
Resistance trends of the Gram-negative ESKAPE pathogens *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. over five years: Northdale, Grey's and Harry Gwala State Hospitals

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BY

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DECLARATION

I hereby declare that the work which is being presented in the thesis titled, "Resistance trends of the Gram-negative ESKAPE pathogens *Klebsiella pneumoniae, Acinetobacter baumannii* complex*, Pseudomonas aeruginosa* and *Enterobacter* spp. over five years: Northdale, Grey's and Harry Gwala Regional Hospitals. This research was carried out in accordance with the regulations of the Central University of Technology, Free State. It is original and is the result of my own work, unless otherwise acknowledged or indicated as referenced work. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Signature:  Date: 18/08/2024

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ABSTRACT

Background and aim

Antimicrobial resistance is one of the greatest risks to human health in the modern era and a major health concern worldwide. Previous studies have shown that certain strains of bacteria have developed resistance to almost all antibiotics. The study aimed to investigate the antimicrobial resistance trends of the Gram-negative ESKAPE (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* species) pathogens in the current era, determine the most prevalent microorganism, and determine which ward harbors the most microorganisms and specific isolates in the Pietermaritzburg (PMB) area of the KwaZulu-Natal (KZN), South Africa (SA), over five years. Antimicrobial resistance is known to be mostly common in patients of long-term acute care (LTAC) facilities due to a multitude of known risk factors. However, there has not been a thorough description and/or investigation of how antimicrobial resistance is at these selected PMB state hospitals.

Methodology

Retrospective data from 1 February 2017 to 31 January 2022 from the Pietermaritzburg National Health Laboratory Service (NHLS) hospitals, namely Grey's, Harry Gwala, and Northdale Hospitals, were collected via the Corporate Data Warehouse (CDW) from 10 303 isolates to assess the resistance trends of Gram-negative ESKAPE (*Enterococcus faecium; Staphylococcus aureus; Klebsiella pneumoniae; Acinetobacter baumannii; Pseudomonas aeruginosa; Enterobacter* species) pathogens from various clinical samples such as wound swabs, blood, sputum, urine, Central Venous Catheter (CVC) tips, soft tissue, and other body fluids (ascitic and synovial fluid, Cerebrospinal fluid).

These samples originated from patients in the hospital wards, intensive care units, and outpatient departments and were previously delivered to the lab for routine diagnostic examinations. Good laboratory practices for microscopy, culture, and susceptibility testing, or isolation, and identification were followed. Once in the NHLS TrakCare database, the test results were retained in the CDW. Information about people diagnosed within the allotted time frame with infections brought on by *Enterobacter* species, *Pseudomonas aeruginosa (P. aeruginosa),*

Klebsiella pneumoniae (K. pneumoniae), and *Acinetobacter baumannii* (*A. baumannii)* complex was sought. The results of microscopy, culture, antibiotic sensitivity testing, and biochemical tests

where specimens were tested, were also received. The NHLS laboratory tests for *K. pneumoniae, A. baumannii complex, P. aeruginosa, and Enterobacter* species were reported. Proper data storage in the Figshare system was always maintained in compliance with the POPIA Act.

Results and Discussion

Among the 10 303 isolates, the *Klebsiella* genus was predominant among all ESKAPE isolates, with n = 4 088 (39,7%). The *Enterobacte*r genus played a limited role, with *E. cloacae* complex being the most frequent in the genus (1 506, 14,6%) and *E. aerogenes* with the least contribution within the ESKAPE pathogen group (240, 2,3%). Overall, most isolates were resistant to third generation cephalosporins, including ceftazidime and ceftriaxone, indicative of extended spectrum beta lactamase (ESBL) production. The findings of this study correlate well with a study done in 2011–2015 in the same area (PMB), which states that ceftriaxone (8 326; 58,3%) and ceftazidime (7 412; 51,9%) were among the third generation cephalosporins that more than 50% of isolates were resistant to (Ramsamy *et al*., 2018). Colistin and tobramycin had the least overall resistance of 2,5% and 8,5%, respectively. This compares well with a 3,1% colistin resistance found in a recent study (Uzairue *et al*., 2022). Ertapenem and nitrofurantoin were the third-most effective antimicrobial agents, with both an overall resistance percentage of 9,5%. Among drugs of beta-lactam inhibitor combinations/beta-lactam, piperacillin/tazobactam (TZP) was better active against the isolates, with a resistance rate of 25,2%, which is indicative of the fact that ESBLs were sensitive to TZP. Even though *P. aeruginosa* showed low resistance to the tested antimicrobial agents during the period of this study, it showed a 98,8% resistance to tigecycline. Tigecycline does not consistently suppress *P. aeruginosa;* however, it appears to be an effective antimicrobial agent in extremely ill and immunocompromised patients. Regarding the prevalence of these resistant organisms according to wards, our data indicated that the ICU and male surgical ward had the highest rates of resistant isolates at 12,1% and 6,9%, respectively. This is in line with recent research findings, which showed that extended hospital stays, and high usage of invasive procedures contribute to the

prevalence of nosocomial and invasive infections in intensive care units (Abubakar and Salman, 2024; Greatorex and Oosthuizen, 2015).

The high resistance trend found in this study supports the need for increased surveillance of hospitalized patients.

Conclusion

This research presents a detailed analysis of the five-year resistance patterns of Gram-negative ESKAPE bacteria in PMB state hospitals. For example, these data show that the *A. baumannii* Complex shows significant fluctuations in resistance rates to numerous antibiotics. These results emphasize the need for effective antibiotic stewardship, robust infection control techniques, and continuous surveillance as antibiotic resistance grows. PMB state hospitals can enhance patient outcomes and public health by implementing these suggestions and controlling and lowering the prevalence of antibiotic-resistant illnesses. The global threat posed by these resistant pathogens is confirmed by the consistency of our results with earlier research, which highlights the importance of collaboration in addressing this public health emergency.

