ANTIMICROBIAL SUSCEPTIBILITY ANALYSIS OF GRAM-NEGATIVE ESKAPE ORGANISMS AT INKOSI ALBERT LUTHULI CENTRAL HOSPITAL AND MAHATMA GANDHI MEMORIAL HOSPITAL FROM 2018 TO 2022

by

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DECLARATION OF INDEPENDENT WORK

I, Ayanda Chiliza, student number _____ hereby declare that this dissertation submitted to the Central University of Technology, Free State; for the degree Master of Health Sciences in Biomedical Technology, is my own independent work that has not been submitted before to any institution, by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

Signature: **AP** **Date: 13/08/2024**

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ABSTRACT

Background: Antimicrobial resistance poses a major challenge to the healthcare system globally due to micro-organisms with the ability to produce enzymes such as Extended-Spectrum Beta-Lactamases (ESBL) and carbapenemase. They have been categorised by the World Health Organisation in 2017 as ESKAPE pathogens, namely *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species, to emphasise their importance as global multidrug-resistant priority pathogens.

Aim: This study assessed the prevalence and sensitivity profiles of gram-negative ESKAPE pathogens at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital over a five-year period.

Methods: Retrospective data were retrieved from the National Health Laboratory service through the Academic Affairs and Research Management Systems. The data was for patients infected by the gram-negative ESKAPE pathogens at Inkosi Albert Luthuli Central and Mahatma Gandhi Memorial Hospital, using analysis for five years. The data were analysed using Microsoft 365 Excel, and the results are presented in tables and figures.

Results: A total of 4 781 patient samples from the two hospitals were analysed, of which 27.51% belonged to the 19–30 year age group, with a median average of 42 years. Most were females at 55.33%, and males accounted for 44.34%. A small percentage did not specify their sex. Most pathogens were isolated from urine samples (38.38%) and blood cultures (37.36%). Isolated pathogens were identified as *K. pneumoniae* (57.31%), *A. baumannii complex* (18.74%), *P. aeruginosa* (17.30%) and *E. cloacae complex* (6.66%). Extended-spectrum Beta-Lactamases-producing microorganisms accounted for only 10.73% of all the isolated pathogens. The highest sensitivity was noted in amikacin (>60%) and carbapenem antimicrobial agents, except for *A. baumannii complex*. Tigecycline sensitivity was >50% for all pathogens except for P. aeruginosa and colistin sensitivity >70% for both *P. aeruginosa* and *A. baumannii complex*. *Klebsiella pneumonia*e was found to be the most isolated pathogen from all the gram-negative ESKAPE pathogens.

These results correlate with results obtained in other studies globally, which report the resistance of *K. pneumoniae* as causing major infections in the ICU. All these microorganisms were mostly isolated in urine and blood culture samples, proving invasive infections. This did not agree with reports from Iran, where only 8% of *K. pneumoniae* was noted, but high sensitivity to tigecycline, amikacin and carbapenems. The overall ESBL production for all Enterobacteriaceae was 10.71%.

Conclusion: Over the five-year period, 2018 to 2022, infections caused by ESKAPE pathogens increased, and their resistance to beta-lactam and carbapenem antimicrobial agents also increased. Tigecycline and amikacin are still effective, but close monitoring will be needed to prevent the loss of the last effective antimicrobial agent.

LIST OF ABBREVIATIONS

A. baumannii complex– Acinetobacter baumannii complex

AARMS – Academic Affairs and Research Management Systems

AMR – Antimicrobial resistance

CDW – Central Data Warehouse

CRE – Carbapenem-Resistant Enterobacteriaceae

E. cloacae complex – Enterobacter cloacae complex

E. coli – Escherichia coli

ENTER – Enterobacteriaceae

ESBL – Extended-Spectrum Beta-Lactamase

ESKAPE – *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacter spp.

HAI – Hospital Acquired Infection

IALCH – Inkosi Albert Luthuli Central Hospital

ICU – Intensive Care Unit

IMP – Imipenem Metallo-beta-lactamase

K. pneumoniae – Klebsiella pneumoniae

MBL – Metallo-Beta-Lactamases

MDR – Multidrug-Resistant

MGMH – Mahatma Gandhi Memorial Hospital

NDM-1 – New Delhi Metallo-beta-lactamase

NFGNB – Non-Fermenting Gram-negative Bacteria

NHLS – National Health Laboratory Service

OXA – Oxacillin hydrolysing Enzymes

P. aeruginosa – *Pseudomonas aeruginosa*

Post-COVID-19 – Period after COVID-19

Pre-COVID-19 – Period before COVID-19

SA – South Africa

STAPH – Staphylococcus species

UTI – Urinary Tract Infection

VIM – Verona Integron encoded Metallo-beta-lactamase

WHO – World Health Organisation

CHAPTER 1: INTRODUCTION

Bacterial resistance to antimicrobial agents poses a treatment challenge and has emerged as a major cause of morbidity and mortality worldwide (Spellberg and Gilbert, 2014). These organisms, which primarily populate hospital areas, are commonly isolated from the urinary tract, gastrointestinal tract and respiratory tract (Paterson, 2006). For bacteria to survive the effects of antibiotics, they are constantly finding new defence mechanisms, "and this has been the case for almost eight decades" (Christaki *et al*., 2020). The bacteria that produce Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases are members of the Enterobacteriaceae (ENTER) family, characterised as Gram-negative organisms.

Beta-lactamases are enzymes causing resistance to beta-lactam antibiotics. They are classified using two main systems, the Ambler and the Bush-Jacoby-Medeiros system, which classifies the most clinically significant beta-lactamases as those produced by Gram-negative bacteria (Bush and Jacoby, 2010). Similarly, carbapenemases are also members of the molecular classes A, B and D, which are used to classify the carbapenemase enzymes produced by these organisms (Kim and Eom, 2021). Ambler class A enzymes include penicillinase, cephalosporinase, broad-spectrum beta-lactamases, ESBLs, and carbapenemases. They can be inhibited by beta-lactamase inhibitors, such as clavulanic acid, sulbactam, or tazobactam (Rice, 2010). Extended-Spectrum Beta-Lactamase class A enzymes include TEM, SHV, and CTX-M types. The TEM-92, SHV-12, and CTX-M-2 and 43 have been described in *Acinetobacter baumannii* (*A. baumannii*) in Italy, China, Japan and Bolivia, respectively (Almasaudi, 2018). Class A carbapenemase can be segregated into different groups, namely KPC, GES, SME, and NMC-A enzymes (Vrancianu *et al*., 2020). They are all produced by certain species of the Gram-negative ESKAPE pathogens, which are members of the ENTER that are regarded as highly virulent and multidrug-resistant (MDR). They include *Enterobacter species, Pseudomonas aeruginosa* (*P. aeruginosa)*, *Acinetobacter baumannii* complex (*A. baumannii*), and *Klebsiella pneumoniae* (*K. pneumoniae*) (Dzidic and Kos, 2008).

Ambler class B are Metallo-Beta-Lactamases (MBL), most commonly, Imipenem Metallo-beta-lactamase (IMP), mostly detected in *P. aeruginosa, A. baumannii, K.*

pneumoniae and *Enterobacter spp*. Verona Integron encoded Metallo-beta-lactamase (VIM) detected in *P. aeruginosa* and *A. baumannii,* the New Delhi Metallo-beta-lactamase (NDM-1) detected in *K. pneumoniae* and *Enterobacter cloacae* (Rice, 2010; Kumarasamy *et al.,* 2010).

Ambler class C includes the AmpC beta-lactamase, which inactivates aztreonam. It is identified in *P. aeruginosa* and *Enterobacter spp* (Bush and Jacoby, 2010). Oxacillin hydrolysing enzymes (OXA) and their members are usually identified in *P. aeruginosa* and belong to the Ambler Class D (Hall *et al*., 1993; Danel *et al*., 1995). OXA type carbapenemases are also found in *A. baumannii* (Thomson and Bonomo, 2005).

Beta-lactamases and most carbapenemase enzymes can hydrolyse antibiotics and render them ineffective against bacterial infections (Tewari *et al*., 2019). Severe infections brought on by carbapenem-resistant Enterobacteriaceae (CRE) have been linked to high fatality rates, typically surpassing 40% (Tzouvelekis *et al*., 2012). *Klebsiella pneumoniae* carbapenemases were first identified in 2001. Since then, genes encoding beta-lactamases have spread among several Gram-negative bacteria (Queenan, Jenkins and Bush, 2001).

Previous studies in the United States have shown that the most prevalent ESBL-producing organisms are *Escherichia coli* (*E. coli*), *Klebsiella*, *Pseudomonas* and *Acinetobacter* (Bradford, 2001; Alekshun and Levy, 2007). These organisms are usually acquired in hospitals, causing infections such as urinary tract infections (UTIs), wounds, and respiratory infections (Shakibaie *et al*., 2014). Among these infections, UTIs are the most common hospital-acquired infections (HAIs), accounting for 40% of nosocomial infections (Kalsi *et al*., 2003; Shakibaie *et al*., 2014).

Treatment of these infections was first done using cephalosporins and penicillins. However, in Saudi Arabia in 2016, >70% of *K. pneumoniae* were resistant to ampicillin, which proved to be ineffective against ESBL-producing bacteria (Al-Wutayd *et al*., 2018). Beta-lactamase resistance to antimicrobial agents began in the early 1940s, even before the first beta-lactam penicillin was developed (Abraham and Chain, 1940). Therefore, new beta-lactam agents like carbapenems have been designed to hydrolyse the beta-lactam ring of beta-lactamases and carbapenemases/ oxacillinases. Still, with every class of antimicrobial agent developed, new hydrolysing

enzymes also emerged (Bradford, 2001). These enzymes cause resistance to that specific class of beta-lactam agents, probably due to the use and/or misuse of these antibiotics. Hence, the cephalosporins discovered in the 1980s were not effective in 1983 (Knothe *et al*., 1983; Bradford, 2001; Queenan and Bush, 2007).

The first-generation cephalosporins, including cephalexin and cephalothin, have moderate activity against several Enterobacteriaceae, such as *E. coli* and *Klebsiella* species (Sykes and Papich, 2013). The second- and third-generation cephalosporins include cefoxitin, cefuroxime, cefixime, ceftriaxone, cefotaxime and ceftazidime being the most effective against *P. aeruginosa* (Balsalobre *et al*., 2019). The fourth-generation cephalosporins include cefepime and cefpirome, which have remarkable stability against plasmid-mediated beta-lactamases (Bush and Bradford, 2016). However, in a study done in Saudi Arabia 2016 to evaluate the antimicrobial susceptibility of isolates in the intensive care unit (ICU), *K. pneumoniae* was found to be 87% to 96% resistant to ceftazidime, cefuroxime and cefotaxime (Azim *et al*., 2019). Europe also reported 15.7% resistance to third- and fourth-generation cephalosporins (Boattini *et al*., 2024).

Furthermore, the organisms started becoming resistant to the current choice of antibiotics, such as ertapenem, imipenem, meropenem and doripenem, which all belong to the carbapenem class (Balsalobre *et al*., 2019). Resistance rates of 6.5% to 31.4% to carbapenems were reported in Saudi Arabia, and only 8.6% towards meropenem (Al-Wutayd *et al*., 2018; Azim *et al*., 2019). Over the years, resistance has increased to 23% for *K. pneumoniae* and 75.6% for *A. baumannii* towards imipenem, including a 23.8% *P. aeruginosa* resistance towards meropenem, as reported in China (Yang *et al*., 2023). As a result, in 2017, the World Health Organisation (WHO) created a list of global MDR priority pathogens defined by the Infectious Diseases Society of America as ESKAPE bacteria, *Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, A. baumannii*, *P. aeruginosa* and Enterobacter spp (WHO, 2017).

These bacteria included the Gram-negative bacteria *K. pneumoniae, A.baumannii*, *P. aeruginosa* and Enterobacter spp., and when they are resistant to three or more antimicrobial agents, it is called MDR (Pendleton *et al*., 2013). A resistance study done in KwaZulu-Natal in 2011–2015 from 64 502 isolated organisms reported a high ESBL resistance in *K. pneumoniae,* accountable for 65%, while carbapenem resistance

increased from 4% in 2014 to 16% in 2015, and multidrug resistance in *K. pneumoniae, w*hile in *A. baumannii* numbers were as high as 70% (Ramsamy *et al*., 2018). It is, therefore, of the utmost importance to monitor resistance in these organisms to practice effective antimicrobial stewardship.

1.1 Research problem

Extended-Spectrum Beta-Lactamases-producing bacteria and carbapenemase-producing bacteria have become a worldwide health issue because of their ability to form resistance against different antimicrobial agents, deeming them challenging to treat. Extended-Spectrum Beta-Lactamase-producing bacteria and CRE have been recognised as the leading causes of HAIs, such as UTIs and respiratory tract infections, since the 1980s and continue to evolve rapidly. Therefore, it necessitates vigilant surveillance. During the COVID-19 pandemic, patients with compromised immune systems were hospitalised for extended periods, raising the likelihood of increased resistance development against beta-lactam and carbapenem antibiotics. This could be due to the increased use of broad spectrum antimicrobial agents during the pandemic and the increased capacity of patients who could be harbouring different stains of resistant microorganisms.

1.2 Aim

To determine the prevalence and sensitivity profiling of ESKAPE pathogens at the Inkosi Albert Luthuli Central Hospital and the Mahatma Gandhi Memorial Hospital from 2018 to 2022.

1.3 Objectives

- Identify the Enterobacteriaceae organisms producing ESBLs and carbapenemases using data from the National Health Laboratory Service (NHLS) for KwaZulu-Natal Mahatma Gandhi Memorial Hospital (MGMH) and Inkosi Albert Luthuli Central Hospital obtained (IALCH) situated In KZN Bellair Durban.

- Determine the number of ESBL- and carbapenemase-producing organisms isolated in clinical settings using data from the NHLS Central Data Warehouse (CDW).
- Determine antimicrobial sensitivity profiles using data from CDW.

1.4 Hypothesis

Infections caused by ESKAPE organisms have increased in both IALCH and MGMH, with high resistance to beta-lactam and carbapenem antibiotics.

CHAPTER 2: LITERATURE REVIEW

Bacterial resistance to antimicrobial agents has been an ongoing issue causing major difficulties in infection treatment and control (Spellberg and Gilbert, 2014). Antimicrobial agents are the primary treatment of fatal bacterial infections, however, their use and inappropriate distribution has led to increased resistance, creating a global health emergency (Lin *et al*., 2003; Tumbarello *et al*., 2007).

Extended-Spectrum Beta-Lactamases and carbapenemase/oxacillinase-producing organisms continue to be a global challenge to the health sector, with an increase in the antimicrobial resistance to the conventional drugs used in the treatment of infections (Paterson and Bonomo, 2005). Since the 1950s, diseases caused by Enterobacteriaceae have been treated with beta-lactam antibiotics. However, the introduction to broad-spectrum, third-generation cephalosporins, ESBLs, and carbapenems caused resistance to emerge in the family of ENTER, particularly *E. coli*, *Klebsiella spp*, *Pseudomonas* and *Acinetobacter* Gram-negative organisms (Korzeniewska *et al*., 2013).

