reader without referral to the main body of the article.
  - Do not include any references in the abstracts.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide evidence of consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.
- Scans/photos showing a specific feature e.g. *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author.
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) consecutively as they are referred to in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
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- Footnotes must be indicated with consecutiveuse of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:
Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:
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NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted.If reference manager software is used, the reference list and citations in text are to be unformatted to plain text before submitting..*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
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Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx/doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references
- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports
11: Page or section number
(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.
NOTE: no . after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
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Appendix H:

Turnitin report

MMED

by Mark Moodley

Submission date: 08-Feb-2021 06:07PM (UTC+0200)
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MMED

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Appendix I:

Copy of the research protocol approved by the HSREC

RESEARCH PROTOCOL

Protocol for the fulfilment of the requirements for the degree Master of Medicine in Paediatrics

Faculty of Health Sciences, University of Free State

The incidence, clinical profile and antimicrobial susceptibility pattern of *Acinetobacter baumannii* sepsis in premature neonates at Universitas Academic Hospital for the period 1 January 2016 to 31 December 2018, a retrospective descriptive study

Principal Investigator

Dr M Moodley, MBChB, DCH (SA)

Registrar: Department of Paediatrics and Child Health

Faculty of Health Sciences, University of Free State

Student Number: 2017556248

Study Leader

Dr M Pienaar, MBChB, MMED(Paed), FC(Paed)SA

Consultant: Paediatric Intensive Care Unit

Department of Paediatrics and Child Health

Faculty of Health Sciences, University of Free State

TABLE OF CONTENTS

1. Summary

Neonatal infections currently cause about 1.6 million deaths annually in developing countries. Antibiotic resistance is emerging and has placed a huge burden on the health sector.

Acinetobacter baumannii is a bacterial organism which displays a high degree of antibiotic resistance and contributes to in-hospital mortality and morbidity amongst premature neonates. There is limited literature to describing the outcomes of neonates admitted to a neonatal unit who are infected with *A.baumannii* in developing and developed countries.

This study aims to investigate the incidence, the clinical characteristics, frequency of risk factors as described in the literature, antibiotic susceptibility and the clinical outcomes of premature new-born babies who are infected with *A.baumannii* in the neonatal unit at Universitas Academic Hospital in Bloemfontein over 36 months (01 January 2016- 31 December 2018).

This research study will be a retrospective descriptive study. A literature review will be done to evaluate the current data on the clinical characteristics and outcomes, risk factors and the antibiotic susceptibility profile of neonates with *A.baumannii* infection.

2. Selected Definitions

Early-Onset Sepsis	Sepsis in the first 72 hours of life [1]
Late-Onset Sepsis	Sepsis after the first 72 hours of life [1]
Nosocomial infection	Infection acquired in a hospital setting [2]
Neonate	First 28 days of life
Prematurity	Premature birth is one that occurs before the 37th week of pregnancy is completed (World Health Organisation)
High-frequency oscillatory ventilation	Mechanical ventilation using respiratory rates greater than four times the normal value and small tidal volumes [3]
Mechanical ventilation	The technique through which gas is moved toward and from the lungs through an external device connected directly to the patient [4]
Meditech	A computerised patient record-keeping system
Positive predictive value	The probability that subjects with a positive screening test truly have the disease [5]
Colonisation	The presence of bacteria on a body surface without causing disease
Infection	The invasion of a host organism's bodily tissues by disease-causing organisms
Catecholamines	Medications that change the force of contractions of the heart [6]
Thrombocytopaenia	A platelet count < 150 x 10^9/L [7]
Neutropaenia	A neutrophil count < 1500/mm^3 [7]
Mortality rate	The number of deaths in a given area or period, or from a particular cause
Antimicrobial susceptibility	The susceptibility of bacteria to antibiotics determined in the microbiological laboratory and reported in the relevant culture result
Central venous catheterisation	Insertion of a cannula into a large vein to give fluids, blood, intravenous nutrition or medication
Multidrug-resistant	Resistant to more than one antimicrobial agent [8]
Hypothermia	Temperature less than 36.5 degrees Celsius
Fever	A body temperature of 38 degrees Celsius or higher
Onset of neonatal sepsis	Onset of neonatal sepsis is characterised by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life [9]
Apnoea	No respiratory effort for more than 20 seconds or a break in respiration of less than 20 seconds but associated with a low heart rate (less than 100 beats a minute)