This analysis reveals significant changes in resistance rates for several antibiotics, particularly in the *A. baumannii* complex. These findings underscore the need for continued surveillance, effective antibiotic stewardship, and robust infection control measures to address the growing challenge of antibiotic resistance.

Keywords: antimicrobial agents, ESKAPE, PMB, resistance trends

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LIST OF ABBREVIATIONS

AARMS = Academic Affairs and Research Management System *A.*

***baumannii = Acinetobacter baumannii* complex**

AMR = Antimicrobial Resistance

CA = Carbapenem-acquired, Community-acquired

CarO = Carbapenem- associated resistance protein

CDW = Central Data Warehouse

CF = Cystic Fibrosis

cIBI = Complicated Intra-abdominal Infections

COVID-19 = Coronavirus disease 2019

CpaA = coagulation targeting metallo-endopeptidase of *Acinetobacter baumannii*

CSF = Cerebrospinal Fluid

CRAB = Carbapenem-Resistant *Acinetobacter Baumannii*

CRE = Carbapenem-Resistant Enterobacteriaceae

cUTI = Complex Urinary Tract Infection

CVC = Central Venous Catheter

E. cloacae = Enterobacter cloacae

Etest = Epsilometer test

ESBLs = Extended Spectrum Beta-Lactamases

ESKAPE = *Enterococcus faecium; Staphylococcus aureus; Klebsiella pneumoniae; Acinetobacter baumannii; Pseudomonas aeruginosa; Enterobacter* species

FDA = Food and Drug Administration

HA = Hospital-Acquired

HAI = Hospital-Acquired Infections

HAP = Hospital-Associated Pneumonia

HSREC = Health Sciences Research and Ethics Committee

HV = Hypervirulent

ICU = Intensive Care Unit

KZN = Kwa-Zulu Natal

K. pneumoniae= Klebsiella pneumoniae

KpSC = *Klebsiella pneumoniae* Species Complex

LPS = Lipopolysaccharide

MDR = Multidrug-Resistant

MIC = Minimum Inhibitory Concentration

MRPA = Multidrug Resistant *Pseudomonas aeruginosa*

NHLS = National Health Laboratory Service

MRSA = Methicillin-resistant *Staphylococcus aureus*

NHLS = National Health Laboratory Services

OmpA = Outer membrane protein A

P. aeruginosa = Pseudomonas aeruginosa

PABA = Para-amino benzoic acid

PMB = Pietermaritzburg

POPIA = Protection of personal information act

PPE = Personal Protective Equipment

SA = South Africa

SMZ = Sulfamethoxazole

T2SS = Type II secretion system

UAE = United Arab Emirates

UFS = University of Free State

USA = United States of America

VAP = Ventilator-Acquired Pneumonia

WHO = World Health Organization

XDR = Extreme-drug resistance

PREAMBLE TO THESIS

This thesis determines the Antimicrobial Resistance (AMR) profiles of the Gram-negative ESKAPE pathogens by reviewing laboratory retrospective data from the Central Data Warehouse (CDW).

CHAPTER 1: INTRODUCTION

Global health is at risk due to the rapidly growing number of untreated and undetected multidrug-resistant (MDR) bacterial organisms, as well as the growing number of incurable infections

(Catalano *et al*., 2023). We cannot afford to ignore antimicrobial resistance (AMR) from 2020 and beyond. More than 2,8 million bacterial infections, where the organisms are MDR, meaning resistant to more than three antimicrobial agents, occur annually in the USA, resulting in at least 35 000 fatalities and $20 billion in healthcare expenses (Strathdee *et al*., 2020). A study conducted by (Matta *et al.,* 2018) also states that hospital institutions have become a threatening environment due to increasing numbers of virulent pathogens brought to hospitals from the community through admitted patients. Due to these pathogens, patients are now exposed to indigenous hospital microbes and the microbes of other patients as well.

Klebsiella pneumoniae (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*) complex, *Enterobacter* spp., and *Pseudomonas aeruginosa* (*P. aeruginosa*), as part of the *Enterococcus faecium; Staphylococcus aureus; K. pneumoniae; A. baumannii; Pseudomonas aeruginosa;* and *Enterobacter* spp. (ESKAPE) organisms were identified by the World Health Organization (WHO) in 2017 as the highest-priority organisms for research and the most common hospital-acquired (HA) pathogens causing morbidity (WHO, 2017; Matta *et al*., 2018). Previous studies showed that nosocomial infections increasingly plague healthcare facilities, especially during and post the coronavirus disease 2019 Coronavirus disease 2019 (COVID-19) era (Sikora and Zahra, 2023; Samira *et al*., 2023). The reasons are associated with the prolonged hospitalization of acuity patients, high admission rates resulting in overcrowded facilities, staff shortages due to illnesses, and lack of personal protective equipment (PPE) (Donà *et al.,* 2020). Moreover, the greatest spike in the number of patients infected with nosocomial organisms was among the elderly with pre-existing comorbidities because they required prolonged hospitalization with ventilation support and a higher intake of broad-spectrum antibiotics (Donà *et al*., 2020). Also, one of many challenges imposed by COVID-19 infection has been the vast number of patients needing intensive care (Bardi *et al*., 2021). Much

focus and attention was on the COVID-19 causative agent, the Coronavirus, and less on other infectious agents, such as those that cause nosocomial and community-acquired (CA) infections. This lack of attention imposes a big challenge, because nosocomial infections are among the most

common causes of hospital morbidity and mortality, affecting many worldwide (Su *et al*., 2021). Not enough information is known about the resistance profiles of the Gram-negative ESKAPE organisms in PMB, and more is needed.