The first plasmid-mediated beta-lactamase was described in the early 1960s, isolated from a blood culture and named TEM 1 after Temonicra in Greece, capable of hydrolysing ampicillin (Bradford, 2001). In the late 1980s, carbapenems were introduced, but resistance emerged in Japan in 1990 (Osano *et al*., 1994). The SHV-1 beta-lactamase was found in *Klebsiella* and *E. coli* species in the 1980s and led to the introduction of the third generation of cephalosporins (Deepthi and Deepti, 2010). In Germany, the SHV-2 beta-lactam enzyme with several mutants from TEM and SHV beta-lactamases, showed resistance to cephalosporins (Knothe *et al*., 1983; Livermore and Paterson, 2006). These organisms have shown an alarming level of antimicrobial resistance (AMR) to ESBL and carbapenems in clinical settings (Manandhar *et al*., 2020).

2.1 Mechanisms of action

Extended-Spectrum Beta-Lactamases can inactivate beta-lactam antimicrobials by binding to their carbonyl group and hydrolysing the beta-lactam ring, thus enabling the

organisms' resistance (Tewari *et al*., 2019). These enzymes can also hydrolyse penicillins, first-, second-, and third-generation of cephalosporins, monobactams and carbapenems (Pitout *et al*., 2005). Three mechanisms, including enzyme production (carbapenemase), efflux pumps, and porin mutation, can lead to carbapenem resistance. Enzyme production is the primary resistance mechanism (Suay-Garcia and Gracia, 2019). Most beta-lactam compounds, including carbapenems, are hydrolysed by carbapenemases, which are frequently encoded by mobile genetic components that are simple to transfer between bacteria, aiding in the rapid spread of resistance (Suay-Garcia and Gracia, 2019). Structurally, the carbapenemase mode of action is initiated by penetrating the bacterial cell wall, binding to penicillin-binding proteins and altering the cell membrane porin channels, reducing permeability and thus preventing beta-lactams from reaching their targets and acquiring resistance (Gasink *et al*., 2009).

2.2 Transmission of resistant organisms

There are several ways that AMR organisms can be transmitted among people in hospitals and communities (Hagel *et al*., 2019). These organisms are said to overspread mostly in hospital settings and are spread easily by healthcare professionals who regularly come into contact with contaminated surfaces such as bed bars, sinks and bed remotes (Guet-Revillet, 2012; Tschudin *et al*., 2017). The spread of ESBL can occur by simply touching someone or leaving the bacteria on surfaces other people touch (Hagel *et al*., 2019). Colonisation can also spread ESBL bacteria, where an individual becomes infected by interacting with an asymptomatic carrier (Schaufler *et al*., 2016).

The spread of these AMR organisms is primarily associated with international clonal lineages CC1, CC2 and CC3 (Lee *et al*., 2012). Most ESBLs in *P. aeruginosa* are VEB and PER types, and they readily mutate to become resistant to carbapenems by inactivating the OprD porin, a family of proteins in the outer membrane of Gram-negative bacteria (Bush and Jacoby, 2010; Bahr *et al*., 2021). In a study in Israel to distinguish the transmission route of different ESBL-*E.coli* clones, out of 125 patients, 52 were colonised with ESBL-*E. coli* on admission, 59 acquired it during their hospital stay, and for 14 patients, the source was undetermined (Adler *et al*., 2012). A higher

increase in nosocomial-acquired ESBL-*E. coli* (2.5%) was reported compared to community-acquired (1.6%) in Sweden from 2004 to 2008 (Helldal *et al*., 2013).

2.3 Predisposing factors

Several risk factors contribute to infections with ESBL-producing organisms, including prolonged use of antibiotics, recent invasive procedures, anaemia, ICU stay, advanced age, immune suppression and permanent urinary catheters (Mendelson, 2015). This poses an issue in the health sector as these ESBL-producing organisms seem to have or form defence mechanisms against commonly used antimicrobial agents (About ESBL-producing Enterobacterales | ESBL-producing Enterobacterales | CDC). Almost all human diseases are sexually dimorphic with regard to prevalence, severity and cause of infection, although genetic, immunity and social roles also contribute to the differences in disease risk (Ober *et al*., 2008).

Acquiring infections such as viral, fungal and bacterial leading to individuals being hospitalised can be influenced by several factors such as sex inequality, social norms, occupational activities, social roles, lifestyle changes and access to healthcare (Fowler *et al*., 2007; Dias *et al*., 2022). Sex inequality plays a vital contribution to social and health outcomes, which include but are not limited to violence against women, men being breadwinners, and women as caregivers, leading to stress and the risk of being unable to fight infections (Shannon *et al*., 2019). Sex also influences susceptibility to infections, immune response, pathophysiology, and clinical presentation as they tend to differ in males and females, respectively (Giefing-Kröll *et al*., 2015).

Several studies have been conducted to assess the influence of sex differences in hospitalised patients. Galligan and Fish note that the females immune system has an increased ability to detect pathogens compared to males due to the expression of pathogen-associated molecular receptors when compared to males (Galligan and Fish, 2015). Females have also been reported to possess a stronger innate and adaptive response than males, which allows better pathogen elimination and response to vaccination, but consequently makes them more prone to inflammatory autoimmune diseases (Klein and Flanagan, 2016). Furthermore, females are said to have higher

neutrophil counts, which are the first immune cells that respond to infections caused by micro-organisms (Bain and England, 1975).

Age also contributes vastly to the ability of an individual's capabilities to fight infections leading to high hospitalisation rates in the older population as compared to the younger population of female patients (Pandey *et al*., 2017). It has been noted that the median age of hospitalisation reported in China in 2019 was 47 years, while in 2020, the median age of deceased patients was 68 years when compared to a median age of 51 years of male patients who recovered from the COVID-19 (Wan *et al*., 2020; Chen *et al*., 2020b). On the contrary, a 56% hospitalisation rate for various diseases for females in the age range 15 to 59 years, which includes the younger age group (Naser *et al*., 2023). Furthermore, the influence of sex and age on the infectivity remains an area of active investigation (Kopel *et al*., 2020).

2.4 Epidemiology

2.4.1 Global reporting of ESBLs and carbapenems since 2004

Antimicrobial resistance is still a major concern globally, and to try and keep up with the increasing resistance patterns caused by the Gram-negative ENTER, different countries worldwide are constantly under surveillance to minimise antimicrobial stewardship. In this case, infection control measures can be enhanced, and all countries will have an insight into the priority pathogens and how they are constantly acquiring resistance.

A study, “Tigecycline Evaluation and Surveillance Trial or TEST”, done for the global surveillance database showed the rate of ESBL production was highest among *K. pneumoniae* isolates collected in Latin America, followed by Asia/Pacific Rim, Europe and North America (Reinert *et al*., 2007). India, with 72%, had the highest ESBL rate, followed by Mexico, with 71.4% (Reinert *et al*., 2007). The prevalence of ESBL among *E. coli* isolates was observed in the exact order between geographical regions but with lower rates compared to *K. pneumoniae* (Reinert *et al*., 2007). A study done in South India in 2004 reported 69% *E. coli* and 73% *K. pneumoniae* to be ESBL-producers. However, another study in India (Coimbatore) reported 41% *E. coli* and 40% *K. pneumoniae* as ESBL-producers (Babypadmini and Appalaraju, 2004; Singhal *et al*.,

2005). Furthermore, according to a study done in Israel, the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was expectedly high in long-term care facilities (Mendelson *et al*., 2005).

A study done in the UK to test for ESBL production revealed that of 213 urine, blood and exudate specimens, 69% of the isolates were ESBL-producers. Of the 117 isolates from exudate specimens tested, 78 (66.7%) were ESBL positive, 69% were *E. coli* and 73% *were K. pneumoniae*. Out of 92 isolates from urine samples, 69 (75%) were ESBL positive and out of 4 isolates from blood samples, 3 were ESBL positive (Umadevi *et al*., 2011). Mexico in 2013 reported 36.9% of *K. pneumoniae* as ESBL-producers (Llaca-Diaz *et al.,* 2013). Twenty percent of isolated *E. coli* produced detectable ESBL enzymes in Iran from 2011 to 2012 (Shakibaie *et al*., 2014). At the Nepali tertiary care hospital from 2012 to 2018, out of 2 153 isolates, 719 were *E. coli,* 532 *Klebsiella spp*, 520 *Enterobacter*, and 383 *Acinetobacter spp*, with half of these species being ESBL-positive (Manandhar *et al*., 2020).

A study done in KZN from 2011 to 2015 revealed an increased ESBL from 54% to 65%, and a study done in Tygerberg Cape Town reported 73.8% of *Klebsiella* species being ESBL positive (Ramsamy *et al.,* 2018; Reddy *et al*., 2021). Crichton conducted the same study in a rural hospital (Khayelitsha District Hospital) and noted a high rate of ESBL-producing *K. pneumoniae* (100%) (Crichton *et al.,* 2018). A study in Pretoria, South Africa (SA) in 2018 showed that 39 patients in ICU for more than five days were ESBL positive, with the majority due to *K. pneumoniae* (Fourie *et al*., 2018). On the contrary, a retrospective review done in an academic complex hospital in Durban, South Africa, documented a decrease in ESBL production, even though the multidrug-resistant organisms included ESBL-producing *K. pneumoniae* (7.6%) and *A. baumannii* complex (7.0%) (Pillay *et al.,* 2021).

2.4.2 Isolation trends of ESKAPE pathogens

To assess the trends for the isolation rates of ESKAPE pathogens, several studies were conducted worldwide, and Iran reported 8% *K. pneumoniae* and 1.81% Enterobacter species isolated from 2011 to 2012 (Shakibaie *et al*., 2014). In a 10-year resistance trend study done in Saudi Arabia on pathogens causing HAI, Gram-negative pathogens accounted for 63% of the infections, with specifically the *Klebsiella*

infection rate higher than *Acinetobacter* (Balkhy *et al.,* 2020). On the contrary, China reported a rather less isolation rate for *K. pneumoniae* (26.2%), being the second-most isolated organism in 2021 (Yang *et al*., 2023). Similarly, in a recent European study, *Enterobacter cloacae* complex (44.8%) was the most prevalent organism isolated (Boattini *et al*., 2024).

Birru found that out of 225 blood samples, only 22 had confirmed bloodstream infections, with 40.9% caused by Gram-negative bacteria in a general South Ethiopia hospital (Birru *et al*., 2021). Contrary to the worldwide study by Shakibaie and team (2014), 78% of *K. pneumoniae* was obtained in a study conducted in South Africa from 2010 to 2012, with 70% of carbapenem-resistant Enterobacteriaceae found to be hospital acquired, with a 38% mortality rate (Perovic *et al.,* 2014). *Klebsiella* species are among the leading causes of sepsis in hospital cases, and *K. pneumoniae* was the most common isolate with 77%, and only 10% *E. coli* found in 36 of the patients on invasive ventilator support with 44% mortality rate in a private hospital in Pretoria from 2013 to 2014 (Fourie *et al*., 2018; Sorsa *et al*., 2019).

2.4.3 Trends in antimicrobial agents

Several studies have been conducted to assess the changes in resistance of ESBLs to different antimicrobial agents used as treatment. A descriptive study done in Saudi Arabia showed that ciprofloxacin, norfloxacin, nalidixic acid, aztreonam, amikacin and tobramycin have great efficacy with more than 83% sensitivity. Only imipenem had 100% sensitivity against *K. pneumoniae* (Ahmad *et al*., 2009). A study by Chaudhary and colleagues (2010) in India showed high resistance to amoxicillin plus clavulanic acid with piperacillin plus tazobactam. However, ESBL-producing strains showed considerable resistance to imipenem with cilastatin (Chaudhary *et al*., 2010). There was sensitivity to elores, colistin and tigecycline with decreased sensitivity against carbapenems, although colistin is regarded as the last resort antibiotic (Falagas *et al*., 2005; Chaudhary *et al*., 2010). However, according to Falagas and Karageorgopoulos (2009), carbapenems were regarded as the antibiotics of choice in treating infections caused by ESBL-producing bacteria. At the same time, a study in the United States indicated that amikacin is the most effective drug against ESBL-producing uropathogens after carbapenems (Falagas and Karageorgopoulos, 2009). To prove

the outcome of amikacin in treating UTI caused by ESBL-producing *E. coli*, an 88.9% cure rate and 100% susceptibility rate were reported in the United Arab Emirates (Al-Zahrouni *et al.,* 2008; Cho *et al*., 2016).

According to Coetzee and Brink, the utilisation of ertapenem, meropenem and imipenem in the private sector in SA more than doubled between January 2009 and June 2011 due to the increased resistance noted in ESBL-producing pathogens (Coetzee and Brink, 2011). However, the emergence of carbapenem resistance was still low in SA (Coetzee and Brink, 2011). Africa as a developing country has not yet illustrated the antimicrobial problem adequately due to insufficient financial resources and a lack of antimicrobial agents in several regions, leaving common infections untreated, reports Tansarli *et al*. (2014). They go on to state that the prevention of infections in Africa is necessary for healthcare systems (Tansarli *et al*., 2014). In 2014, sensitivity to amikacin, imipenem, and meropenem in Iran was reported as 20% of isolated *E. coli* (Shakibaie *et al*., 2014). Also, in 2017, amikacin and imipenem were found to be the most active antibiotics against ESBL-positive *K. pneumoniae* in Asia, while other Gram-negative ESKAPE organisms demonstrated a reduced susceptibility to commonly prescribed third-generation cephalosporins (Yang *et al.,* 2017). A study done in KwaZulu-Natal, South Africa, revealed carbapenem resistance to be 6% between 2011 and 2014, but it increased to 16% in 2015 (Ramsamy *et al.,* 2018). The World Health Organisation (WHO) then launched the Global Antimicrobial Resistance Surveillance System (GLASS) in 2015 as an action plan against antimicrobial resistance (WHO, 2018). This included resistance against penicillins, third- and fourth-generation cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, tetracyclines, polymyxins, macrolides and co-trimoxazole (WHO, 2018).

ESKAPE organisms have shown an enormous change in antimicrobial resistance to most antibiotics, including vancomycin, penicillin, cefazolin, ceftriaxone and piperacillin/tazobactam (Marturano and Lowery, 2019). However, SA has shown low occurrence of Gram-negative pathogens being resistant to vancomycin, while private and public sectors revealed an increased carbapenem resistance against these pathogens (Ismail *et al.,* 2019; Mogokotleng *et al*., 2022).