3. List of Abbreviations

AMS	Antimicrobial stewardship
CRP	C-reactive protein
CDC	Centre for Disease Control and Prevention
CSF	Cerebrospinal fluid
DOH	Department of Health
EOS	Early-onset sepsis
ETA	Endotracheal aspirate
ICU	Intensive care unit
LOS	Late-onset sepsis
MDR	Multi-drug resistant
NHLS	National Health Laboratory Services
NICU	Neonatal Intensive Care Unit
TPN	Total Parenteral Nutrition
VLBW	Very low birth weight
WHO	World Health Organisation
WCC	White cell count

4. Introduction

Acinetobacter baumannii is a leading cause of nosocomial sepsis in the neonatal unit. The bacterium often displays multi-drug resistance which contributes to higher morbidity and mortality mainly amongst premature infants. [10] There are only a few reports in the literature on *Acinetobacter baumannii* sepsis in neonatal units. *A.baumannii* can be endemic in several neonatal units worldwide and it is difficult to eradicate once established. [10]

There have been numerous studies to show the increased prevalence of Gram-negative sepsis such as *A.baumannii* sepsis following multiple courses of broad-spectrum antibiotics and the irrational use of antibiotics.

Multiple exposures and a relatively compromised immune system are factors that predispose a neonate to infections. This, together with multi-resistant organisms and overwhelming sepsis also impact these neonates. [11]

Anecdotal evidence in our setting is that we have seen a high number of neonates with *A.baumannii* sepsis. We have also observed that many of these neonates will eventually require catecholamine infusions and escalation of respiratory support. Many of these neonates developed refractory shock and required high-frequency oscillatory ventilation. A significant number of neonates who developed refractory shock did not survive. It is pertinent to note that these neonates eventually demised from *A.baumannii* septicaemia and not from their primary diagnosis requiring admission to the neonatal unit.

Acinetobacter spp. are often transmitted to patients from transient colonisation of the hands of health care workers and persistence on environmental surfaces due to its intrinsic resistance to desiccation. [12]

Acinetobacter sepsis places a substantial burden in neonatal units. Antibiotic resistance is also emerging with many cases being pan-resistant. [13]

5. Antibiotic Stewardship

The core principles common to all antimicrobial stewardship (AMS) programmes are (i) correctly identifying patients who need antimicrobial therapy; (ii) using local and regional antibiograms to guide prescribing; (iii) refraining from the use of antimicrobials with overlapping activity; (iv) administering the correct dose at the correct intervals; (v) regularly reviewing culture results and adjusting antibiotics accordingly; (vi) monitoring drug levels and adjusting the dose accordingly, and (vii) stopping antibiotics when guided by negative cultures. [14]

6. Background

6.1 Literature Review

6.1.1 Microbiology

Acinetobacter baumannii is an aerobic Gram-negative bacillus that is pleomorphic and non-motile. Members of the genus Acinetobacter are ubiquitous and free-living that prefer a moist environment and can easily be found in soil, water, food and sewage. [15]

It is an opportunistic pathogen, commonly infecting immunocompromised individuals. Its pathological potential includes the ability to form biofilms, adhere to surfaces and acquire genetic material from unrelated genera making it a versatile, difficult bacterium to control and eliminate. [15]

In recent years, this microbe has caused alarm amongst the medical fraternity, arising mainly from its extensive resistance to antibiotics. The World Health Organisation (WHO) has identified antimicrobial resistance as one of the 3 most important issues concerning human health. [15]

Premature new-borns admitted to the neonatal intensive care unit have an increased risk of acquiring infection due to their immature immune systems and barrier functions of the skin and gastrointestinal tract. Invasive diagnostic and therapeutic procedures also place them at an increased risk. Nosocomial infections are those that are acquired in a hospital setting. The Centre for Disease Control and Prevention (CDC) defines ICU infections as those that occur 48 hours after admission to ICU or within 48 hours after discharge from an ICU. [15]

6.1.2 Neonatal Sepsis

Neonatal sepsis is a clinical syndrome resulting in non-specific signs and symptoms of infection accompanied by bacteraemia in the first 28 days of life. The non-specific features of sepsis may include irritability, poor feeding or feed intolerance, temperature instability, lethargy, tachycardia or bradycardia, glucose disturbances, jaundice, apnoea, poor circulation and bleeding tendencies. [16]