1.1 RESEARCH PROBLEM

Antimicrobial resistance has become a worldwide problem due to the escalating evolution of resistance combined with a reduced antimicrobial pipeline. AMR is estimated to be the leading primary cause of mortality worldwide by 2050 if preventive measures are not implemented (Tang et al., 2023). Global estimates show that the number of fatalities directly linked to AMR increased to over 1,2 million in 2019, and if insufficient action is taken to manage AMR, this number is expected to rise to almost 10 million deaths annually by 2050 (Tang *et al*., 2023). However, AMR modeling and prediction of future scenarios were challenged due to the need for more detailed data on resistant profiles of organisms. Hence, accessing the worldwide prevalence of AMR of the pathogen and investigating its resistance developmental pattern will help us interpret what is happening and make an appropriate assessment of future situations. The microorganisms mainly associated with AMR are the ESKAPE pathogens, *K. pneumoniae, A. baumannii, P. aeruginosa, and* Enterobacteriaceae, capable of "escaping" from common antimicrobial treatments (Xie *et al*., 2018). This research will determine the AMR profiles of infection caused by the Gram-negative ESKAPE family pathogens by reviewing laboratory results on the TrakCare laboratory information system.

1.2 AIM OF THE STUDY

To determine the resistance trends of Gram-negative ESKAPE pathogens, namely *K. pneumoniae, A. baumannii* complex, *P. aeruginosa,* and *Enterobacter* spp. in Northdale, Grey's and Harry

Gwala state hospitals over five years.

1.3 SPECIFIC OBJECTIVES

✓ To determine the resistance trends of *K. pneumoniae, Enterobacter* spp*., P. aeruginosa* and A. *baumannii* complex for patients in PMB state hospitals.

✓ To determine the most common ward from which these resistant organisms are isolated.

✓ To determine the antimicrobial profile of these organisms.

CHAPTER 2: LITERATURE REVIEW

In 2017, the WHO released its list of pathogens for which new antimicrobial development is

urgently required to focus and direct research and development on new antimicrobial agents. The ESKAPE Gram-negative pathogens *K. pneumoniae, A. baumannii, P. aeruginosa,* and *Enterobacter* species were given "priority status" on this list (WHO, 2017). During a five-year (2011–2015) study conducted in KZN state hospitals, high levels of antimicrobial resistance were detected among the ESKAPE pathogens, with more than 70% of the *A. baumannii complex* isolates being resistant to all tested antimicrobial agents, except for amikacin, gentamicin, and colistin, which exhibited a resistance of 18%, 68% and 4%, respectively (Ramsamy *et al*., 2018). Also, between 2011 and 2015, more than 50% of *K. pneumoniae* isolates were resistant to third generation cephalosporins, such as ceftazidime at 51,9% (Ramsamy *et al*., 2018). *Enterobacter* spp. was shown to have an average accumulative resistance rate of 5% to available carbapenems. *Enterobacter cloacae* (*E. cloacae*) showed a higher resistance of 16% to ciprofloxacin than *E. aerogenes,* with only 8% (Ramsamy *et al.,* 2018). Further research also suggested that the most common resistance pattern noted in the Gram-negative ESKAPE pathogens was resistance to ceftriaxone and amoxicillin-clavulanate (Ramsamy *et al.,* 2018).

2.1 STRUCTURAL OVERVIEW OF BACTERIA

Two broad categories classify most bacteria, Gram-negative and Gram-positive, as presented in Figure 1. Their reaction to the Gram stain test is based on the composition of the cell wall. The Gram staining method was developed by Hans Christian Gram in 1884, and it identifies bacteria based on how their cell wall reacts to certain chemicals and dyes (Wang *et al.,* 2022).

Gram-negative bacteria have a thin peptidoglycan layer and an outer lipid membrane, whereas Gram-positive bacteria have a thick peptidoglycan layer, impenetrable to the stain (Steward, 2019). The peptidoglycan layer possessed by Gram-positive bacteria makes it possible to retain the crystal violet dye, while the Gram-negative bacteria lose it during decolorization with acetone. The Gram-negative cell wall is then stained red/pink by the counterstain, Safranin dye.

or Carbol Fuchsin (Steward, 2019).

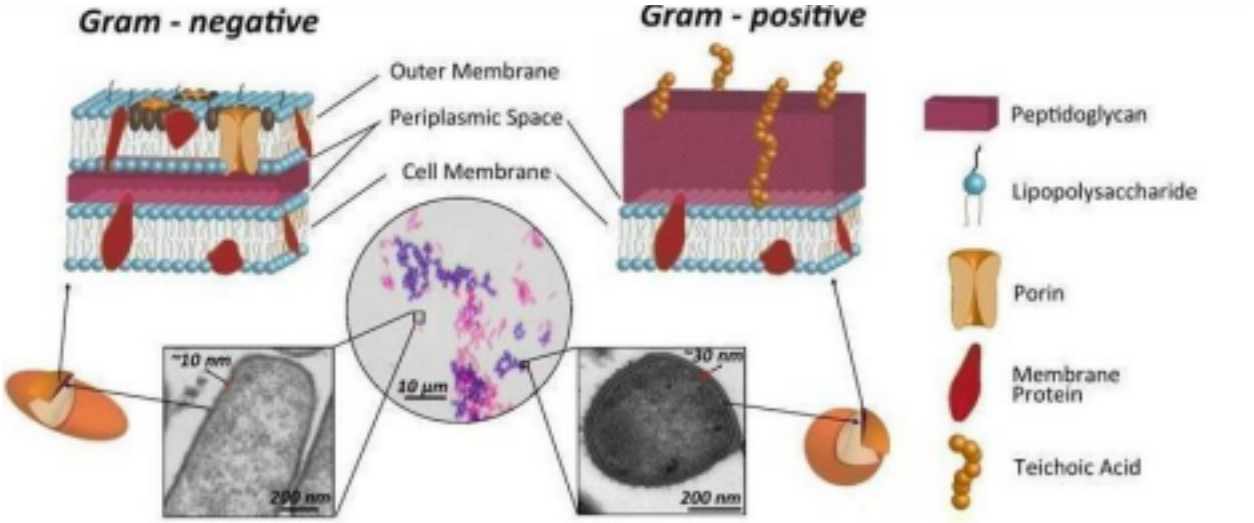


Figure 1: Structural cross-section of Gram-negative vs Gram-positive cell composition (Navid, 2020)

2.2 BACTERIAL ANTIMICROBIAL RESISTANCE MECHANISMS

Gram-negative and Gram-positive bacteria have a variety of defense mechanisms against many of the antimicrobials used in clinical medicine**,** such as alteration of the drug-binding site and membrane permeability, efflux pumps, degradation enzymes, and the drug's conformational change that result in its inactivation and represent a few of the resistance mechanisms (Oliveira and Reygaer*,* 2023).