Increased resistance over the years has spread to carbapenems, bactericidal beta-lactam antimicrobial agents with an extremely broad spectrum, presumed to be the

last resort antimicrobial treatment against ESBL-producing pathogens (Mills and Lee, 2019). A study done in the US showed 65 isolates out of 322 in wastewater were imipenem-resistant *E. coli,* which poses an environmental risk of dissemination to humans (Hoelle *et al*., 2019). However, Saudi Arabia noted a higher carbapenem resistance in *Acinetobacter* and *Pseudomonas* (Balkhy *et al.,* 2020). China reported *K. pneumoniae* to have a higher resistance rate of 23.4% to cefotaxime, 7.7% and 7.6% to imipenem and meropenem, respectively, an average resistance of 17.4% to gentamicin and 6.6% to amikacin (Yang *et al*., 2023). Furthermore, ciprofloxacin has recently been reported to have a much higher resistance rate (23.3%) in China (Yang *et al*., 2023). *Enterobacter cloacae* complex accounted for a 15.7% resistance rate to third- and fourth-generation cephalosporins and carbapenems (Boattini *et al*., 2024). Gram-negatives were resistant to third-generation cephalosporins, with increased susceptibility to ciprofloxacin and amikacin, respectively, in a study done in Southeast Ethiopia (Sorsa *et al.,* 2019). However, in 2021, in a study done in Ethiopia, it was found that 40.9% of Gram-negative isolates were sensitive to meropenem (Birru *et al*., 2021).

In a study conducted in Tygerberg, *A. baumannii* had low susceptibility to aminoglycosides, carbapenems and cephalosporins, while a retrospective review done in IALCH drew a conclusion that there were high resistance rates to first- and second-line antimicrobial agents (Reddy *et al*., 2021; Pillay *et al.,* 2021). However, meropenem with or without vancomycin still provides optimal susceptibility, but requires ongoing surveillance (Pillay *et al.,* 2021).

A study done in 2020 at tertiary academic hospitals in South Africa also indicated an increased resistance in CRE-causing bacteraemia, with 76.8% carbapenem-hydrolysing oxacillinase, followed by NDM 21.1% and lastly VIM with 1.3% (Lowe *et al*., 2022). As a result, this study will determine the prevalence of ESBLs over the past five years, which included before and during the pandemic of COVID-19, and possibly recommend detecting ESBL using the double-disc synergy test (DDST) as a screening routine test in all medical microbiology laboratories and to also further enhance infection control measures to decrease the resistance rate of Gram-negative ESKAPE pathogens.

CHAPTER 3: METHODOLOGY

3.1 Study location

The study was conducted with data from Mahatma Gandhi Memorial Hospital at the NHLS in KwaZulu-Natal Durban Phoenix and Inkosi Albert Luthuli Central Hospital NHLS situated in KZN Bellair Durban.

3.2 Research design

This is a retrospective, quantitative study where a systemic investigation was conducted on quantifiable data and statistical techniques performed with existing data. Data were obtained from the NHLS CDW for samples processed on the Vitek®2 automated system (bioMérieux, Marcy l'Etoile, France).

3.3 Population and sample

The data used do not include patient personal information, except for sex and age. It includes tests performed, antibiotics tested in the laboratory for susceptibility, and the site of the laboratory where testing was performed. Permission to use such data was obtained from the NHLS by applying to the Academic Affairs and Research Management System (AARMS). The data in all NHLS laboratories are stored in the laboratory information system referred to as LIS and controlled at the CDW, where the data were extracted.

Random sampling was done to minimise selection bias and to have an equal distribution of all characteristics. The method of randomisation that was used is stratified randomisation, where data were stratified in terms of sample type and then type of infection. A total of 4 782 samples were analysed.

3.4 Inclusion criteria

All specimens from patients aged 18 and above admitted at IALCH and MGMH. Immunocompromised patients were included. Duplicates were also included if a different diagnosis was confirmed for the same patient to assess if the infection was

hospital acquired. Only urine, sputum, stool, wound and blood culture samples were included. The length of stay in the hospital did not influence the inclusion criteria.

3.5 Exclusion criteria

The non-ESKAPE organisms and contaminants were excluded. All outpatients were excluded.

3.6 Lab analysis methodology

The diagnostic laboratory isolated the organisms using the following methods of detection, described briefly in sections 3.6.1 to 3.6.3. Data of results from these analysis methods were then captured on the LIS and stored in CDW.

3.6.1 Double-disk diffusion

An overnight suspension of the cultured isolate adjusted to 0.5 McFarland standards is inoculated using a cotton swab on a Mueller Hinton agar plate. A disk of cefotaxime 30 µg and cefotaxime-clavulanic acid 30 µg/10 µg are placed 20 mm apart. The zone diameter is measured between the two disks, and >/= 5 mm zone is interpreted as ESBL production.

3.6.2 Vitek analyser

A pure culture or well-isolated colonies of the organism are used. On a labelled test tube, 3 ml of saline is dispensed and using an inoculating swab, a few colonies of the test organism are removed and carefully emulsified in the saline. The suspension tube is then placed into the Densichek, rotated through 360˚, and the reading should be adjusted to a 0.5 McFarland standard. The suspension tube (ID tube) is placed into the cassette alongside an empty tube to test for sensitivity. The isolate data are entered on the Vitek®2 automated system (bioMérieux, Marcy l'Etoile, France) analyser, and the ID and sensitivity card are scanned. The cards are placed into the corresponding slots on the cassette with the suspension or the blank tube and loaded into the Vitek®2 analyser. Results are generated after four to six hours for the ID and 24 hours for the sensitivity where, if an organism is ESBL positive, the Vitek®2

analyser would state that the organism is a beta-lactamase producer and populate the sensitivity and resistance of different antibiotics of choice.

3.6.3 Chromogenic cephalosporin method

The reagent used is Nitrocefin, which is reconstituted by adding one vial of rehydration fluid, since it comes lyophilised. A drop of Nitrocefin reagent ID is placed on a strip of filter paper on a dry glass slide. Using an applicator stick, one to three colonies fresh from subculture are swabbed onto the paper strip, and a colour change is observed. No colour change indicates negative for ESBL, and a colourless to red/pink colour indicates Beta-Lactamase production.

3.7 Data management plan

3.7.1 Data collection

This research included data from 1 January 2018 to 31 December 2022. The requested data were for patients in the MGMH and IALCH hospitals with infections caused by Gram-negative ESKAPE organisms between January 2018 and December 2022. Documented NHLS laboratory results recorded for the antimicrobial sensitivities were received.

3.7.2 Documentation and metadata

Data were received in CSV file format to ensure integrity and stored in a double password-protected file/computer on the Figshare storage system as original. Figshare is a secure web application that POPIA security policies require. It is password protected, and passwords are unique for each investigator. The analysis of original data deidentification was done in Excel files that were password protected and completely deidentified and stored with a reference to a folder different from that with the deidentified and pseudo-anonymous information. The data were stored in a different file that does not have the metadata of the original study data and identifier. The anonymous data were analysed in Excel to create documents with my initials as principal investigator, the date created, and protected with passwords.

3.7.3 Data analysis

Statistical data analysis done by the researcher and presented in the form of figures and tables. Microsoft 365 Excel was used to analyse the data and determine the significance of the results by calculating p-values using the T.TEST method. The results use different graphs and tables illustrating the difference in infection types between specimen types versus infections, ages, sex and hospitals, as well as the different antibiotic profiles for the different antibiotics of each of the Gram-negative ESKAPE organisms. Data did not indicate the immune status of the patients and thus were not considered in this study.

3.7.4 Data storage

This research is low risk, but certain measures have been put in place to protect original personal information in accordance with the Protection of Personal Information Act (POPIA) (POPIA, 2013). Data have been anonymised and de-identified before being shared among the research team. Data are stored on Figshare, which is POPIA-approved to store all research data securely on the cloud and enable sharing of data privately among the research team. The device is scanned extensively for malware using reputable antivirus software, and regular backups are performed to ensure the safe storage of data.

3.7.5 Ethics clearance

Permission to use NHLS data was obtained via AARMS (PR2232466-see **Appendix A**) from CDW, ethical approval was obtained from the Health Science Research Ethics Committee (UFS-HSD2023/0400/2908-see **Appendix B**). Since NHLS is the data owner, no approval from the KwaZulu-Natal Department of Health was needed. Approval from the Central University of Technology (CUT) (FHES 3/13/03-see **Appendix C**) was also obtained.

According to POPIA, for researchers, any research involving human participants requires ethical approval from a recognised or constituted research ethics committee, and that information is to be protected according to sections 14, 15 and 17 of the

National Health Act 61 of 2003 (RSA, 2003) with regard to confidentiality, access to health records and protection of health records.

Data supplied by the NHLS were used ethically and solely for the purpose of this research, and confidentiality measures are maintained at a participant and institutional level with no disclosure of personal or confidential information as described by the NHLS policy and the POPIA Act (POPIA, 2013).

The data were deidentified to avoid traceability to any patient. Only the information required for the research was supplied. The data used in this study are from routine clinical care investigations, and no further investigations were required.

3.7.6 Data sharing and accountability

Data will not be shared with anyone else outside the research group. The primary investigator is responsible for storing and protecting the research data according to the POPIA Act (POPIA, 2013). The principal investigator will be liable for any non-compliance.

Data will be stored for five years and thereafter destroyed. If the principal investigator leaves the NHLS/CUT, data and files under my protection will be deleted, and final control will be with the supervisor/CUT or an employee appointed if the supervisor is no longer available.

CHAPTER 4: RESULTS

4.1 Demographic profile of patients presenting to the hospital from 2018 to 2022

A total of 4 782 isolates identified at the two hospitals, IALCH and MGMH, were analysed, and the results are presented in this chapter. Demographic profiles of patients presenting to healthcare facilities IALCH and MGMH according to age, sex and facility are presented between Figure 1 to 2.

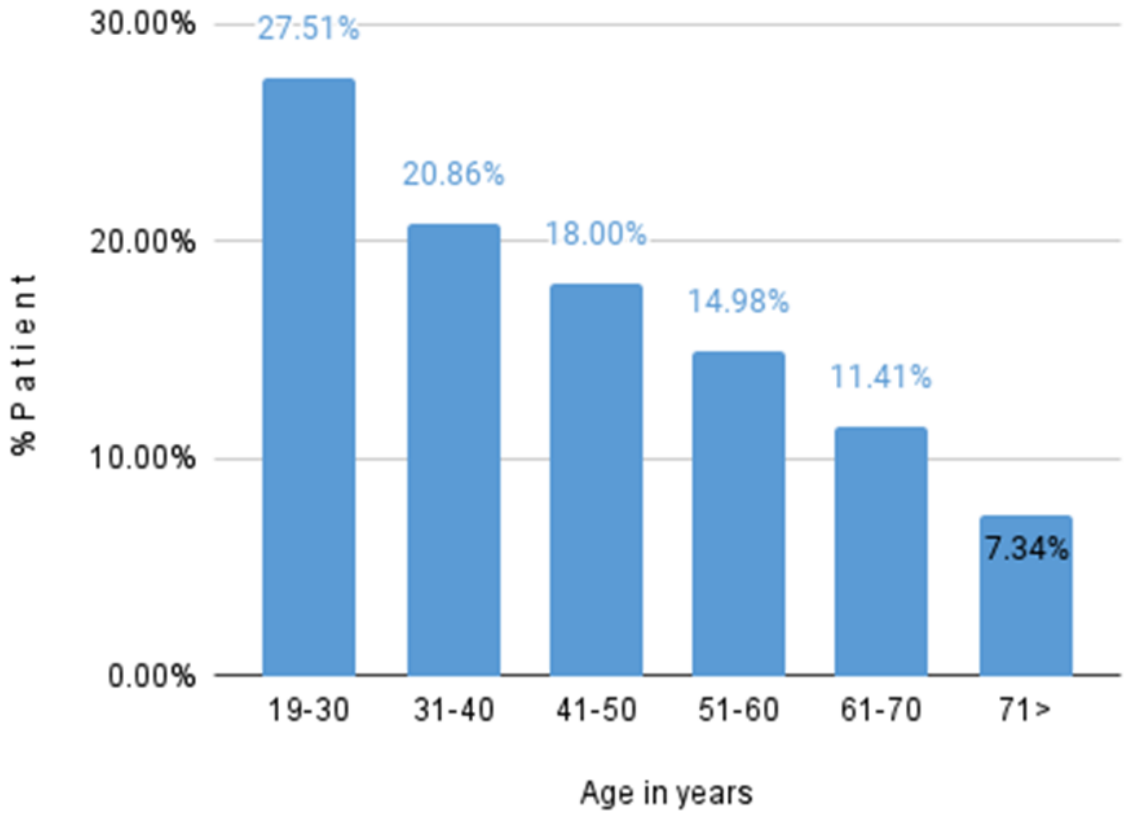

Figure 1: Age distribution of patients admitted at IALCH and MGMH from 2018 to 2022

Figure 1 indicates that the majority of patients presenting to the hospitals range between the ages of 19 to 30 years, accounting for 27.51%, followed by the age group 31 to 40 years, with 20.86%. The median age of patients presenting to either hospital is 42 years old.

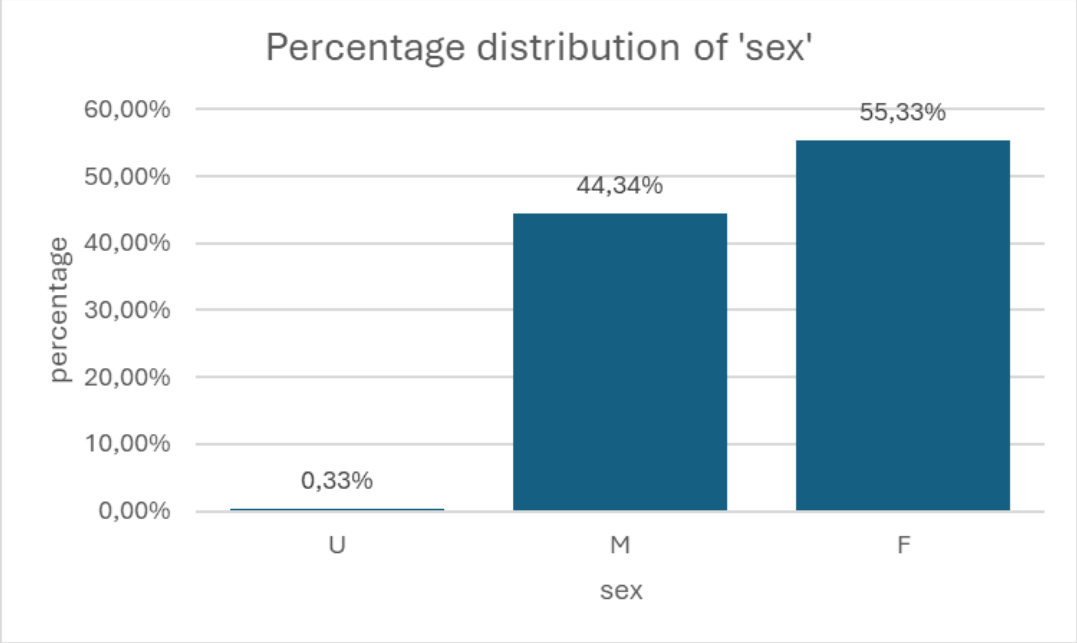

Figure 2: Sex distribution of patients who were treated at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital from 2018 to 2022

Figure 2 indicates that the majority of patients presenting to IALCH and MGMH during five years are females (55.33%). Males only accounted for 44.34%, which is not too different from females, indicating that both sexes are almost equally affected by infections caused by Gram-negative ESKAPE pathogens.