Early-onset sepsis is defined as sepsis occurring in the first 72 hours of life. It is mainly due to intrapartum vertical transmission of bacteria from the mother to the neonate which can occur from ascending infection in the genital tract or trans-placentally. Late-onset sepsis is defined as sepsis occurring after 72 hours of life. It is primarily the result of horizontal transmission of bacteria from the environment and most often from the hands of the healthcare worker. [16]

6.1.3 Incidence and Mortality

Decreasing gestational age and birth weight has been shown to have a direct relationship with regards to increased risk for acquiring sepsis and death from sepsis. The overall incidence of neonatal

sepsis from South African data is 8.5 to 10 % with many of these infections resulting from Late-Onset Sepsis (LOS)- 83.2-94.3 %. [16]

According to South African data, the mortality rate from sepsis was found to vary from 24.2 and 40% with 19.7 % accounting for early-onset sepsis and 22.5 % for LOS. Gram-negative sepsis was associated with significant mortality with a rate of 69.2 to 80 %. [16]. In this literature study, no publications describing the true incidence of *Acinetobacter baumannii* sepsis in premature neonates were found.

6.1.4 Diagnosis of Neonatal Sepsis

A positive culture from a sterile site (blood, cerebrospinal fluid or urine) is the gold standard for diagnosing neonatal sepsis [17]. The positive predictive value for true bacteraemia improves when multiple cultures grow the same organism. Cultures that flag positive within 72 hours are more likely to be contaminants. At least 1ml of blood should be collected for sterile blood cultures with appropriate measures taken to avoid contamination. [18] There are very few clinical studies on the effect of blood volume alone on blood culture outcomes in neonates. Looking at quantitative blood culture data from the 1970s with high loads of *E.Coli* found in 80 % of infected neonates, it has traditionally been thought unnecessary to draw more than small amounts of blood. [19] There have however been changes in the spectrum of organisms since then which has prompted investigators to revisit this question.

Majority of blood culture bottles utilised in paediatrics are optimised for 1-4 ml of blood with limited neonatal comparative data supporting increased sensitivity compared with adult bottles. [20]

There are no neonatal data on the timing of blood cultures. [21]

Trials assessing skin preparation before culture suggest that alcoholic chlorhexidine, iodine tincture, 70 % isopropyl alcohol are superior to povidone-iodine in reducing contamination rates [22]. The maximal killing of skin organisms can be achieved by delaying for one minute before drawing the specimen. [23] The practice in our unit is to obtain cultures via sterile techniques. A minimum of 1ml of blood is generally used when obtaining a blood culture specimen. The physician collecting the specimen needs to wear a sterile gown and the infant's skin is cleaned with alcoholic chlorhexidine.

6.1.5 Risk Factors for Sepsis

Gestational age and birth weight have strong associations with risk of nosocomial sepsis. In a study conducted by Joseph et al, 255 neonates were investigated out of the 263 neonatal admissions. [24]

The incidence of nosocomial infection was found to be 7.8 % per NICU admission or 2.3 per 100 hospital days. Among VLBW infants enrolled in this study, the most common risk factors for acquiring sepsis were mechanical ventilation, central line insertion and chest tube insertion. [24]

Those neonates with LOS had longer durations of mechanical ventilation and longer duration of a central line in situ. The impacts on hospital stay and mortality were also investigated. These neonates had a significantly prolonged hospital stay and the mortality rate was 5 %. [24]

6.1.6 Studies of Acinetobacter baumannii Sepsis in Neonatal Units

6.1.7 South African Studies

The burden of *A.baumannii* sepsis in developing countries has not been well reported. A South African study was conducted by Thomas et al from the University of Witwatersrand in 2018. Medical records

of neonates admitted to Chris Hani Baragwanath Academic Hospital from 1 October 2007 to 31 October 2011 with positive blood or cerebrospinal fluid cultures due to *A.baumannii* were reviewed for demographic characteristics, clinical presentation, laboratory findings, antibiotic susceptibility patterns and outcomes. [25]

The study also aimed to show the outcomes of neonates infected with *A.baumannii* in a developing country. *A.baumannii* was cultured from 399 sterile sites with a prevalence of 4.3/1000 live births or 22.8/1000 admissions which accounted for 13 % of all culture-confirmed sepsis. 91 % of neonates who cultured positive for *A.baumannii* were preterm with a mean gestational age of 30 weeks and birth weight of 1400 grams. Antimicrobial susceptibility to isolates was 64 % to cephalosporins, 21 % to aminoglycosides and 17 % were extremely drug-resistant, only susceptible to Colistin. [25]