The Gram-negative bacteria have both an internal and an external membrane (Oliveira and Reygaert*,* 2023). The outer membrane of the Gram-negative bacteria has a major glycolipid layer, the lipopolysaccharide (LPS) capsule, as visible in Figure 1 (Farhana and Khan, 2023). Three sections make up the LPS: The O-antigen polysaccharide, which is linked to the core oligosaccharide and in direct touch with the external environment, and the outer membrane are all held in place by Lipid A, which also serves as an anchor for the molecule (Farhana and Khan, 2023). This LPS enables the antimicrobial intrinsic resistance of Gram-negative bacteria (Jacobson *et al*., 2015; Mobed and Hasanzadeh., 2022).

2.2.1 Treatment of the gram-negative organisms *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species

Resistance microorganisms are difficult to treat, necessitating higher or different doses of

antibiotics, or there may be a scarcity of available effective antibiotics. This has a negative impact on countries at all stages of development (Parmanik *et al.,* 2022). The WHO claims that multidrug-resistant microorganisms, sometimes known as "superbugs", are one of the main threats to public health and cause several million fatalities worldwide each year (Bloom *et al*., 2018)

The WHO released its list of priority pathogens (antibiotic-resistant bacteria) in 2021, with a focus on resistant gram-negative bacteria that represent the greatest risk to human health (WH0, 2024). The list is divided into three categories based on the need for new antibiotics: medium priority, high, and critical. *Enterobacteriaceae, P. aeruginosa,* and *A. baumannii* are among the key groups of MDR bacteria that cause bloodstream infections and pneumonia in hospitalized patients (WHO, 2024).

Pathogen-directed treatments and host-directed therapeutics are the two main therapeutic options available to tackle superbugs. Through modifications to their virulence components, pathogen-directed treatments reduce the toxicity of microorganisms (Parmanik *et al*., 2022). On the other hand, HDT is described as a treatment method based on tactics meant to limit immunopathology and/or enhance the innate or adaptive protective response required for pathogen management. In this regard, the vaccination might be viewed as the quintessential host-directed strategy that prevents bacterial illnesses and combats antibiotic resistance (Tagliabue and Rappuoli., 2018). Moreover, HDT may include any medication that might down-modulate tissue-damaging immune responses and/or activate antimicrobial response effector mechanisms such as autophagy, phagolysosome maturation, ROS formation, and antimicrobial peptide production (Kaufmann *et al.,* 2017).

Carbapenem-resistant *Enterobacteriaceae* (CRE)'s resistance to carbapenem antibiotics is primarily caused by its β-lactamase activity, which works in conjunction with structural alterations to produce carbapenemases, which are enzymes that hydrolyze the antibiotics (Parmanik *et al*., 2022).

A viable option for treating CRE is ursolic acid, a medication that is indicated for nosocomial infections brought on by *Enterobacter cloacae*. It most likely works by preventing the formation of biofilms and interfering with the integrity of bacterial cell membranes (Rajput *et al*., 2022). Additionally, several medication combinations have been effectively used to treat CRE. For instance, meropenem and amikacin were combined to boost the effectiveness against CRE. The

anti-CRE effects of meropenem and polymyxin combination were also investigated (Kulengowski *et al.*, 2018).

Multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) has been refuted successfully by several methods. Because they alter the peptidoglycan structure of the bacteria's outer membrane, lysozyme enzymes such as endolysins and lysins have been shown to be effective against MRPA (Gondil *et al*., 2020). The combination of the β-lactamase inhibitor (tazobactam) and third generation cephalosporin (ceftolozane) demonstrated a low minimum inhibitory concentration (MIC) against *P. aeruginosa* in Etest strips, indicating potential antimicrobial action against MRPA (Kulengowski *et al*., 2018).

The top 50 pharmaceutical companies are developing around 43 novel drugs with strong antibacterial capabilities to treat serious or potentially fatal bacterial infections (WHO, 2019). Of these drugs, 18 have demonstrated the ability to operate as an antibacterial against Gram negative resistant organisms, including *Enterobacteriaceae* and *P. aeruginosa.* The Food and Drug Administration (FDA) has recently approved cefiderocol and eravacycline, respectively, for the treatment of intra-abdominal infections brought on by Gram-negative pathogens such as *P. aeruginosa* and complex urinary tract infections (cUTI). There were previously few alternatives for treating these illnesses (Parmanik *et al*., 2022). Treating patients with severe community acquired pneumonia is extremely challenging. In response, the FDA approved xenleta for use in parenteral and oral forms to treat mild to severe lung disease in people of all ages (Eraikhuemen *et al*., 2021). Furthermore, the FDA authorised the intravenous administration of recarbrio, a combination of relebactam and imipenem–cilastatin, to treat a range of severe infections resistant to antibiotics. These infections include complicated intra-abdominal infections (cIBI), ventilator-acquired and HA bacterial pneumonia caused by Gram-negative bacteria (Heo, 2021).

2.2.2 Antimicrobial resistance mechanisms of the Gram-negative ESKAPE pathogens *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species*

There are primarily four ways that Gram-negative ESKAPE bacteria show antibiotic resistance.