4.2 Representation of the samples tested and Gram-negative ESKAPE pathogens isolated

The following Figure 3 to 5 represent the specimen type used for the analysis of the different types of bacteria for both hospitals from 2018 to 2022.

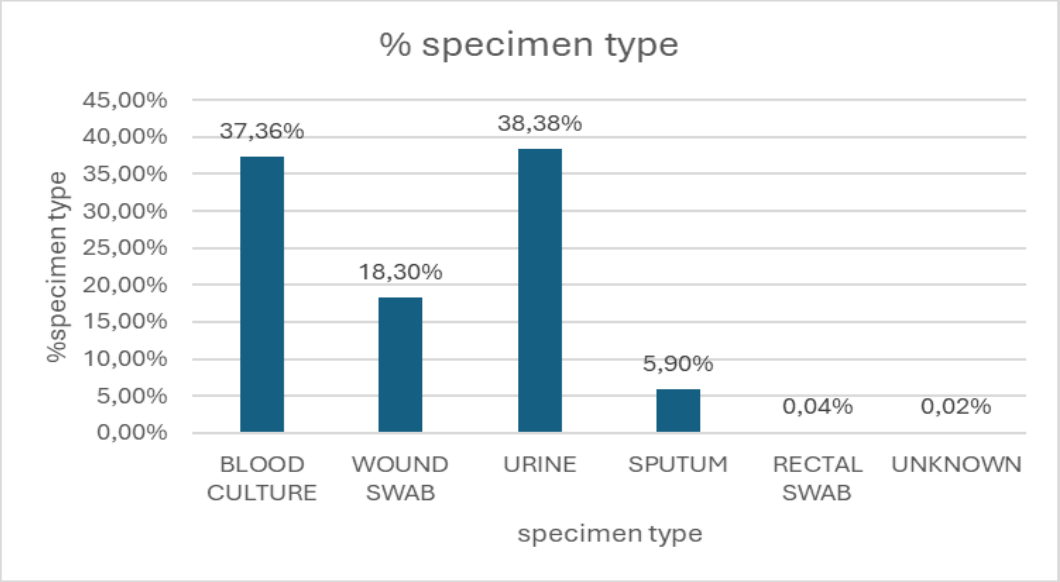

Figure 3: Specimen type tested from patients treated at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital 2018 to 2022

Figure 3 presents the distribution of the types of specimens collected for testing to aid in the diagnosis of a variety of medical conditions. Urine samples were the most predominant specimen type collected for analysis and accounted for 38.38%. Blood culture samples accounted for 37.36%, being the second-most frequently collected specimen for testing. The diagnoses were aided by laboratory tests, depending on the specimen received at the laboratory.

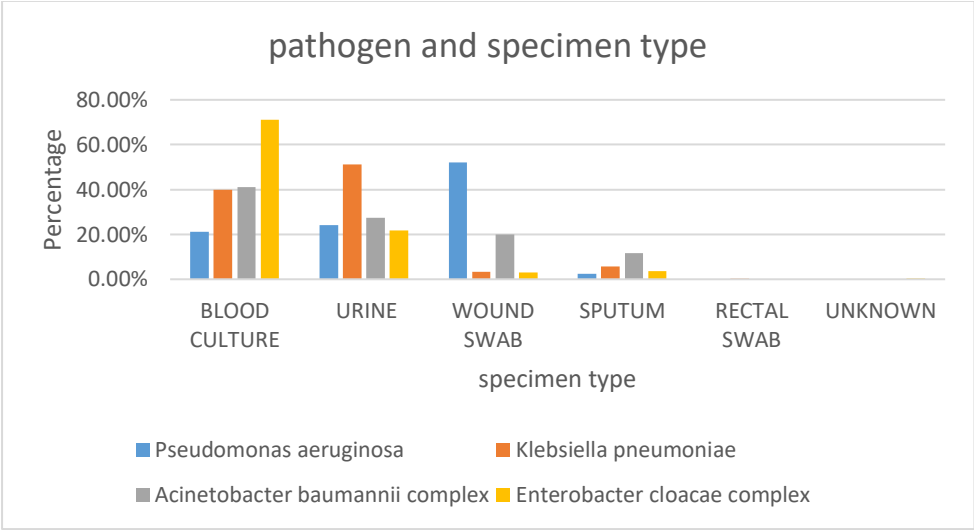

Figure 4: Specimen type tested from patients treated at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital 2018 to 2022

The above Figure 4 illustrates which specimen type the Gram-negative ESKAPE pathogens were mostly isolated from. *Klebsiella pneumoniae* was mostly isolated from urine samples, *P. aeruginosa* from wound swabs, *A. baumannii* complex and *E. cloacae* complex from blood culture samples, respectively. A p-value of 0.0267 was obtained, which proves that the data are statistically significant.

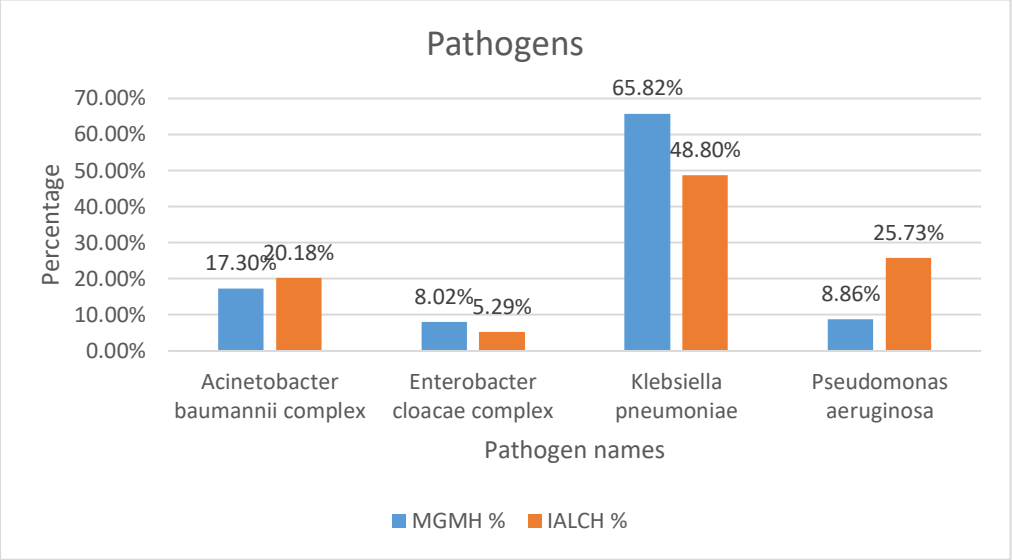

Figure 5: Pathogen groups isolated from patients at Mahatma Gandhi Memorial and Inkosi Albert Luthuli Central Hospitals, 2018 to 2022

The most isolated pathogen is *K. pneumoniae*, with 65.82% and 48.80% at MGMH and IALCH, respectively, as illustrated in figure 5. *Pseudomonas aeruginosa* (25.73%) was the second-most isolated pathogen at IALCH, but at MGMH, *A. baumannii* complex (17.30%) was the second-most isolated pathogen. The p-value obtained was 0,0316, which is <0.05, indicating a statistically significant association between the pathogen name and the facilities.

4.3 Antimicrobial susceptibility patterns for the Gram-negative ESKAPE organisms isolated from Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital from 2018 to 2022

Figures 6 and 7, and Tables 1 and 2 illustrate the percentage of antimicrobial resistance patterns and ESBL production obtained in both hospitals for the five-year period.

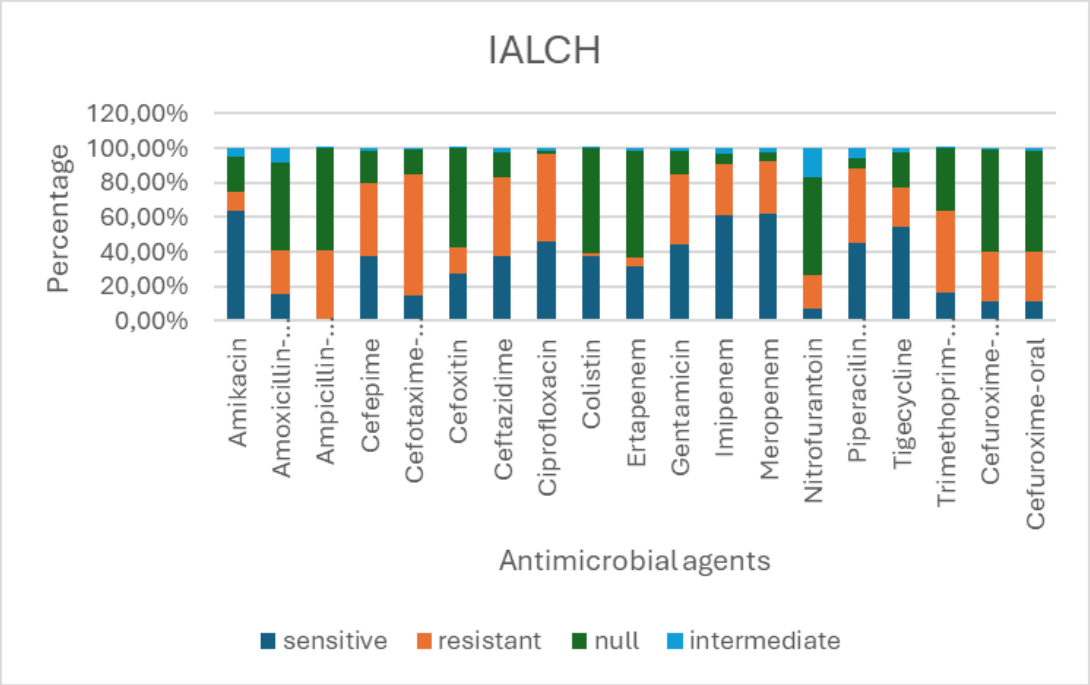

Figure 6: Inkosi Albert Luthuli Central Hospital antimicrobial susceptibility pattern from 2018 to 2022

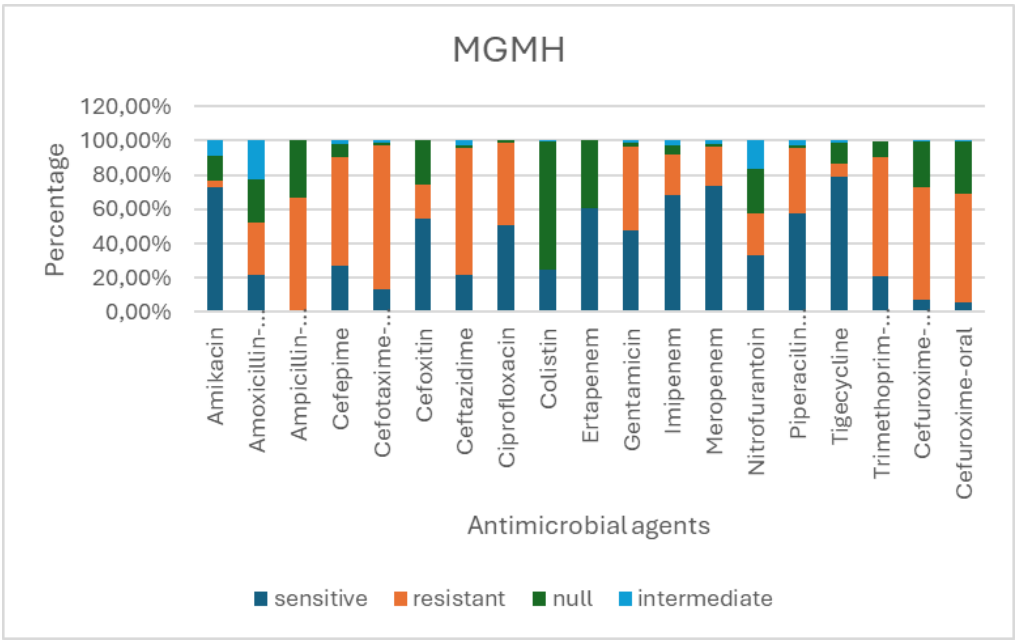

Figure 7: Mahatma Gandhi Memorial Hospital antimicrobial susceptibility pattern from 2018 to 2022

Figures 6 and 7 illustrate the antimicrobial susceptibility patterns obtained for both IALCH and MGMH. Results in both graphs represented by null indicate all the samples that were not tested for susceptibility for each type of antibiotic thus not classified as

either sensitive, intermediate or resistant. The highest sensitivity at IALCH was seen in amikacin (63.99%), followed by meropenem (62.11%) and imipenem (61.43%), while the highest resistance was noted in cefotaxime-ceftriaxone (70.17%). At MGMH, tigecycline (78.86%) had the highest sensitivity rate, followed by meropenem (73.36%) and amikacin (72.52%), while the highest resistance rate was noted in trimethoprim-sulfamethoxazole (69.98%).

Table 1: Extended-spectrum beta-lactamase production at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital

Pathogen name	ESBL producers	%	Total isolated
K. pneumoniae	462	19,14%	2414
P. aeruginosa	17	1,48%	1150
A. baumannii complex	6	0,63%	951
E. cloacae complex	28	10,53%	266
Total	512		
p-value = 0,028			

Table 1 indicates the ESBL production by the gram-negative ESKAPE pathogens isolated at IALCH and MGMH. Out of 2414 *K. pneumoniae* isolates, 462 (19.14%) were ESBL positive, followed by *E. cloacae complex* with 10.53% ESBL production. *Pseudomonas aeruginosa* and *A. baumannii complex* with only 1.48% and 0.63% respectively. The p-value was <0.05, which indicates that these results are statistically significant.