The case fatality rate was found to be 32 %. The use of central venous lines and parental nutrition before the onset of sepsis was high, 36 % and 33 % respectively. [25] The study showed that the most commonly affected system at the onset of infection was the respiratory system(tachypnoea and apnoeas), followed by the gastrointestinal tract (abdominal distension and large gastric aspirates). [25]

South African literature on *A.baumannii* infection in the neonate is limited. A study conducted by Pillay et al was published in 1999. The study investigated an outbreak of multi-drug resistant *Acinetobacter spp.* in the neonatal unit at King Edward Hospital in Durban, South Africa. [26]

Out of a total of 218 neonates, 9 were infected with *A.Baumannii* during the outbreak. The outbreak was characterised by EOS in pre-term babies with an attributable mortality of 22 %. Ribotyping was used to determine the source of the outbreak and it was presumed that colonisation was mainly in suction bottles and catheters in the neonatal admission room. [26]

Five of the infected neonates were successfully treated with ciprofloxacin and amikacin. The outbreak was curtailed by reinforcement of strict infection control measures. [26]

6.1.8 Studies in other countries

A study was conducted by Nazir et al a teaching hospital in Northern India. Prospective data analysis was conducted over one year of all neonates admitted with Acinetobacter sepsis and their antibiotic susceptibility pattern was carried out.

The incidence of *A.baumannii* septicaemia was 13.7 % (49/357). The major symptoms were lethargy and poor feeding. The major signs were tachypnoea, intercostal retractions, and respiratory distress. The major risk factors were low birth weight and prematurity. Of the isolates, 95.9 % were MDR while 93.68 % were resistant to carbapenems. All the strains were noted to be susceptible to Colistin. [27]

A retrospective case-series study was conducted at the Children's Hospital of China Medical University which is a tertiary-level medical centre in central Taiwan with a total of 20 beds. [28]

Neonates with multi-drug resistant *A.baumannii* sepsis were identified from January 2010 till December 2013. A total of 67 isolates from 59 neonatal patients were positive for *A.baumannii*. Of the 67 isolates, 38 were from blood (56.72 %), 16 from sputum (23.88 %), 7 from pus (10.45 %), 2 from CSF (2.99 %) and 1 from pleural fluid (1.49 %). [28]

The mortality rate due to *A.baumannii* sepsis was 20.34 %. Statistically, significant risk factors for mortality were being infected within 7 days of NICU admission, use of umbilical vein catheters, absolute neutrophil count of less than 1500/mm, platelet count of less than 100 000/mm and a delay in initiating adequate antibiotic treatment.[28]

The study concluded that multi-drug resistant *A.baumannii* infection is responsible for a high mortality rate among neonates in the NICU, particularly those neonates with thrombocytopenia or neutropenia.

Strict infection control measures and appropriate choice of antibiotics play an important role in reducing mortality. [28]

In a study done by Mishra A et al in 1998, 79 neonates with reviewed with positive blood cultures for *A.baumannii*. The study was conducted in a level 2 neonatal care unit. [29]

The study aimed to look at the epidemiological and clinical profile of *A.baumannii*. The incidence of *Acinetobacter* sepsis was 11.1/1000 live births. 64.6 % of the babies were at term and 40.5 % had a birth weight of 2500 grams and more. [29] Common signs observed, were that of fever (66%), tachypnoea (68%), poor feeding (58%) and abdominal distension (35%). Hypothermia was found in a small number of babies (11%).

Complications observed were that of meningitis, bleeding manifestations and necrotising enterocolitis. The overall mortality was 13.9 %.[29]

A study conducted by Akter et al in 2011 to determine the sources of *A.baumannii* in an outbreak situation. The study was conducted over 12 months in the NICU of Addin Women's Medical College Hospital which is in Bangladesh, India which is a referral centre for babies born outside the hospital. [30]

A.baumannii was shown to be isolated from incubator door handles, suction tubes and the body surface of neonates in NICU. Those organisms isolated from the suction water and tubing were found to be XDR. [30]

Enforcement of hospital infection control measures was conducted and in the following six months, only 5 *Acinetobacter spp* were isolated from the blood of the neonate. Institutional birth, prematurity and low birth weight were identified as the most common risk factors for *A.Baumannii* infection. [30]