During the antibiotic resistance mechanism, the antibiotics are rendered inactive in cell walls by the bacterial protective enzymes. For example, penicillin-resistant bacteria release the β lactamase enzyme, which inactivates penicillin G (Gaglio *et al*., 2016). These enzymes destroy the amide bond of the beta-lactam ring, rendering the antimicrobial ineffective. When the Gram-negative ESKAPE bacteria alter a metabolic pathway, they typically protect or modify the target site and limit the ability of antibiotics to attach to it, which lowers the affinity of antibiotic molecules. From the precursor para-aminobenzoic acid (PABA), bacteria produce vital folic acid and nucleic acid (Lade and Kim, 2021). Sulfanilamide was the first synthetic broad-spectrum member of the sulfonamide group. With their bacteriostatic effect, these antibiotics interfere with the metabolism of both Gram-positive and Gram-negative bacteria by blocking the production of folic acid, a crucial growth factor for the synthesis of nucleic acids. One of the three components that are joined enzymatically to form folic acid is PABA, a chemical that shares structural similarities with sulfamethoxazole (SMZ), a sulfonamide (Griffith *et al*., 2018). SMZ and PABA are in competition for the active site of the bacterial enzyme. DNA replication is ultimately stopped by this competitive inhibition, which stops nucleic acid synthesis. Certain bacterial species, however, have recently developed mutations that enable them to absorb folic acid from external sources and confer resistance to sulfonamides (Griffith et al., 2018). However, when an antimicrobial agent targets this process, the bacteria use folic acid that has already been prepared to adapt. This limits the effectiveness of antimicrobials that reduce bacterial growth by blocking the PABA pathway, such as sulphonamide. Additionally, bacteria use a drug efflux mechanism to either limit drug permeability from the cell surface or enhance the expression of active efflux pumps, which leads to low-level antibiotic susceptibility. Fluoroquinolone resistance usually results from changes in drug entry and efflux as well as changes in the target enzymes, DNA gyrase and topoisomerase IV. The more vulnerable target is chosen first for mutations. Gram-positive bacteria use topoisomerase IV, while gram-negative bacteria use DNA gyrase (Davin-Regli *et al*., 2013).

The topoisomerases II and IV mutations, particularly in fluoroquinolone antimicrobial drugs, or genetic changes are the primary causes of the *P*. *aeruginosa* pathogen's resistance (Ferreira *et al*., 2020). Type II topoisomerases work by creating a temporary double-strand break that allows them to pass an independent, undamaged double helix (Ferreira *et al.,* 2020).

2.3 MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA

It is crucial to note that MDR bacterial groups are the largest issue facing the healthcare system, given the cosmopolitan nature of Gram-negative bacteria and their capacity to cause infections in humans. MDR Gram-negative infections are among the most significant health problems facing the world today since these organisms do not react well to antimicrobials, which have been all but destroyed by the synthesis of Extended Spectrum Beta-Lactamases (ESBLs) and carbapenemases (Oliveira and Reygaert, 2023).

2.3.1 *Klebsiella pneumoniae*

K. pneumoniae is a Gram-negative, non-motile, lactose-fermenting, aerobic, rod-shaped bacterium that Edwin Klebs first isolated in the late-19th century, and ever since then, this bacterium has always been a known human pathogen (Wen-Liang *et al*., 2023). *Klebsiella pneumoniae* is a highly diverse species, termed *K. pneumoniae* Species Complex (KpSC), which has been introduced to include seven closely related taxa, the most frequently reported in human clinical samples being *Klebsiella quasipneumoniae*, *Klebsiella variicola*, and *K. pneumoniae sensu stricto* (Fostervold *et al*., 2022). *Klebsiella pneumoniae* is a direct hazard to humans as it keeps evolving and becomes more resistant to drugs (Li *et al*., 2024). The widespread acquisition of genes encoding enzymes, such as ESBLs and carbapenemases, which mediate the respective resistance to these crucial drugs, has compromised the efficacy of cephalosporins and carbapenem-class antibiotics, which have been the mainstay of treatment of severe infections caused by Enterobacteriaceae, such as *K. pneumoniae.* Severe infections caused by CRE have been linked to high mortality rates, frequently exceeding 40% (David *et al*., 2020).

2.3.1.1 Klebsiella pneumoniae infections

Over the past few decades, *K. pneumoniae* has been a major human pathogen implicated in healthcare infections (Sharma *et al*., 2023). *K. pneumoniae* was found in serious nosocomial infections such as bloodstream infections, pneumonia, meningitis and wound or surgical site infections, especially in critically ill patients admitted to the Intensive Care Unit (ICU) (Sharma *et*

al., 2023). Whilst hospitalized patients with severe infections are usually the target of K. *pneumoniae* infections, certain hypervirulent (HV) clones can produce invasive infections in the community, frequently in healthy individuals (Aasmund *et al*., 2022). These are referred to as community-acquired (CA) infections. The increase and spread of *K. pneumoniae* strain resistant to several antimicrobials have made the antimicrobial treatment of these illnesses more challenging (Della-Rocca *et al*., 2022).

2.3.1.2 Klebsiella pneumoniae epidemiology

The WHO reported that more than 30% of *K. pneumoniae* infections worldwide and more than 60% in some countries have resistance to third generation cephalosporins (WHO, 2014). A 2004-2009 Study for Monitoring Antimicrobial Resistance Trends (SMART) showed that *K. pneumoniae* has been shown to produce ESBLS at rates of 23,1% in Thailand, 31% in China, and 55,0% in India (Brink *et al*., 2012). An alarming third-generation cephalosporin resistance rate in *K. pneumoniae*, ranging from 8% to 77%, has been reported throughout Africa (WHO, 2014). A study done in KZN reported that data collected between 2011 and 2015 indicated that 54,5% of *K. pneumoniae* isolates were found to be resistant to amoxicillin-clavulanate (Ramsamy *et al*., 2018). Likewise, more than 50% of isolates were found to be resistant to third generation cephalosporins, including ceftazidime (51,9%) and ceftriaxone (58,3%) (Ramsamy *et al*., 2018).