Central University of Technology, Free State

Table 2: Antimicrobial susceptibility patterns

ORGANISM_NAME	*KLEBSIELLA PNEUMONIAE*		*PSEUDOMONAS AERUGINOSA*		*ACINETOBACTER BAUMANNII* COMPLEX		*ENTEROBACTER CLOACAE* COMPLEX	
	Resistance	**Sensitivity**	**Resistance**	**Sensitivity**	**Resistance**	**Sensitivity**	**Resistance**	**Sensitivity**
Amikacin	8,66%	75,52%	10,61%	85,13%	13,67%	13,25%	2,26%	64,66%
Amoxicillin- clavulanic acid	42,54%	31,44%	0,43%	0,09%	0,21%	0,11%	73,68%	2,26%
Ampicillin-amoxicillin	84,80%	0,08%	0,43%	0%	0,32%	0%	6,02%	0,38%
Cefepime	48,76%	30,74%	10,09%	71,39%	80,13%	10,09%	17,67%	36,47%
Cefotaxime-ceftriaxone	64,42%	24,18%	85,04%	0,17%	85,70%	0,31%	29,32%	32,33%
Cefoxitin	24,07%	59,86%	0,43%	0,09%	0,21%	0,11%	59,77%	1,13%
Ceftazidime	57,21%	25,39%	8,52%	78,43%	80,97%	12,62%	25,94%	32,71%
Ciprofloxacin	52,48%	42,58%	18,61%	78,17%	86,96%	11,78%	30,83%	67,67%
Colistin	0%	0,04%	3,91%	79,65%	4,21%	86,01%	0%	0%
Ertapenem	8,78%	62,47%	0,09%	0,17%	0,21%	0%	1,88%	47,37%
Gentamicin	45,48%	41,30%	11,57%	73,56%	73,50%	17,87%	22,18%	41,73%
Imipenem	16,11%	72,58%	13,57%	78,00%	84,75%	11,78%	4,89%	78,19%
Meropenem	18,48%	74,81%	11,74%	76,17%	86,12%	12,09%	3,76%	84,96%
Nitrofurantoin	37,28%	16,94%	0,43%	0,09%	0,32%	0%	6,77%	25,56%
Piperacillin-tazobactam	38,03%	45,86%	16,26%	80,78%	89,38%	8,83%	21,80%	38,72%
Tigecycline	1,53%	73,99%	84,95%	0,08%	1,05%	83,60%	1,50%	49,25%
Trimethoprim-sulfamethoxazole	61,35%	25,68%	0,70%	0,35%	84,23%	9,78%	31,58%	33,08%
Cefuroxime-p	58,86%	20,75%	0,17%	0%	0,21%	0%	35,71%	10,15%
Cefuroxime-o	58,82%	21,25%	0,52%	0%	0,21%	0,11%	36,47%	10,53%

Table 1 shows the different pathogens and their resistance patterns to different types of antimicrobial agents; however, the resistance and sensitivity do not amount to 100%, due to the intermediate susceptibility and some samples that were not tested for certain antimicrobial agents, but are shown in **Appendix D**. *Klebsiella pneumoniae* resistance is seen the highest against ampicillin-amoxicillin (84.80%), followed by cefotaxime-ceftriaxone, with 64.42% over the period of five years. The lowest resistance is noted against tigecycline (1.53%) and amikacin (8.66). *Acinetobacter baumannii* complex is highly resistant to piperacillin-tazobactam (89.38%), ciprofloxacin (86.96%), meropenem (86.12%), cefotaxime-ceftriaxone (85.70%), imipenem (84.75%) and trimethoprim-sulfamethoxazole (84.23%). The lowest resistance is seen against tigecycline (1.05%) and colistin (4.21%), with a sensitivity of 83.60% and 86.01% for tigecycline and colistin, respectively.

Pseudomonas aeruginosa resistance was highest against cefotaxime-ceftriaxone (85.04%) and lowest against colistin (3.9%), but sensitive to amikacin (85.13%). *Enterobacter cloacae* complex is highly resistant to amoxicillin-clavulanic acid (73.68%) and less resistant to amikacin (2.26%) and tigecycline (1.50%), respectively. These findings demonstrate a statistically significant positive correlation between pathogens and antimicrobial agents, with a p-value of 0.037 which is <0.05.

CHAPTER 5:
DISCUSSION AND CONCLUSION

As shown in graph 1, the IALCH has the greatest number of patients with bacterial infection, which is explained by the capacity of patients this hospital can accommodate as a referral hospital. MGMH, as a district hospital, has fewer patients presenting with infections, as it can accommodate a smaller number of patients over the period of five years. These results were also affected by the COVID-19 era that occurred from 2020 to 2022, where patients were hospitalised for extended periods and being immunocompromised and the restriction measures that were enforced.

A total of 4 782 patients were admitted in both these hospitals with different bacterial infections, 27.51% of these patients were 19–30 years of age. The median age was 42 years, which differs from a study that reported a median age of 47 years for patients hospitalised (Wan *et al*., 2020). On the contrary, China reported a median age of 68 years, which is expected, since elderly people are more prone to infections as they have weakened immune systems (Chen *et al*., 2020b). Furthermore, India's reported elderly population had a much higher rate of hospitalisation (Pandey *et al*., 2017), but on the contrary, this study indicated young adults are mostly affected by bacterial infections, which may be further distinguished with regard to which sex is mostly affected. The age gap of a study in China indicated widespread age, with most patients being elderly, with a median age of 47 years (Wan *et al.,* 2020; Chen *et al.*, 2020b and Pandey *et al.*, 2017). Graph 1 shows right-skewed data, which could predict that the mode age range (19–30) could possibly be less than the median of the population of the study. Indeed, it is true that the average median age of the study population was found to be 42 years which is represented by adults in South Africa's life expectancy, which is currently between 60 and 65 years. Therefore, the study results correlate with a study by Wan et al. (2020), so this could indicate that the older population was more prone to the infection.

The infection rates, including COVID-19, have been reported not to be sex-dependent but mostly associated with the underlying health status of an individual (Kopel *et al*., 2020). China reported a mortality rate of 75% males and only 27% of females during the pandemic This was explained by the fact that males are smokers, which affects

their lungs. Similarly, COVID-19 caused respiratory difficulties (Chen *et al*., 2020a). The results of the study indicated that out of 4 782 patients, 55.33% were females and 44.34% were males, indicating a significant difference of 10.99% as reflected in Graph 2. These results correlate with the common view that infections, including COVID-19, are based on an individual's health status and not sex. Different specimen types were collected and tested to ascertain the cause of these bacterial infections, and further analysis was performed.

The most frequently tested specimen was urine, and therefore the most used test set was reported to be urine culture (38.38%). Blood culture samples (37.36%) were the second-most used specimen type, as indicated in Graph 4. These results correlated with the study by Sorsa *et al.* (2019), which reports that most of these infections are caused by pathogens associated with bacteraemia and are the leading cause of sepsis; therefore, the best specimen type was blood culture (Sorsa *et al.,* 2019). This can be attributed to the health-deteriorating and shocked state experienced during COVID-19, where people were immunocompromised, and due to a high use of sanitisation and use masks, which provided less exposure to circulation of organisms, and possibly the aftereffects of COVID-19 infection, leading to prolonged hospitalisations.

The most isolated pathogen for both hospitals is *K. pneumoniae,* with 48.80% and 65.82% at IALCH and MGMH, respectively, which correlate with the fact that historically this organism was one of the pathogens that led to the introduction of third-generation cephalosporins in the 1980s due to its prevalence and increasing resistance patterns (Deepthi *et al*., 2010). However, in this study, *K. pneumoniae* for both hospitals was attributed to 57.31%, which correlates with Balkhy, who reported a 63% *K. pneumoniae* infection rate (Balkhy *et al*., 2020). The *P. aeruginosa*, *E. cloacae* complex and *A. baumannii* complex results were 25.73%, 5.29%, and 20.18%, respectively, at IALCH. On the contrary, in a recent European study, *Enterobacter cloacae* complex (44.8%) was the most prevalent organism isolated, which does not correlate with this study (Boattini *et al*., 2024).

Most studies have reported considerably high ESBL percentages, as opposed to the overall 10.71% obtained in this study. For example, of 117 exudate isolates reported by Umadevi *et al*., 66.75% were ESBL-positive. India reported 72%, followed by

Mexico with 71.4% in 2007 (Reinert *et al*., 2007; Umadevi *et al*., 2011). In 2013, Mexico only reported 36.9% of *K. pneumoniae* isolates being ESBL producers (Llaca-Diaz *et al*., 2013). In South Africa, 7.6% ESBL-producing *K. pneumoniae* and 7.0% ESBL-producing A. *baumannii* complex were reported in 2011 on neonates (Pillay *et al*., 2021). However, this study reported a 19.14% ESBL-producing *K. pneumoniae*, 10.53% *E. cloacae* complex, 1.48% *P. aeruginosa* and 0.63% *A. baumannii* complex, which indicates increasing rates of ESBL production in adults. Individual antimicrobial resistance was done for each pathogen against commonly used antibiotics.

IALCH, together with MGMH, noted a very high sensitivity rate against amikacin, tigecycline, and colistin, which correlates with a study done in India that reported colistin and tigecycline to have the highest sensitivity (Chaudhary *et al*., 2010). Colistin, however, shows increased sensitivity for *A. baumannii* complex (86.01%) and has not been tested for other pathogens, since it is normally considered the last-resort antibiotic for the treatment of multi-drug-resistant gram-negative infections (Falagas *et al*., 2005). *A. baumannii* complex has high resistance against most carbapenem (>80%) antimicrobial agents, leading to the use of colistin.

On the contrary, most organisms have been reported to be more sensitive to carbapenem antimicrobial agents in some studies, although some degree of resistance has also been noted. In Asia, amikacin and imipenem were noted as the most active antibiotics, while Saudi Arabia as well as Ethiopia reported a greater than 83% sensitivity to amikacin (Ahmed *et al*., 2009; Yang *et al*., 2017; Sorsa *et al*., 2019). Similarly, correlation was found in this study that at IALCH, a greater than 60% sensitivity rate was obtained for meropenem and amikacin. The results of this study proved the efficacy meropenem still has against gram-negative ESKAPE pathogens by showing optimal susceptibility (Pillay *et al*., 2021). At MGMH, most carbapenems had a sensitivity rate greater than 60%, including ertapenem (60.89%) and imipenem (68.08%). On the contrary *A. baumannii* had 75.6% resistance to imipenem reported in China (Yang *et al*., 2023).

The highest resistance was noted against cefotaxime-ceftriaxone antimicrobial agents, with greater than 70% for both hospitals. IALCH showed a 45.90% resistance to ciprofloxacin, which differ from the findings of high sensitivity to ciprofloxacin that

was noted in Saudi Arabia in 2009, thus showing how quickly these organisms become resistant to antimicrobial agents (Ahmed *et al*., 2009; Sorsa *et al*., 2019). Most penicillins and third-generation cephalosporins had a greater than 30% resistance rate in both hospitals. Although these patterns clearly indicate the resistance to antimicrobial agents, each of the pathogens belonging to the gram-negative ESKAPE group was also individually assessed.

Klebsiella pneumoniae was highly resistant to an ampicillin-amoxicillin combination (84.80%), with a greater than 40% resistance rate to cephalosporins ,which include ceftazidime, cefepime, cefuroxime and cefotaxime-ceftriaxone combination, but sensitive to amikacin (75.52%). A less than 20% resistance rate to carbapenem antimicrobial agents was noted compared to the 4% noted in 2014 to 16% in 2015, which proves the slowly increasing carbapenem resistance rates in these organisms dating back from 2015 in other studies to the >20% obtained in this study (Ramsamy *et al.*, 2018).

A greater than 80% resistance to piperacillin-tazobactam, ciprofloxacin, gentamicin, imipenem, meropenem and third-generation cephalosporins was noted in *A. baumannii* complex. *Acinetobacter baumannii* complex has shown high sensitivity towards tigecycline and colistin. *Pseudomonas aeruginosa* was highly resistant to cefotaxime-ceftriaxone (85.04%) and tigecycline (84.95%), with <12% resistance towards carbapenems and amikacin, proving a low carbapenem resistance, as opposed to the high resistance noted in Saudi Arabia (Balkhy *et al*., 2020).

Resistance to amoxicillin-clavulanic acid combination was noted in *Enterobacter cloacae* complex (73.68%), with a less than 5% resistance rate to carbapenem antimicrobial agents, particularly meropenem (84.96), showing much greater efficacy in treating infections caused by *E. cloacae* complex. *Enterobacter cloacae* complex accounted for a 15.7% resistance rate to third- and fourth-generation cephalosporins and carbapenems in Europe, which does not correlate with this study (Boattini *et al*., 2024)

5.1 Study limitations

Only two hospitals were analysed in KZN; therefore, the study did not show an in-depth analysis of the situation of antimicrobial resistance in KZN province.

5.2 Recommendations

Screening for the production of ESBL in all organisms should be routinely done at all district and provincial hospital laboratories to provide optimum patient treatment. Resistance patterns of ESKAPE priority pathogens should be monitored to improve infection control measures and limit the spread of HAI. As resistance has been noted in most commonly used antimicrobial agents, penicillins and first-generation cephalosporins have shown very little efficacy towards ESBL-producing Enterobacteriaceae. It is recommended that third-generation cephalosporins and carbapenems be used instead. *Acinetobacter baumannii* complex also requires more surveillance, as it has shown concerning resistance patterns towards some carbapenem antibiotics.

5.3 Conclusion

The most isolated gram-negative ESKAPE pathogen is *K. pneumoniae*, with the highest sensitivity towards amikacin in both hospitals. The infection rates over the five-year period have increased for most pathogens, which proves the hypothesis that infections caused by ESKAPE organisms are on the increase. The resistance noted in this study further proves the increasing resistance patterns, although they were considerably lower increments, probably due to the extra precautions that were put in place to curb the spread of COVID-19. However, some of these organisms were still able to thrive and increase their resistance to most anti-microbial agents. Carbapenem resistance in these two hospitals is still low, but a concern for *A. baumannii* complex, which shows very high resistance to carbapenem antimicrobial agents, further proves my hypothesis of increased resistance rates to beta-lactam and carbapenem antimicrobial agents. Antimicrobial resistance has proven to be an area of concern that requires more research, as these pathogens seem to have more of an impact than what current studies portray. The ability of these organisms to constantly adapt and

evolve is concerning, especially in South Africa, which is a developing country without enough resources to overcome antimicrobial stewardship. Therefore, constant surveillance is required.

REFERENCES

1. Abraham, E.P. and Chain, E., 1940. An enzyme from bacteria able to destroy penicillin. *Nature*, *146*(3713), pp.837-837.

2. Adler, A., Gniadkowski, M., Baraniak, A., Izdebski, R., Fiett, J., Hryniewicz, W., Malhotra-Kumar, S., Goossens, H., Lammens, C., Lerman, Y. and Kazma, M., 2012. Transmission dynamics of ESBL-producing *Escherichia coli* clones in rehabilitation wards at a tertiary care centre. *Clinical Microbiology and Infection*, *18*(12), pp. E497-E505.

3. Ahmad, S., Al-Juaid, N.F., Alenzi, F.Q., Mattar, E.H. and Bakheet, O.E.S., 2009. Prevalence, antibiotic susceptibility pattern and production of Extended-Spectrum β-Lactamases amongst clinical isolates of *Klebsiella pneumoniae* at Armed Forces Hospital in Saudi Arabia. *Journal of the College of Physicians and Surgeons Pakistan*, *19*(4), pp.264-5.

4. Alekshun, M.N. and Levy, S.B., 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell*, *128*(6), pp.1037-1050.

5. Almasaudi, S.B., 2018. *Acinetobacter* spp. as nosocomial pathogens: Epidemiology and resistance features. *Saudi Journal of Biological Sciences*, *25*(3), pp.586-596.

6. Al-Wutayd, O., Al Nafeesah, A., Adam, I. and Babikir, I., 2018. The antibiotic susceptibility patterns of uropathogens isolated in Qassim, Saudi Arabia. *The Journal of Infection in Developing Countries*, *12*(11), pp.946-952.

7. Al-Zarouni, M., Senok, A., Rashid, F., Al-Jesmi, S.M. and Panigrahi, D., 2008. Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the United Arab Emirates. *Medical Principles and Practice*, *17*(1), pp.32-36.