The study concluded that *Acinetobacter spp*. can reach outbreak proportions in a neonatal unit and may involve babies of normal birth weight. Most of the *Acinetobacter spp*. are multidrug-resistant. Colistin is the drug of choice for these multidrug-resistant infections. [30]

6.1.9 Antimicrobial susceptibility

In another study conducted in Indonesia by Tjoa et al, a total of 24 *A.baumannii* isolates were tested for susceptibility to antibiotics. Susceptibility to antibiotics overall was low ranging between 0 % and 21 %. All isolates originating from blood showed multi-drug resistance while of the environmental isolates, 82 % were MDR. The following table shows some of the susceptibility patterns that were obtained from this study. [31]

Antibiotic	**Susceptibility**
Ceftazidime	4 %
Amikacin	21 %
Bactrim	8 %
Meropenem	16 %
Imipenem	16 %
Gentamycin	16 %
Piperacillin-tazobactam	8 %

Table 1: Table showing antibiotic susceptibility patterns according to an Indonesian study, Tjoa et al

7. Problem Statement

From these studies, it is evident that the literature on *A.baumannii* sepsis in neonatal units in South Africa and internationally is limited and no report is available from neonatal units in the Free State. Neonatal sepsis is a major contributor to neonatal mortality. The burden that *A.baumanni* places on the neonatal unit in South African hospitals is not known, further research is needed.

8. Universitas Academic Hospital- Facilities

Universitas Academic Hospital is a tertiary level hospital located in Bloemfontein, Free State. The hospital receives referrals from the Free State, Northern Cape and Lesotho.

The neonatal unit is sub-divided into 3 units- neonatal ICU, neonatal high-care, and neonatal ward. The NICU is a 12-bed unit and consists of medical, surgical and isolation sections. The isolation cubicle can accommodate 2 neonates with culture-proven sepsis or suspected sepsis. The neonatal high care unit is a 16-bed unit but can accommodate up to 20 neonates. There is an isolation section that has a 2-bed capacity which accommodates patients with neonatal sepsis. The neonatal ward serves as a transition unit for neonates > 1200 grams, neonates whose mothers are admitted to maternal high care or too ill to take care of the baby, neonates awaiting specialist consults, eg. neonates with congenital anomalies awaiting genetic review and babies for palliative care as decided by the neonatal team. *A.baumannii* sepsis has been prevalent mainly in the neonatal ICU often with many babies infected at the same time and occasionally in the neonatal high care unit. Many of the culture results were obtained retrospectively after the baby had demised. Seldom are cases with culture-proven sepsis in the neonatal ward. If a neonate is found to be septic in the neonatal ward, he is moved to the isolation section at the high care unit or NICU if required, depending on the clinical condition of the baby.

9. Aims of the Study

This study aims to determine the incidence of *Acinetobacter baumannii* sepsis in premature neonates over 36 months and to describe the clinical profile and antimicrobial susceptibility patterns of these infected neonates.

9.1 Objectives

- To determine the incidence of *Acinetobacter baumannii* sepsis in premature neonates admitted to the neonatal Unit at Universitas Academic Hospital over 36 months.
- To describe the clinical profile of neonates with *A.Baumannii* sepsis- in terms of demographics, clinical presentation and course, laboratory results (WCC, neutrophil and platelet count) and outcome of the neonate(did the neonate die or was the neonate discharged)
- To evaluate the frequency of the known risk factors as described in the literature
- To describe the antibiotic susceptibility profiles of *A.Baumannii* cultured from different sites and look at the common sites isolated

10. Methodology

10.1 Study Design

The study will be a retrospective descriptive study.

10.2 Study Population

The population of this study will consist of premature neonates infected with *A.baumannii* who were admitted to the Neonatal Intensive Care Unit, High Care Unit and Neonatal Ward at Universitas Academic Hospital over 36 months (January 2016-December 2018).

10.3 Sampling and sample size

The researcher will obtain a list of all positive *A.Baumannii* cultures for the period 01 January 2016 to 31 December 2018 from the National Health Laboratory Services. Approval has been granted already by the NHLS for use of this data. The researcher will then go through the patient records of these positive cultures and patients that showed no clinical signs of sepsis, normal infective markers and not started on antibiotics will be excluded from the study. These results will be viewed as possible contaminants. The estimated study size for this study is 80 patients. No sample size calculation is required as this is a retrospective descriptive study. The incidence will be determined using the total number of admissions in the neonatal unit as the denominator (for the period 1 January 2016 to 31 December 2018).