2.3.2 *Acinetobacter baumannii* complex

A. baumannii complex, a Gram-negative coccobacillus, is strictly aerobic, non-fastidious, non-fermenting, and found increasingly in hospitalized patients (Howard *et al*., 2012). The most recent scientific literature has identified it as the second-most common non fermenting Gram-negative microbe isolated from clinical samples after *P. aeruginosa* (Cerqueira and Peleng, 2011). The medical community has been alarmed by its designation as a "red alert" human pathogen in recent years, primarily due to its wide range of antibiotic resistance (Cerqueira and

Peleng, 2011). Patients' isolates habitually show colonization rather than infection (Monteiro-Neto *et al*., 2017).

2.3.2.1 Acinetobacter baumannii infections

Infections caused by *Acinetobacter* mainly occur in wars, tropical environments, and natural disasters. It is found commonly in the burns and intensive care units in hospitals (Monteiro-Neto *et al*., 2017). In addition to hospital-acquired *A. baumannii* complex infections, community-acquired infections due to *A. baumannii* complex are steadily increasing (Monteiro-Neto *et al*., 2017). *Acinetobacter baumannii* complex causes a wide range of infections. Most reported cases involve the respiratory tract, but wound infection, bacteremia and meningitis may also occur. A survey conducted in US hospitals showed that most (57,6%) were isolated from the respiratory tract, followed by 23,9% of the bloodstream and wound or skin of 9,1% in 2010 (Monteiro-Neto *et al*., 2017). *Acinetobacter* species are ranked fifth as ventilator-associated pneumonia (VAP) causative organisms at 6,6% and thirteenth as central-line associated bloodstream-infection causative agents at 2,1% (Monteiro-Neto *et al*., 2017). The WHO ranked carbapenem-resistant CRAB as the priority for antibiotic research and development in 2019 (WHO, 2019). Carbapenem was chosen as a marker because its resistance is often associated with a wide range of co-resistance to other antibiotic classes (Lim *et al*., 2018).

The prevalence of multidrug-resistant strains in patients with *A. baumannii* complex hospital acquired pneumonia (HAP) and VAP is estimated to be 79,9%, ranging from 56,5% in Argentina and 61,8% in Taiwan to 100% in Central America, Pakistan, Lebanon, Qatar, and Croatia (Lim *et al*., 2018).

2.3.2.2 Taxonomy of Acinetobacter baumannii

Martinus Willem Beijerinck, a Dutch bacteriologist, discovered *A. baumannii* in 1911 and described it as a *Micrococcus calcoaceticus* (Torres *et al*., 2010; Asif *et al*., 2018). The same bacteria were isolated numerous times over the next fifty years and published under various names, including *Alcaligens hemolysans, Moraxella lwoffii, Herellea vaginosis,* and Mirococcuscalco-*aceticus* (Asif *et al*., 2018). Due to its non-pigmented nature and incapacity to

move, Brisou and Prevot intended to include it in the genus Achromobacter forty years later (Asif *et al*., 2018). Four years later, the committee on the taxonomy of *Moraxella* and *Allied* Bacteria accepted the classification that Baumann and colleagues had made in 1968, grouping all these isolates into the genus *Acinetobacter* (Bouvet and Grimont, 1987; Asif *et al*., 2018). In 1986, Bouvet and Grimont further divided it into 12 categories based on similarities in their DNA pattern. They are currently taxonomically categorised as y-proteobacteria in the order Pseudomonadales, family Moraxellaceae (Bouvet *et al*., 1987; Nemec *et al*., 2016). Based on their biochemical profile, the Acinetobacter species underwent their first identification test. Using molecular techniques, particularly DNA–DNA hybridisation, at least 33 distinct genospecies of *Acinetobacter* have been identified (Vázquez-López, 2020). Refined taxonomy has been updated continuously since the 1980s, coinciding with the growing awareness and development of *Acinetobacter* as a nosocomial infection (Dijkshoorn, 2007).

There are 59 species in the genus *Acinetobacter*, of which 15 have tentative descriptions, and 11 have defined names (Vijayakumar *et al*., 2019). VITEK technology is still unable to distinguish between *A. baumannii*, *A. nosocomialis*, and *A. pittii* due to their very similar microbiological traits (Ayoub and Hammoudi., 2020). As a result, this study will collectively refer to these three species as the *A. baumannii* complex.

2.3.2.3 Virulence mechanism of Acinetobacter baumannii

Before the mid-1990s, *A. baumannii* was considered a low-virulence bacteria, and its severity was not fully recognized (Wu et al., 2023). However, the *A. baumannii* complex has now emerged as one of the most virulent and significant sources of nosocomial infections, posing a serious threat

to public health (Ma and McClean, 2021). The understanding of *A. baumannii* drug-resistant mechanisms has significantly improved due to advancements in modern medicine and microbiological research equipment and methods. A summary of the known mechanisms underlying *A. baumannii* antibiotic resistance is presented in Figure 2, along with potential avenues for future research. The most common form of resistance in *A. baumannii* is fluoroquinolone resistance, which is caused by spontaneous mutations in the gyrA, gyrB, and parC

genes, encoding topoisomerase IV and gyrase (Wu *et al*., 2023).