8. Azim, N.S.A., Al-Harbi, M.A., Al-Zaban, M.I., Nofal, M.Y. and Somily, A.M., 2019. Prevalence and antibiotic susceptibility among gram-negative bacteria isolated from intensive care units at a tertiary care hospital in Riyadh, Saudi Arabia. Journal of Pure and Applied Microbiology, *13*, pp.201-208.

9. Babypadmini, S. and Appalaraju, B., 2004. Extended-spectrum β-lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* prevalence and susceptibility pattern in a tertiary care hospital. *Indian Journal of Medical Microbiology*, *22*(3), pp.172-174.

10. Bahr, G., Gonzalez, L.J. and Vila, A.J., 2021. Metallo-β-lactamases in the age of multidrug resistance: from structure and mechanism to evolution, dissemination, and inhibitor design. *Chemical Reviews*, *121*(13), pp.7957-8094.

11. Bain, B.J. and England, J.M., 1975. Normal haematological values: sex difference in neutrophil count. *British Medical Journal*, *1*(5953), pp.306-309.

12. Balkhy, H.H., El-Saed, A., Alshamrani, M.M., Alsaedi, A., Al Nasser, W., El Gammal, A., Aljohany, S.M., Almunif, S., Arabi, Y., Alqahtani, S. and Bonnie, H.B., 2020. Ten-year resistance trends in pathogens causing healthcare-associated infections; reflection of infection control interventions at a multi-hospital healthcare system in Saudi Arabia, 2007–2016. *Antimicrobial Resistance & Infection Control*, *9*(1), pp.1-12.

13. Balsalobre, L., Blanco, A. and Alarcón, T., 2019. Beta-lactams. *Antibiotic Drug Resistance*, *Wiley Online Library,* pp.57-72.

14. Bell, J.M., Turnidge, J.D., Gales, A.C., Pfaller, M.A., Jones, R.N. and Sentry APAC Study Group, 2002. Prevalence of extended-spectrum β-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998–99). *Diagnostic Microbiology and Infectious Disease*, *42*(3), pp.193-198.

15. Birru, M., Woldemariam, M., Manilal, A., Aklilu, A., Tsalla, T., Mitiku, A. and Gezmu, T., 2021. Bacterial profile, antimicrobial susceptibility patterns, and associated factors among bloodstream infection suspected patients attending Arba Minch General Hospital, Ethiopia. *Scientific Reports*, *11*(1), p.15882.

16. Boattini, M., Bianco, G., Llorente, L.I., Acero, L.A., Nunes, D., Seruca, M., Mendes, V.S., Almeida, A., Bastos, P., Rodríguez-Villodres, Á. and Gascón, A.G., 2024. Enterobacterales carrying chromosomal AmpC β-lactamases in Europe (EuESCPM): epidemiology and antimicrobial resistance burden from a

cohort of 27 hospitals, 2020–2022. *International Journal of Antimicrobial Agents*, *63*(5), p.107115.

17. Bolikas, E., Astrinaki, E., Panagiotaki, E., Vitsaxaki, E., Saplamidou, S., Drositis, I., Stafylaki, D., Chamilos, G., Gikas, A., Kofteridis, D.P. and Kritsotakis, E.I., 2023. Impact of SARS-CoV-2 Preventive Measures against Healthcare-Associated Infections from Antibiotic-Resistant ESKAPEE Pathogens: A Two-Center, Natural Quasi-Experimental Study in Greece. *Antibiotics*, *12*(7), p.1088.

18. Bradford, P.A., 2001. Extended-spectrum β-lactamases in the 21st century: characterisation, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*, *14*(4), pp.933-951.

19. Bush, K. and Bradford, P.A., 2016. β-Lactams and β-lactamase inhibitors: an overview. *Cold Spring Harbor Perspectives in Medicine*, *6*(8), p.a025247.

20. Bush, K. and Jacoby, G.A., 2010. Updated functional classification of β-lactamases. *Antimicrobial Agents and Chemotherapy*, *54*(3), pp.969-976.

21. Chaudhary, M., Kumar, S. and Payasi, A., 2013. Prevalence and antimicrobial sensitivity of Extended-Spectrum β-Lactamase producing gram-negative bacteria from clinical settings in India from 2010–2012. *International Journal of Medicine and Medical Sciences*, *46*(2), p.1212.

22. Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y. and Yu, T., 2020a. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet*, *395*(10223), pp.507-513.

23. Chen, T., Wu, D.I., Chen, H., Yan, W., Yang, D., Chen, G., Ma, K., Xu, D., Yu, H., Wang, H. and Wang, T., 2020b. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *British Medical Journal*, p.*368*.

24. Cho, S.Y., Choi, S.M., Park, S.H., Lee, D.G., Choi, J.H. and Yoo, J.H., 2016. Amikacin therapy for urinary tract infections caused by extended-spectrum β-

lactamase-producing *Escherichia coli*. *The Korean Journal of Internal Medicine*, *31*(1), p.156.

25. Christaki, E., Marcou, M. and Tofarides, A., 2020. Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. *Journal of Molecular Evolution*, *88*(1), pp.26-40.

26. Coetzee, J. and Brink, A., 2011. The emergence of carbapenem resistance in Enterobacteriaceae in South Africa. *Southern African Journal of Epidemiology and Infection*, *26*(4), pp.239-240.

27. Crichton, H., O'Connell, N., Rabie, H., Whitelaw, A.C. and Dramowski, A., 2018. Neonatal and paediatric bloodstream infections: Pathogens, antimicrobial resistance patterns and prescribing practice at Khayelitsha District Hospital, Cape Town, South Africa. *South African Medical Journal*, *108*(2), pp.99-104.

28. Danel, F., Hall, L.M., Gur, D. and Livermore, D.M., 1997. OXA-15, an extended-spectrum variant of OXA-2 beta-lactamase, isolated from a *Pseudomonas. aeruginosa* strain. *Antimicrobial Agents and Chemotherapy*, *41*(4), pp.785-790.

29. Deepthi, N. & Deepti , R. 2010. Extended Spectrum Beta Lactamases in Gram Negative Bacteria. *Journal of Global Infectious Diseases*, *2*(3), pp.263-274.

30. Dias, S.P., Brouwer, M.C. and Van de Beek, D., 2022. Sex and gender differences in bacterial infections. *Infection and Immunity*, *90*(10), pp. e00283-22.

31. Doi, Y., Adams, J., O'Keefe, A., Quereshi, Z., Ewan, L. and Paterson, D.L., 2007. Community-acquired extended-spectrum β-lactamase producers, United States. *Emerging Infectious Diseases*, *13*(7), pp.1121-1124.

32. Džidić, S., Šušković, J. and Kos, B., 2008. Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology & Biotechnology*, *46*(1).

33. Falagas, M.E. and Karageorgopoulos, D.E., 2009. Extended-spectrum β-lactamase-producing organisms. *Journal of Hospital Infection*, *73*(4), pp.345-354.*Chemotherapy*, *56*(1), pp.52-59.

34. Falagas, M.E., Kasiakou, S.K. and Saravolatz, L.D., 2005. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clinical Infectious Diseases*, *40*(9), pp.1333-1341.

35. Fourie, T., Schellack, N., Bronkhorst, E., Coetzee, J. and Godman, B., 2018. Antibiotic prescribing practices in the presence of extended-spectrum β-lactamase (ESBL) positive organisms in an adult intensive care unit in South Africa – A pilot study. *Alexandria Journal of Medicine*, *54*(4), pp.541-547.

36. Fowler, R.A., Sabur, N., Li, P., Juurlink, D.N., Pinto, R., Hladunewich, M.A., Adhikari, N.K., Sibbald, W.J. and Martin, C.M., 2007. Sex-and age-based differences in the delivery and outcomes of critical care. *Canadian Medical Association, 177*(12), pp.1513-1519.

37. Galligan, C.L. and Fish, E.N., 2015. Sex differences in the immune response. In *Sex and gender differences in infection and treatments for infectious diseases* (pp. 1-29). Cham: *Springer International Publishing*.

38. Gasink, L.B., Edelstein, P.H., Lautenbach, E., Synnestvedt, M. and Fishman, N.O., 2009. Risk factors and clinical impact of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. *Infection Control & Hospital Epidemiology*, *30*(12), pp.1180-1185.

39. Giefing-Kröll, C., Berger, P., Lepperdinger, G. and Grubeck-Loebenstein, B., 2015. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Ageing Cell*, *14*(3), pp.309-321.

40. Guet-Revillet, H., Le Monnier, A., Breton, N., Descamps, P., Lecuyer, H., Alaabouche, I., Bureau, C., Nassif, X. and Zahar, J.R., 2012. Environmental contamination with extended-spectrum β-lactamases is there any difference between *Escherichia coli* and Klebsiella spp. *American Journal of Infection Control*, *40*(9), pp.845-848.

41. Hagel, S., Makarewicz, O., Hartung, A., Weiß, D., Stein, C., Brandt, C., Schumacher, U., Ehricht, R., Patchev, V. and Pletz, M.W., 2019. ESBL colonisation and acquisition in a hospital population: The molecular epidemiology and transmission of resistance genes. *Plos One*, *14*(1), p.e0208505.

42. Hall, L.M., Livermore, D.M., Gur, D., Akova, M. and Akalin, H.E., 1993. OXA-11, an extended-spectrum variant of OXA-10 (PSE-2) beta-lactamase from Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy*, *37*(8), pp.1637-1644.

43. Helldal L, Karami N, Florén K, Welinder-Olsson C, Moore ER, Åhrén C., 2013. Shift of CTX-M genotypes has determined the increased prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* in south-western Sweden. *Clinical Microbiology and Infection*. 2013 Feb;19(2): E87-90. doi: 10.1111/1469-0691.12086. Epub 2012 Nov 27. PMID: 23190133.

44. Hoelle, J., Johnson, J.R., Johnston, B.D., Kinkle, B., Boczek, L., Ryu, H. and Hayes, S., 2019. Survey of US wastewater for carbapenem-resistant Enterobacteriaceae. *Journal of Water and Health*, *17*(2), pp.219-226.

45. https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/antibiotic-resistant-bacteria accessed on 6 June 2022.

46. About ESBL-producing Enterobacterales | ESBL-producing Enterobacterales | CDC

47. https://www.healthline.com/health/esbl accessed on 6 June 2022.

48. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance accessed on 6 June 2022.

49. Ismail, H., Lowman, W., Govind, C.N., Swe Swe-Han, K., Maloba, M.R.B., Bamford, C. and Perovic, O., 2019. Surveillance and comparison of antimicrobial susceptibility patterns of ESKAPE organisms isolated from patients with bacteraemia in South Africa, 2016-2017. *South African Medical Journal*, *109*(12), pp.934-940.

50. Kalsi, J., Arya, M., Wilson, P. and Mundy, A., 2003. Hospital-acquired urinary tract infection. *International Journal of Clinical Practice*, *57*(5), pp.388-391.

51. Kim, H.R. and Eom, Y.B., 2021. Synergistic activity of equal and meropenem against carbapenem-resistant *Escherichia coli*. *Antibiotics*, *10*(2), p.161.

52. Klein, S.L. and Flanagan, K.L., 2016. Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), pp.626-638.

53. Knothe, H., Shah, P., Krcmery, V., Antal, M. and Mitsuhashi, S., 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection*, *11*(6), pp.315-317.

54. Kopel, J., Perisetti, A., Roghani, A., Aziz, M., Gajendran, M. and Goyal, H., 2020. Racial and gender-based differences in COVID-19. *Frontiers in Public Health*, *8*, p.418.

55. Korzeniewska, E. and Harnisz, M., 2013. Beta-lactamase-producing Enterobacteriaceae in hospital effluents. *Journal of Environmental Management*, *123*, pp.1-7.

56. Kumarasamy, K.K., Toleman, M.A., Walsh, T.R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S. and Krishnan, P., 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *The Lancet Infectious Diseases*, *10*(9), pp.597-602.

57. Lee, J.H., Bae, I.K. and Hee Lee, S., 2012. New definitions of extended-spectrum β-lactamase conferring worldwide emerging antibiotic resistance. *Medicinal Research Reviews*, *32*(1), pp.216-232.

58. Lin, Y.H., Mickel, A.K. and Chogle, S., 2003. Effectiveness of selected materials against *Enterococcus faecalis*: part 3. The antibacterial effect of calcium hydroxide and chlorhexidine on *Enterococcus faecalis*. *Journal of Endodontics*, *29*(9), pp.565-566.

59. Livermoore, D.M. and Paterson, D.L., 2006. Pocket guide to extended-spectrum Beta-lactamases in resistance. *J Antimicrob Chemother*, *58*, pp.231-237.

60. Llaca-Díaz, J.M., Mendoza-Olazarán, S., Camacho-Ortiz, A., Flores, S. and Garza-González, E., 2013. One-year surveillance of ESKAPE pathogens in an intensive care unit of Monterrey, Mexico. *Chemotherapy*, *58*(6), pp.475-481.

61. Lowe, M., Shuping, L. and Perovic, O., 2022. Carbapenem-resistant Enterobacterales in patients with bacteraemia at tertiary academic hospitals in

South Africa, 2019-2020: An update. *South African Medical Journal*, *112*(8), pp.545-552.

62. Manandhar, S., Zellweger, R.M., Maharjan, N., Dongol, S., Prajapati, K.G., Thwaites, G., Basnyat, B., Dixit, S.M., Baker, S. and Karkey, A., 2020. A high prevalence of multi-drug-resistant Gram-negative bacilli in a Nepali tertiary care hospital and associated widespread distribution of Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemase-encoding genes. *Annals of Clinical Microbiology and Antimicrobials*, *19*(1), pp.1-13.

63. Marturano, J.E. and Lowery, T.J., 2019. ESKAPE pathogens in bloodstream infections are associated with higher cost and mortality but can be predicted using diagnoses upon admission. *In Open Forum Infectious Diseases 6*(12), p. ofz503). US: Oxford University Press.

64. Mendelson, G., Hait, V., Ben-Israel, J., Gronich, D., Granot, E. and Raz, R., 2005. Prevalence and risk factors of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in an Israeli long-term care facility. *European Journal of Clinical Microbiology and Infectious Diseases*, *24*, pp.17-22.

65. Mendelson, M., 2015. Role of antibiotic stewardship in extending the age of modern medicine. *South African Medical Journal*, *105*(5), pp.414-419.

66. Mills, M.C. and Lee, J., 2019. The threat of carbapenem-resistant bacteria in the environment: Evidence of widespread contamination of reservoirs at a global scale. *Environmental Pollution*, *255*, p.113143.

67. Mogokotleng, R., Ismail, H., Perovic, O. and Jallow, S., 2022. A Retrospective Analysis of Culture-Confirmed Enterococci Bloodstream Infections in South Africa, 2016–2020: A Cross-Sectional Study. *Tropical Medicine and Infectious Disease*, 8(1), p.19.