10.4 Inclusion Criteria

- All premature neonates (<37 completed weeks of gestation) with confirmed *A.baumannii* that were either started on antibiotics, or with clinical signs of infection, or raised infection markers (CRP, WCC count) .
-

10.5 Exclusion Criteria

- Patients who acquire *A.baumannii* sepsis after 28 days of life
- Neonates who have positive cultures for A.baumanni but not treated with antibiotics due to the neonate not having clinical signs of sepsis and normal infective markers.
- Neonates with multiple organisms cultured from the same site
- Gestational age of > 37 completed weeks

	Day 1	Day 3	Day 12-14	Day 24-28
White cell count (x 10^9/L or /mm^3)	5-19	4-33.8	7.5-22.1	6.4-15.8
Total neutrophil	2-9	1-23.1	1.8-12.4	0.8-6.1
Platelet count (x10^9/L)	112-380	75-40	110-543	128-600
C-reactive protein (mg/dl)	<1	<1	<1	<1

Table 2 : Haematological values for pre-term infants (5^{th} to 95^{th} centile), adapted from Neonatal Guidelines and drug doses 2015, Alan Horn

10.6 Measurement

The investigator will obtain consent from National Health Laboratory Services (NHLS) regarding use of culture results that were positive for *A.baumannii* from 1 January 2016 to 31 December 2018. These results will be obtained from NHLS Microbiology. Resistance and susceptibility patterns will be captured.

The investigator will then obtain further information on those patients who had positive *A.baumannii* cultures from computerised patient summaries, viz Meditech. Any outstanding information from these computerised summaries can be supplemented by patient statistic forms. These forms are compulsory and completed for every neonate admitted to the unit. The information gathered will be captured on the Redcap system.

The NHLS will be notified before the publication of results/findings are made. The NHLS will be recognised in all publications.

Once all data has been captured, the information will be submitted to the Department of Biostatistics who will assist in extrapolating statistical data.

11. Ethics

Approval for this study will be obtained externally from the Free State Department of Health (DOH) and internally from the Health Sciences Research Ethics Committee of the University of Free State.

Each patient record will be assigned a numerical value and will be reviewed only by the investigator and study supervisor. This retrospective study will be conducted on patient records. All data records will be confidential and depersonalised and thus, no further consent will be sought. Appropriate measures will be taken to ensure the security of all patient data.

12. Statistics

12.1 Pilot study

Once approval has been granted for this study by the Ethics Committee, a pilot study will be conducted by the investigator. The pilot study aims to identify any significant issues with the data capturing that may arise. The first 5 cases to be utilized in this study will serve as the pilot population.

12.2 Data Capturing and Analysis

The data will be stored on a password-protected computer. Only the investigator and study supervisor will have access to the data. The data will be captured daily on a data capture form ***(Appendix A and Appendix B).***

The data will then be captured on the Redcap system and will be sent to the Department of Biostatistics at the University of Free State for analysis. The results will then be summarised as categorical and numerical variables as per the Department of Biostatistics. The researcher will conduct this study over 3 months.

12.3 Value of this Study

On completion of this study, the results will be made available to the relevant departments:

- **The University of Free State, Department of Paediatrics and Child Health**
- **Free State Department of Health**
- **Universitas Academic Hospital**
- **Pelonomi Hospital**

These departments would be able to utilise the data obtained to improve patient management and care in the neonatal setting. Problem areas contributing to *A.baumannii* sepsis can be identified and the relevant interventions and preventative strategies carried out. The clinicians involved with the care of neonates would attain a better understanding of the impact of *A.baumannii* sepsis on the premature baby and its contribution to neonatal morbidity and mortality. It would serve to reiterate the importance of infection control in a neonatal unit and the reinforcement of antibiotic stewardship practices.

With regards to parents whose premature babies become infected with *A.baumannii*, it would allow for the clinician to provide better advice regarding prognosis and outcomes and an ***evidence-based approach*** in counselling these parents.

This project will provide us with a basis for randomised control trials in the future and interventional studies involving infection control strategies.