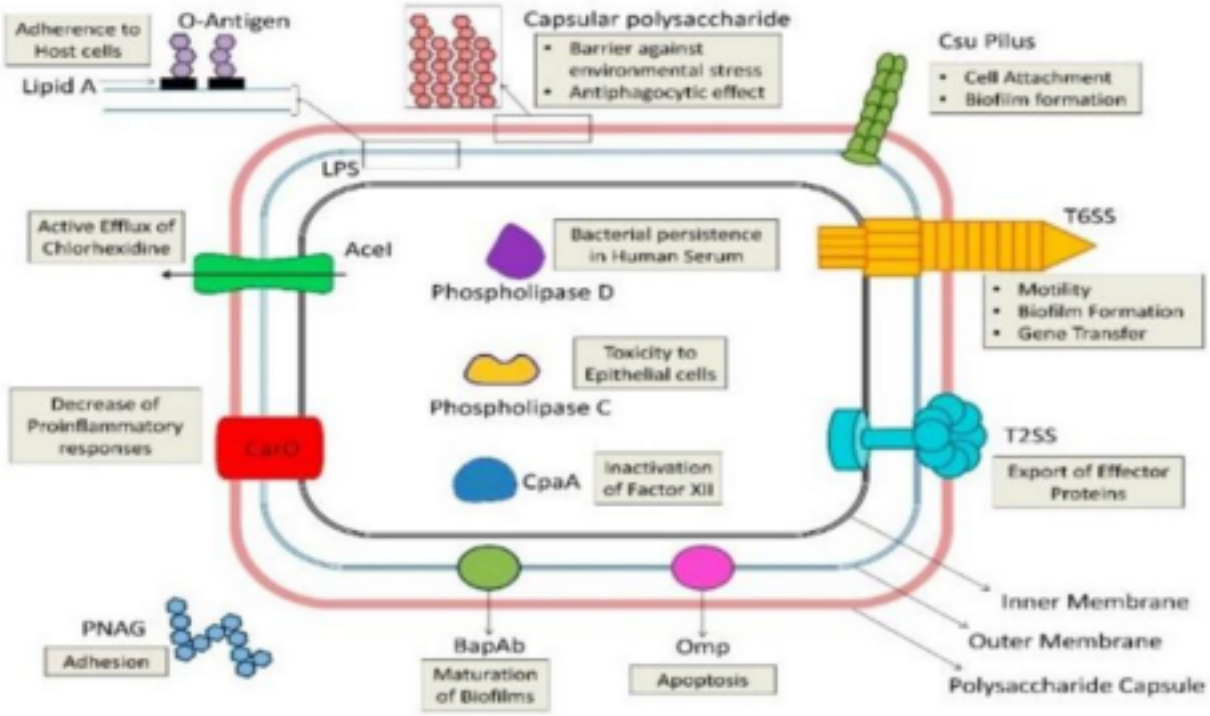


Figure 2: A schematic image of virulence factors possessed by *Acinetobacter baumannii* complex (Ayoub and Hammoudi, 2020). The function of each factor is shown in the adjacent box. CpaA = glycan-specific adamalysin-like protease; AceI = *Acinetobacter* chlorhexidine efflux protein; LPS = lipopolysaccharide; Csu = chaperon/usher pilus system; PNAG = poly-β-1,6-N-acetylglucosamine; Omp = outer membrane protein; T6SS = type VI secretion system; T2SS = type II secretion system

Both apoptosis and cell invasion are mediated by the primary outer membrane protein A (OmpA) of the *A. baumannii* complex (Ayoub and Hammoudi, 2020). OmpA as a non-specific porin which allows passive transportation of many tiny chemical molecules (Choi and Lee*,* 2019). This 38kDA makes the penetration of tiny solutes possible. It attaches itself to the surface of the host cell, localizes itself in the nucleus and mitochondria, and causes cell death (Choi *et al*., 2005). The Omp

33- to 36-kDa protein, which functions as a water channel and whose expression is linked to resistance to carbapenem drugs, is another outer membrane protein of *A. baumannii* (Smani *et al*., 2013). Like Omp 33–36, carbapenem-associated resistance protein (CarO) is involved in *A. baumannii's* resistance to carbapenems (Smani *et al*., 2013).

The CarO protein is a transmembrane beta-barrel that facilitates the entry of carbapenem drugs into *Acinetobacter baumannii* and antibiotic resistance to carbapenem may result from disruption of the carO gene (Kyriakidis *et al.,* 2021). Research has demonstrated that elevated CarO expression postpones pulmonary neutrophil infiltration by reducing proinflammatory reactions in the trachea and lungs, which permit bacterial growth and lead to severe pneumonia (Sato *et al*., 2017).

Gram-negative bacteria's outer membrane contains an LPS, which binds to cells' Toll-like receptor 4 (TLR-4) to cause inflammation. The MyD88/NF-κB signaling pathway is activated when LPS binds to TLR-4. Proinflammatory cytokines like interleukin 1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α) are produced because of this activation response (Ayoub and Hammoudi, 2020). The LPS of *A. baumannii* is critical for resistance to antibodies and provides an advantage over competitors for in vivo survival (Ayoub and Hammoudi, 2020). Additional virulence factors of *A. baumannii* that are known to exist are phospholipases, which are essential hydrolytic enzymes that have a lipolytic action against the phospholipids found in human cell membranes (Camarena *et al*., 2010). A murine pneumonia model has demonstrated that the enzyme phospholipase D facilitates the persistence of *A. baumannii* in human serum, but phospholipase C is harmful to epithelial cells (Camarena *et al*., 2010).

Coagulation targeting metallo-endopeptidase of *Acinetobacter baumannii* (CpaA) is an adamalysin-like protease that is specific to glycans and inhibits blood coagulation by deactivating factor XII. Therefore, CpaA increases the ability of *A. baumannii* to spread by attenuating the production of thrombi in intravascular locations (Waack *et al*., 2018). It has been demonstrated that *A. baumannii* actively pumps chlorhexidine, an antibiotic that breaks down bacterial cell

membranes to combat a variety of microorganisms (Ayoub and Hammoudi, 2020). Such pumping is carried out by the Acinetobacter chlorhexidine efflux protein (AceI), which may enhance survival in harsh environments (Hassan *et al*., 2013). By releasing antimicrobial cells beyond the cell, efflux pumps lower intracellular drug concentrations and decrease sensitivity to certain antibiotics (Bialek-Davenet *et al*., 2015). Among all virulence factors, the development of biofilms has emerged as a key pathogenic characteristic for *A. baumannii*, conferring multidrug resistance to the organism. Biofilms are a group of bacteria that are, by definition, encased in an extracellular material, which makes the bacteria resistant to various stresses such as immune system clearance, desiccation, and antibiotics (Doi *et al*., 2015).