68. Mzimela, H.M.M. and Izegaegbe, J.I., 2021. Metal behaviour in water, sediment and fish from the St Lucia system: implications for public health and ecosystem impact. *Environmental Monitoring and Assessment*, *193*(12), p.807.

69. Naser, A.Y., 2023. Hospitalisation profile in England and Wales, 1999 to 2019: an ecological study. *BMJ Open*, *13*(4), p.e068393

70. Ober, C., Loisel, D.A. and Gilad, Y., 2008. Sex-specific genetic architecture of human disease. *Nature Reviews Genetics*, *9*(12), pp.911-922.

71. Osano, E., Arakawa, Y., Wacharotayankun, R., Ohta, M., Horii, T., Ito, H., Yoshimura, F. and Kato, N., 1994. Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of Serratia marcescens that shows imipenem resistance. *Antimicrobial agents and chemotherapy*, *38*(1), pp.71-78.

72. Pandey, A., Ploubidis, G.B., Clarke, L. and Dandona, L., 2017. Hospitalisation trends in India from serial cross-sectional nationwide surveys: 1995 to 2014. *BMJ Open*, *7*(12), p.e014188.

73. Paterson, D.L., 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. *American Journal of Infection Control*, *34*(5), pp.S20-S28. Assessed on 6 June 2022

74. Paterson, D.L. and Bonomo, R.A., 2005. Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology Reviews*, *18*(4), pp.657-686.

75. Pendleton, J.N., Gorman, S.P. and Gilmore, B.F., 2013. Clinical relevance of the ESKAPE pathogens. *Expert Review of Anti-infective Therapy*, *11*(3), pp.297-308.

76. Perovic, O., Singh-Moodley, A., Dusé, A., Bamford, C., Elliott, G., Swe-Han, K.S., Kularatne, R., Lowman, W., Whitelaw, A., Nana, T. and Wadula, J., 2014. National sentinel site surveillance for antimicrobial resistance in *Klebsiella pneumoniae* isolates in South Africa, 2010-2012. *South African Medical Journal*, *104*(8), pp.563-568.

77. Pillay, D., Naidoo, L., Swe Swe-Han, K. and Mahabeer, Y., 2021. Neonatal sepsis in a tertiary unit in South Africa. *BMC Infectious Diseases*, *21*(1), pp.1-10.

78. Pitout, J.D., Nordmann, P., Laupland, K.B. and Poirel, L., 2005. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy, 56*(1), pp.52-59.

79. POPIA., 2013. Government Gazette, Republic of South Africa, 912(581), p.2 https://www.gov.za/documents/protection-personal-information-act accessed on the 6 June 2022.

80. Queenan, A.M. and Bush, K., 2007. Carbapenemases: the versatile β-lactamases. *Clinical Microbiology Reviews*, *20*(3), pp.440-458.

81. Queenan, A.M., Jenkins, S. and Bush, K., 2001. Cloning and biochemical characterisation of FOX-5, an AmpC-type plasmid-encoded β-lactamase from a New York City Klebsiella pneumoniae clinical isolate. *Antimicrobial Agents and chemotherapy*, *45*(11), pp.3189-3194.

82. Ramsamy, Y., Essack, S.Y., Sartorius, B., Patel, M. and Mlisana, K.P., 2018. Antibiotic resistance trends of ESKAPE pathogens in Kwazulu-Natal, South Africa: a five-year retrospective analysis. *African Journal of Laboratory Medicine*, *7*(2), pp.1-8.

83. Reddy, K., Bekker, A., Whitelaw, A.C., Esterhuizen, T.M. and Dramowski, A., 2021. A retrospective analysis of pathogen profile, antimicrobial resistance and mortality in neonatal hospital-acquired bloodstream infections from 2009–2018 at Tygerberg Hospital, South Africa. *PLoS One*, *16*(1), p.e0245089.

84. Reinert, R.R., Low, D.E., Rossi, F., Zhang, X., Wattal, C. and Dowzicky, M.J., 2007. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. *Journal of Antimicrobial Chemotherapy*, *60*(5), pp.1018-1029.

85. Rice, L.B., 2010. Progress and challenges in implementing the research on ESKAPE pathogens", *Infection Control and Hospital Epidemiology*, *31*(suppl 1), pp. S7–S10.

86. RSA., 2003. https://www.gov.za/documents/acts/national-health-act-61-2003-23-jul-2004 accessed on the 06 July 2023.

87. Shakibaie, M.R., Adeli, S. and Salehi, M.H., 2014. Antimicrobial susceptibility pattern and ESBL production among uropathogenic *Escherichia coli* isolated from UTI children in the pediatric unit of a hospital in Kerman, Iran. *British Microbiology Research Journal*, *4*(3), pp.262-271.

88. Shannon, G., Jansen, M., Williams, K., Cáceres, C., Motta, A., Odhiambo, A., Eleveld, A. and Mannell, J., 2019. Gender equality in science, medicine, and global health: where are we at and why does it matter? *The Lancet*, *393*(10171), pp.560-569.

89. Singhal, S., Mathur, T., Khan, S., Upadhyay, D.J., Chugh, S., Gaind, R. and Rattan, A., 2005. Evaluation of methods for AmpC β-lactamase in gram-negative clinical isolates from tertiary care hospitals. *Indian Journal of Medical Microbiology*, *23*(2), pp.120-124.

90. Sorsa, A., Früh, J., Stötter, L. and Abdissa, S., 2019. Blood culture result profile and antimicrobial resistance pattern: a report from neonatal intensive care unit (NICU), Asella teaching and referral hospital, Asella, south East Ethiopia. *Antimicrobial Resistance & Infection Control*, *8*, pp.1-6.

91. Spellberg, B. and Gilbert, D.N., 2014. The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. *Clinical Infectious Diseases*, *59*(suppl_2), pp. S71-S75.

92. Suay-García, B. and Pérez-Gracia, M.T., Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. *Antibiotics* (Basel). 2019; 8: 122.

93. Sykes, J.E. and Papich, M.G., 2013. Anti-infective Therapy. *Canine and Feline Infectious Diseases*, p.46.

94. Tansarli, G.S., Poulikakos, P., Kapaskelis, A. and Falagas, M.E., 2014. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among Enterobacteriaceae in Africa: evaluation of the evidence—systematic review. *Journal of Antimicrobial Chemotherapy*, *69*(5), pp.1177-1184.

95. Tewari, R., Mitra, S., Venugopal, N., Das, S., Ganaie, F., Sen, A., Shome, R., Rahman, H. and Shome, B.R., 2019. Phenotypic and molecular

characterization of extended spectrum β-lactamase, ampc β-lactamase and metallo β-lactamase producing Klebsiella spp. from farm animals in India. *Indian Journal of Animal Research*, *53*(7), pp.938-943.

96. Thomson, J.M. and Bonomo, R.A., 2005. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: β-lactams in peril!. *Current Opinion in Microbiology*, *8*(5), pp.518-524.

97. Tschudin-Sutter, S., Lucet, J.C., Mutters, N.T., Tacconelli, E., Zahar, J.R. and Harbarth, S., 2017. Contact precautions for preventing nosocomial transmission of extended-spectrum β-lactamase–producing *Escherichia coli*: a point/counterpoint review. *Clinical Infectious Diseases*, *65*(2), pp.342-347.

98. Tumbarello, M., Sanguinetti, M., Montuori, E., Trecarichi, E.M., Posteraro, B., Fiori, B., Citton, R., D'Inzeo, T., Fadda, G., Cauda, R. and Spanu, T., 2007. Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-β-lactamase-producing Enterobacteriaceae: importance of inadequate initial antimicrobial treatment. *Antimicrobial Agents and Chemotherapy*, *51*(6), pp.1987-1994.

99. Tzouvelekis, L.S., Markogiannakis, A., Psichogiou, M., Tassios, P.T. and Daikos, G.L., 2012. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clinical Microbiology Reviews*, *25*(4), pp.682-707.

100. Umadevi, S., Kandhakumari, G., Joseph, N.M., Kumar, S., Easow, J.M., Stephen, S. and Singh, U.K., 2011. Prevalence and antimicrobial susceptibility pattern of ESBL-producing gram-negative bacilli. *Journal of Clinical and Diagnostic Research*, *5*(2), pp.236-239.

101. Vrancianu, C.O., Gheorghe, I., Czobor, I.B. and Chifiriuc, M.C., 2020. Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of *Acinetobacter baumannii*. *Microorganisms*, *8*(6), p.935.

102. Wan, S., Xiang, Y.I., Fang, W., Zheng, Y., Li, B., Hu, Y., Lang, C., Huang, D., Sun, Q., Xiong, Y. and Huang, X., 2020. Clinical features and treatment of COVID-19 patients in northeast Chongqing. *Journal of Medical Virology*, *92*(7), pp.797-806.

103. World Health Organization, 2017. Prioritisation of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis (No. WHO/EMP/IAU/2017.12). World Health Organization.

104. World Health Organization, 2018. *Global Antimicrobial Resistance Surveillance System (GLASS): the detection and reporting of colistin resistance* (No. WHO/WSI/AMR/2018.4). World Health Organization.

105. Yang, Q., Xu, Y.C., Kiratisin, P. and Dowzicky, M.J., 2017. Antimicrobial activity among gram-positive and gram-negative organisms collected from the Asia-Pacific region as part of the Tigecycline Evaluation and Surveillance Trial: comparison of 2015 results with previous years. *Diagnostic Microbiology and Infectious Disease*, *89*(4), pp.314-323.

106. Yang, W., Ding, L., Han, R., Yin, D., Wu, S., Yang, Y., Zhu, D., Guo, Y. and Hu, F., 2023. Current status and trends of antimicrobial resistance among clinical isolates in China: a retrospective study of CHINET from 2018 to 2022. *One Health Advances*, *1*(1), p.8.

Appendix A

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

10 August 2023

Applicant: Ayanda Chiliza
Institution: Central University of Technology
E-mail Address: ayachiliza.ac@gmail.com
Tel: 066 255 2441 **Cell:** 078 587 7188

Project Title: ANTIMICROBIAL SUSCEPTIBILITY ANALYSIS OF GRAM-NEGATIVE ESKAPE ORGANISMS AT INKOSI ALBERT LUTHULI CENTRAL HOSPITAL AND MAHATMA GANDHI MEMORIAL HOSPITAL FROM 2018 TO 2022.
Reference Number: PR2232466

Research Application Type(s):
1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

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

Appendix B

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

Health Sciences Research Ethics Committee

07-Oct-2024

Dear **Ms Ayanda Chiliza**

Ethics Number: UFS-HSD2023/0400/2908-0001

Ethics Clearance: **Antimicrobial susceptibility analysis of Gram-negative ESKAPE organisms at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital from 2018 to 2022**

Principal Investigator: **Ms Ayanda Chiliza**

Department: **CUT - Central University of Technology**

Submission Page

SUBSEQUENT SUBMISSION APPROVED

With reference to your recent submission for ethical clearance from the Health Sciences Research Ethics Committee. I am pleased to inform you on behalf of the HSREC that you have been granted ethical clearance for your request as stipulated below:

- Annual re-approval: The ethical clearance of this project is extended to **06 October 2025.**

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(2020); 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; International Council for Harmonisation (ICH) Harmonised Guideline, Integrated Addendum to ICH E6(R1), Guideline for Good Clinical Practice (GCP) E6(R2), 2016, SAHPRA Guidelines as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

The Principal Investigator (PI) bears final responsibility for the RIMS application. In the event of any misconduct or improper activities perpetuated by a third party, the PI will be held vicariously liable. The HSREC will bear no responsibility or liability for any actions of a PI and/or third party or breach of confidentiality caused by the PI and/or third party.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4012650/9860 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this request for ethical clearance and we wish you continued success with your research.

Yours Sincerely

Dr. C. Armour (Barrett)
Chairperson : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
T: +27 (0)51 401 2650/9860 | E: ethicsfhs@ufs.ac.za
IRB 00011992; REC 230408-011; IORG 0010096; FWA 00027947
Block D, Dean's Division, Room D104 | P.O. Box 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

Appendix C

Central University of Technology, Free State

FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES

March 14, 2023

ATTN: UFS Ethics Committee

Re: Scientific Review

Student: Ayanda Precious Chiliza
Student No: 223052462

To Whom it may concern

This letter serves to confirm that the research protocol, titled, ***"Antimicrobial susceptibility analysis of Extended-Spectrum Beta-Lactamase and Carbapenemase-producing Gram-negative ESKAPE organisms at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital from 2018 to 2022."*** has been reviewed the Faculty Research and Innovative Committee (FRIC) of the Faculty of Health and Environmental Sciences, Central University of Technology on the 13th March 2023 and has been judged to be relevant, designed in accordance with accepted scientific practices and norms.

FRIC resolution reference: FHES 3/13/03

Should you require additional information, please contact Prof TJ Makhafola at jmakhafola@cut.ac.za

Sincerely;

Tel: +27 51 507 3369
Prof TJ Makhafola
Assistant Dean; Research, Innovation and Engagement
Faculty if Health and Environmental Sciences

• Private Bag X20539 • Bloemfontein • SOUTH AFRICA • 9300 •
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Central University of Technology, Free State

FACULTY OF HEALTH AND ENVIRONMENTAL SCIENCES:
DEPARTMENT OF HEALTH SCIENCES

Programme: MHBIO - Biomedical Technology

30 March 2023

Confirmation Letter for AP Chiliza: student number: 223052462

This letter is to certify that AP. Chiliza of student number 223052462 is a student in MHBIO in Biomedical Technology at the Central University of Technology (CUT). I hereby confirm that as her supervisor, all evaluation comments were implemented.

Title of research project

"Antimicrobial susceptibility analysis of Gram-negative ESKAPE organisms at Inkosi Albert Luthuli Central Hospital and Mahatma Gandhi Memorial Hospital from 2018 to 2022".