12.4 Limitations of the Study

There are limitations to this study. Firstly, this is a retrospective study. It is, therefore, possible that the prevalence of *Acinetobacter baumannii* infection in the unit is not a true reflection of the actual prevalence as adequate cultures may not have been taken at the onset of infection with *A.baumannii* and therefore contributing to a lower yield. Taking into consideration that it would be difficult to isolate contaminated specimens from true infection, neonates that were not treated with antibiotics (these are likely neonates who do not have clinical signs of sepsis i.e poor feeding, apnoeas, temperature instability, respiratory distress, vomiting, loose stools, abdominal distension, seizures and jaundice as described in the literature, and have normal infective markers and follow up cultures) will be excluded. The poor technique of taking culture specimens could result in contaminated specimens. Incomplete statistic sheets and incomplete/incorrect information on patient summaries may contribute to the overall accuracy of results attained from this study. Criteria for the exclusion of possible contaminants has been included in the data collection sheet.

13. Time Frame

Protocol and Literature Review Planning	***August 2019-December 2019***
Submission to Ethics Committee	***July 2020***
Submission to the Free State Department of Health	***August 2020***
Pilot study and Data Collection	***September 2020***
Data and Statistical Analysis	***October 2020***
Report Writing and Submission	***November 2020***

14. Budget

All costs incurred by this study will be covered by the investigator conducting the study. The investigator will budget for stationery, paper, the printing of documentation and transport fees. Estimated cost for this study is R1000-00. No additional funding will be required by the investigator.

Item	***Estimated Cost***
Stationery	R200
Printing	R200
Photocopying	R150
Travel Fees	R200
Miscellaneous	R250

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DATA COLLECTION SHEET- APPENDIX A

DEMOGRAPHICS

Patient number	
Gestation at birth (weeks)	
Age of patient at the onset of sepsis (days)	
Sex of Infant	
Birth weight (grams)	

RISK FACTORS BEFORE ONSET OF SEPSIS

1=yes, 2=no, 3=unknown

Use of central venous line	
Administration of TPN	
Thrombocytopenia	
Neutropenia	

CLINICAL PRESENTATION

1=yes, 2=no, 3=unknown

Respiratory distress	
Apnoea	
Abdominal distension	
Fever	
Hypothermia	

LABORATORY RESULTS AFTER ONSET OF SEPSIS

White Cell Count ($x10^9$/L)	
Platelet count ($x10^9$/L)	
C-reactive protein (mg/L)	

ANTIBIOTIC PROFILE

Antibiotic	Sensitive	Resistant	Unknown
Amikacin			
Cefepime			
Ceftazidime			
Ciprofloxacin			
Gentamycin			
Imipenem			
Levofloxacin			
Meropenem			
Piperacillan/tazobactam			
Tigecycline			
Tobramycin			
Colisitin			
Trimethoprim-sulfamethoxazole			

CULTURE SITE

1=yes, 2=no

Site	Result
Blood	
Cerebrospinal Fluid	
Catheter specimen- urine	
Endotracheal Aspirate	

CLINICAL PROGRESSION

1=yes, 2=no, 3=unknown

Infant required catecholamine support after onset of sepsis

Infant required mechanical ventilation after onset of sepsis

Infant required high-frequency oscillatory ventilation after onset of sepsis

OUTCOME OF PATIENT

Infant died =1, Infant discharged=2, unknown=3

EXCLUSION OF POSSIBLE CONTAMINANT (In an infant with a positive A.Baumannii culture who was not started on antibiotics and did not meet both of the following criteria, the result will be considered a contaminant and the infant will be excluded from the study).

1=yes,2=no

Criteria	*Result*
Infant has clinical signs of sepsis	
Raised infection markers (white cell count or C-reactive protein	

APPENDIX B -DATA COLLECTION TO CALCULATE THE INCIDENCE

ADMISSIONS for 1 JANUARY 2016-31 DECEMBER 2016

Month	**Total number of admissions**	**Total number of *A.Baumannii* cases**	**Total number of premature neonates with *A.Baumannii***
January			
February			
March			
April			
May			
June			
July			
August			
September			
October			
November			
December			

ADMISSIONS for 1 JANUARY 2017-31 DECEMBER 2017

Month	**Total number of Admissions**	**Total Number of *A.Baumannii* cases**	**Total number of premature neonates with *A.Baumannii***
January			
February			
March			
April			
May			
June			
July			
August			
September			
October			
November			
December			

ADMISSIONS for 1 JANUARY 2018-31 DECEMBER 2018

Month	**Total number of admissions**	**Total number of *A.Baumannii* cases**	**Total number of premature neonates with *A.Baumannii***
January			
February			
March			
April			
May			
June			
July			
August			
September			
October			
November			
December			