Kind regards

Dr. P. Makhoahle
Programme: Medical Laboratory Sciences
Senior Lecture: Department of Health Science
Contact Details:
(051) 507 2075
pmakhoahle@cut.ac.za

Appendix D

Count of ORGANISM_NAME	AMIKACIN								
ORGANISM_NAME	SENSITIVE	%	NULL	%	RESISTANT	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1823	75,52%	161	6,67%	209	8,66%	221	9,15%	2414
PSEUDOMONAS AERUGINOSA	979	85,13%	48	4,17%	122	10,61%	1	0,09%	1150
ACINETOBACTER BAUMANNII COMPLEX	126	13,25%	675	70,98%	130	13,67%	20	2,10%	951
ENTEROBACTER CLOACAE COMPLEX	172	64,66%	75	28,19%	6	2,26%	13	4,88%	266

Count of ORGANISM_NAME	AMOXICILLIN_CLAVULANIC_ACID								
ORGANISM_NAME	NULL	%	RESISTANT	%	SENSITIVE	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	153	6,34%	1027	42,54%	759	31,44%	475	19,68%	2414
PSEUDOMONAS AERUGINOSA	1143	99,39%	5	0,43%	1	0,09%	1	0,09%	1150
ACINETOBACTER BAUMANNII COMPLEX	948	99,68%	2	0,21%	1	0,11%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	63	23,68%	196	73,68%	6	2,26%	1	0,38%	266

Count of ORGANISM_NAME	AMPICILLIN_AMOXICILLIN								
ORGANISM_NAME	NULL	%	RESISTANT	%	SENSITIVE	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	365	15,12%	2047	84,80%	2	0,08%	0	0%	2414
PSEUDOMONAS AERUGINOSA	1145	99,56%	5	0,43%	0	0%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	948	99,68%	3	0,32%	0	0%	0	0%	951

ENTEROBACTER CLOACAE COMPLEX	248	93,23%	16	6,02%	1	0,38%	1	0,37%	266

Count of ORGANISM_NAME	CEFEPIME								
ORGANISM_NAME	RESISTANT	%	SENSITIVE	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1177	48,76%	742	30,74%	474	19,63%	21	0,87%	2414
PSEUDOMONAS AERUGINOSA	116	10,09%	821	71,39%	169	14,69%	44	3,83%	1150
ACINETOBACTER BAUMANNII COMPLEX	762	80,13%	96	10,09%	86	9,04%	7	0,74%	951
ENTEROBACTER CLOACAE COMPLEX	47	17,67%	97	36,47%	121	45,49%	1	0,37%	266

Count of ORGANISM_NAME	CEFOTAXIME_CEFTRIAXONE								
ORGANISM_NAME	RESISTANT	%	SENSITIVE	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1555	64,42%	591	24,18%	261	10,81%	7	0,29%	2414
PSEUDOMONAS AERUGINOSA	978	85,04%	2	0,17%	170	14,78%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	815	85,70%	3	0,31%	92	9,67%	41	4,31%	951
ENTEROBACTER CLOACAE COMPLEX	78	29,32%	86	32,33%	98	36,84%	4	1,50%	266

Count of ORGANISM_NAME	CEFOXITIN								
ORGANISM_NAME	NULL	%	SENSITIVE	%	RESISTANT	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	385	15,95%	1445	59,86%	581	24,07%	3	0,12%	2414
PSEUDOMONAS AERUGINOSA	1144	99,48%	1	0,09%	5	0,43%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	948	99,68%	1	0,11%	2	0,21%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	104	39,10%	3	1,13%	159	59,77%	0	0%	266

Count of ORGANISM_NAME	CEFTAZIDIME								
ORGANISM_NAME	RESISTANT	%	SENSITIVE	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1381	57,21%	613	25,39%	367	15,20%	53	2,19%	2414
PSEUDOMONAS AERUGINOSA	98	8,52%	902	78,43%	110	9,56%	40	3,48%	1150
ACINETOBACTER BAUMANNII COMPLEX	770	80,97%	120	12,62%	31	3,26%	30	3,15%	951
ENTEROBACTER CLOACAE COMPLEX	69	25,94%	87	32,71%	106	39,85%	4	1,50%	266

Count of ORGANISM_NAME	CIPROFLOXACIN								
ORGANISM_NAME	RESISTANT	%	SENSITIVE	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1267	52,48%	1028	42,58%	62	2,57%	57	2,36%	2414
PSEUDOMONAS AERUGINOSA	217	18,61%	899	78,17%	13	1,13%	21	1,83%	1150
ACINETOBACTER BAUMANNII COMPLEX	827	86,96%	112	11,78%	8	0,84%	4	0,42%	951
ENTEROBACTER CLOACAE COMPLEX	82	30,83%	180	67,67%	3	1,13%	1	0,37%	266

Count of ORGANISM_NAME	COLISTIN								
ORGANISM_NAME	NULL	%	SENSITIVE	%	RESISTANT	%	INTERMEDIATE	%	Grand Total

KLEBSIELLA PNEUMONIAE	2413	99,96%	1	0,04%	0	0%	0	0%	2414
PSEUDOMONAS AERUGINOSA	172	14,96%	916	79,65%	45	3,91%	17	1,48%	1150
ACINETOBACTER BAUMANNII COMPLEX	93	9,78%	818	86,01%	40	4,21%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	266	100%	0	0%	0	0%	0	0%	266

Count of ORGANISM_NAME	ERTAPENEM								
ORGANISM_NAME	NULL	%	SENSITIVE	%	RESISTANT	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	625	25,89%	1508	62,47%	212	8,78%	69	2,86%	2414
PSEUDOMONAS AERUGINOSA	1147	99,74%	2	0,17%	1	0,09%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	949	99,79%	0	0%	2	0,21%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	128	48,12%	126	47,37%	5	1,88%	7	2,63%	266

Count of ORGANISM_NAME	GENTAMICIN								
ORGANISM_NAME	SENSITIVE	%	RESISTANT	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	997	41,30%	1098	45,48%	289	11,97%	30	1,24%	2414
PSEUDOMONAS AERUGINOSA	846	73,56%	133	11,57%	161	14,00%	10	0,87%	1150
ACINETOBACTER BAUMANNII COMPLEX	170	17,87%	699	73,50%	47	4,94%	35	3,68%	951
ENTEROBACTER CLOACAE COMPLEX	111	41,73%	59	22,18%	96	36,09%	0	0%	266

Count of ORGANISM_NAME	IMIPENEM

ORGANISM_NAME	SENSITIVE	%	RESISTANT	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1752	72,58%	389	16,11%	145	6,01%	128	5,30%	2414
PSEUDOMONAS AERUGINOSA	897	78,00%	156	13,57%	94	8,17%	3	0,26%	1150
ACINETOBACTER BAUMANNII COMPLEX	112	11,78%	806	84,75%	16	1,68%	17	1,79%	951
ENTEROBACTER CLOACAE COMPLEX	208	78,19%	13	4,89%	32	12,03%	13	4,89%	266

Count of ORGANISM_NAME	MEROPENEM								
ORGANISM_NAME	SENSITIVE	%	RESISTANT	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1806	74,81%	446	18,48%	112	4,64%	50	2,07%	2414
PSEUDOMONAS AERUGINOSA	876	76,17%	135	11,74%	91	82,73%	48	4,17%	1150
ACINETOBACTER BAUMANNII COMPLEX	115	12,09%	819	86,12%	13	1,37%	4	0,42%	951
ENTEROBACTER CLOACAE COMPLEX	226	84,96%	10	3,76%	26	9,77%	4	1,50%	266

Count of ORGANISM_NAME	NITROFURANTOIN								
ORGANISM_NAME	NULL	%	RESISTANT	%	INTERMEDIATE	%	SENSITIVE	%	Grand Total
KLEBSIELLA PNEUMONIAE	365	15,12%	900	37,28%	740	30,65%	409	16,94%	2414
PSEUDOMONAS AERUGINOSA	1144	99,48%	5	0,43%	0	0%	1	0,09%	1150
ACINETOBACTER BAUMANNII COMPLEX	948	99,68%	3	0,32%	0	0%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	107	40,22%	18	6,77%	73	27,44%	68	25,56%	266

Count of ORGANISM_NAME	PIPERACILLIN_TAZOBACTAM								
ORGANISM_NAME	SENSITIVE	%	RESISTANT	%	INTERMEDIATE	%	NULL	%	Grand Total
KLEBSIELLA PNEUMONIAE	1107	45,86%	918	38,03%	249	10,31%	140	5,80%	2414
PSEUDOMONAS AERUGINOSA	929	80,78%	187	16,26%	13	1,13%	21	1,83%	1150
ACINETOBACTER BAUMANNII COMPLEX	84	8,83%	850	89,38%	6	0,63%	11	1,16%	951
ENTEROBACTER CLOACAE COMPLEX	103	38,72%	58	21,80%	13	4,89%	92	34,59%	266

Count of ORGANISM_NAME	TIGECYCLINE								
ORGANISM_NAME	SENSITIVE	%	RESISTANT	%	NULL	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	1786	73,99%	37	1,53%	551	22,82%	40	1,66%	2414
PSEUDOMONAS AERUGINOSA	1	0,08%	977	84,95%	170	14,78%	2	0,17%	1150
ACINETOBACTER BAUMANNII COMPLEX	795	83,60%	10	1,05%	92	9,67%	54	5,68%	951
ENTEROBACTER CLOACAE COMPLEX	131	49,25%	4	1,50%	127	47,74%	4	1,50%	266

Count of ORGANISM_NAME	TRIMETHOPRIM_SULFAMETHOXAZOLE										
ORGANISM_NAME	RESISTANT	%	NULL	%	SENSITIVE	%	INTERMEDIATE	%	SENSITIVE DOSE DEPENDANT	%	Grand Total
KLEBSIELLA PNEUMONIAE	1481	61,35%	308	12,76%	620	25,68%	4	0,16%	1	0,04%	2414
PSEUDOMONAS AERUGINOSA	8	0,70%	1137	98,87%	4	0,35%	1	0,09%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	801	84,23%	55	5,78%	93	9,78%	2	0,21%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	84	31,58%	94	35,33%	88	33,08%	0	0%	0	0%	266

Count of ORGANISM_NAME	CEFUROXIME_ORAL								
ORGANISM_NAME	NULL	%	RESISTANT	%	SENSITIVE	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	457	18,93%	1421	58,86%	501	20,75%	35	1,45%	2414
PSEUDOMONAS AERUGINOSA	1148	99,83%	2	0,17%	0	0%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	949	99,79%	2	0,21%	0	0%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	120	45,11%	95	35,71%	27	10,15%	24	9,02%	266

Count of ORGANISM_NAME	CEFUROXIME_PARENTERAL								
ORGANISM_NAME	NULL	%	RESISTANT	%	SENSITIVE	%	INTERMEDIATE	%	Grand Total
KLEBSIELLA PNEUMONIAE	453	18,76%	1420	58,82%	513	21,25%	28	1,16%	2414
PSEUDOMONAS AERUGINOSA	1144	99,48%	6	0,52%	0	0%	0	0%	1150
ACINETOBACTER BAUMANNII COMPLEX	948	99,68%	2	0,21%	1	0,11%	0	0%	951
ENTEROBACTER CLOACAE COMPLEX	118	44,36%	97	36,47%	28	10,53%	23	8,65%	266

Appendix E (Proof if linguistic editing)

CORNELIA GELDENHUYS

✆083 2877088
corrieg@mweb.co.za

15 August 2024

TO WHOM IT MAY CONCERN

Herewith I, **Cornelia Geldenhuys (ID 521114 0083 088)** declare that I am a qualified, accredited language practitioner and that I have edited the following Master's dissertation:

ANTIMICROBIAL SUSCEPTIBILITY ANALYSIS OF GRAM-NEGATIVE ESKAPE ORGANISMS AT INKOSI ALBERT LUTHULI CENTRAL HOSPITAL AND MAHATMA GANDHI MEMORIAL HOSPITAL FROM 2018 TO 2022

by

Ayanda Chiliza
Student number: 223052462

All changes were indicated by track changes and comments **for the author to verify, clarify aspects that are unclear, make the necessary adjustments and finalise.** The editor takes no responsibility in the instance of this not being done. The document remains the final responsibility of the author.

.....
C GELDENHUYS
MA (Lin) cum laude, MA (Mus), BA Hons (French), HED, HDL, UELM

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Appendix F (Turn it in summary)

turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

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By

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Thesis submitted for the fulfilment of the requirements for the Master of Health Sciences in Biomedical Technology

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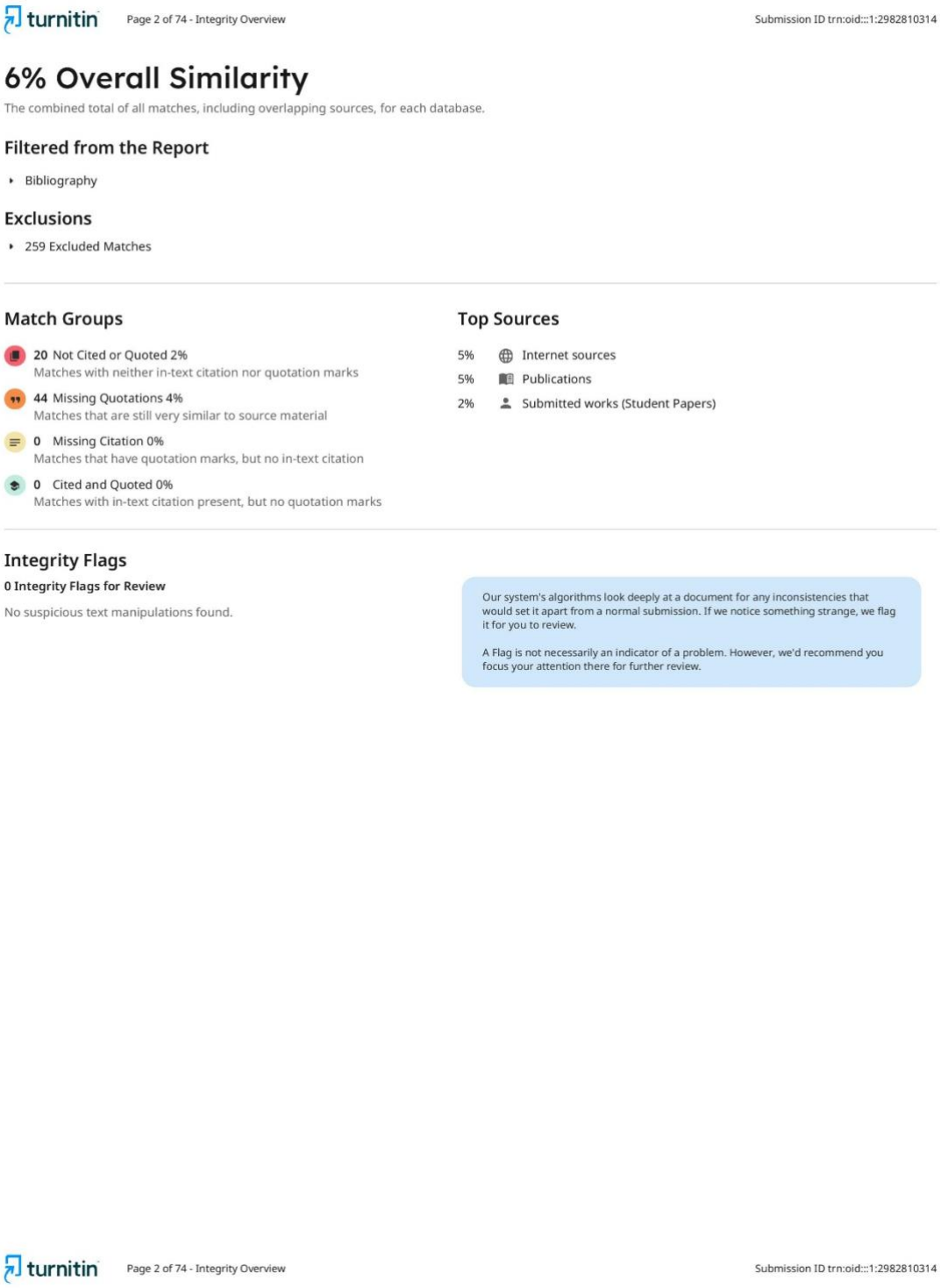

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