Additionally, *A. baumannii* exports several effector proteins through a type II secretion system (T2SS), which is a protein secretion nanomachine that transports folded proteins through Gram-negative bacteria's outer membrane (Ayoub and Hammoudi, 2020). *A. baumannii* is significant in polymicrobial illnesses because, like many other Gram-negative bacteria, it encodes the type VI secretion system (T6SS), a multi-component secretion mechanism that may inject protein toxins into other bacteria in a contact-dependent manner (Hood *et al*., 2010).

2.3.2.4 Acinetobacter baumannii epidemiology

A five-year retrospective survey done in Hungary states that all 15 *A. baumannii* tested isolates.

were highly resistant to all tested antimicrobials, except for colistin, to which all isolates were fully susceptible (Benkő *et al*., 2020). About 90% of clinical isolates of *A. baumannii* in Southern Europe, the Middle East, Asia, and North Africa are resistant to carbapenems (Wu *et al*., 2023). Over 70% of isolates from Latin America and the Middle East and approximately 45% of isolates worldwide are multidrug-resistant *A. baumannii* (Wu *et al*., 2023). In comparison, its overall mortality can be as high as 56,2%. The patterns of carbapenem resistance differ

throughout Europe and within the countries of the Arab League. An increased incidence of CRAB isolates has been observed in Northern and Eastern Europe and in the Levant countries of the Arab League (Iraq, Jordan, Lebanon, Palestinian territories, and Syria) (Lim *et al*., 2018). The virulence of Acinetobacter is low but can infect those who have chronic infections, are immunocompromised, or have extended hospital stays. *A. baumannii* complex infections are

known for being difficult to treat because of their ability to acquire multiple resistance genes and become multidrug resistant. The prevalence of *A. baumannii* complex infection varies based on the patient's socio-economic status and geographic location.

This opportunistic pathogen's resistance and formidable adaptability have hampered the development of antibiotic therapies, which consequently limits treatment options. Carbapenem resistant *A. baumannii* complex is widespread throughout America and Asia (Ma and McClean, 2021). Vaccines represent a sensible alternative strategy to tackle this pathogen; hence, research into anti-*A. baumannii* complex vaccines have been enhanced in the previous ten years, and different antigens have been researched preclinically with fluctuating outcomes (Ma and McClean, 2021).

Since the 1970s, the *A. baumannii* complex has become progressively typical in mild environments, a shift generally credited to further developed natural steadiness systems and MDR improvements. The local area obtained pneumoniae due to the *A. baumannii* complex, which has been depicted in tropical locales of Asia and Australia among people with a background marked by liquor misuse. *A. baumannii* complex disease rates are similarly low, contrasted with those of other ESKAPE microbes (David *et al*., 2020). Roughly 45% of all worldwide *A. baumannii* complex disconnects are viewed as MDR, with rates surpassing 60% in the US, Latin America, and the Middle East (David et al., 2020). Turkey and Greece have detailed MDR rates exceeding 90%. These degrees of MDR for *A. baumannii* are north of four times higher than those seen in *K. pneumoniae* and *P. aeruginosa* (David *et al*., 2020). A vital part of *A. baumannii's* complex physiology is the inclination to foster fast opposition (David *et al*., 2020).

2.3.3 *Enterobacter* species

The *Enterobacter* genus is a member of the Enterobacteriaceae family and is mainly linked to nosocomial infections and, less commonly, community-acquired infections (Ramirez and Giron, 2023). *Enterobacter* currently comprises 22 species: *E. cloacae, E. cowanii, E. dissolvans, E. arachidis, E. asburiae, E. carcinogenus, E. aerogenes, E. amnigenus, E. gergoviae, E. helveticus, E. hormaechei, E. nimipressuralis, E. oryzae, E. pulveris, E. kobei, E. ludwigii, E. mori, E. pyrinus, E. radicincitans, E. soli, E. turicensis, and E. taylorae*. Seven of these species belong to the group *E.*

cloacae complex: *E. hormaechei, E. kobei, E. cloacae, E. asburiae, E. nimipressuralis, E. ludwigii, and E. mori*. However, not every species is considered harmful to humans (Davin-Regli *et al*., 2019). *E cloacae* is the most frequently isolated species of this genus (Rizi *et al*., 2020).

Enterobacter is a Gram-negative, facultatively anaerobic, rod-shaped bacteria. It is also described as lactose fermenting, flagella-containing, urease-positive, and non-spore-forming (Ramirez and Giron, 2023). This bacterium's virulence is dependent on several factors. Enterobacter attaches itself to host cells using adhesions, just as other Gram-negative enteric bacilli. Bacteria that possess a LPS capsule can evade opsonophagocytosis (Ramirez and Giron, 2023). The LPS capsule can potentially cause sepsis by starting a chain reaction of inflammation in the host cell (Ramirez and Giron, 2023).

When treated with antimicrobials, Enterobacter can regulate several resistance mechanisms through a variety of local and global regulator genes and the alteration of protein expressions, such as that of enzymes (beta-lactamases, etc.) or membrane transporters such as efflux pumps and porins (Davin-Regli *et al*., 2019).

2.3.3.3 Enterobacter species epidemiology

By the 1970s, it was known that Enterobacter species might be nosocomial pathogens, even though strains of Klebsiella and *Escherichia coli (E. coli)* were far more frequently isolated (Patel and Patel, 2016). The genus Enterobacter has a wide range of antimicrobial susceptibility due to its various species. During the year 2022 in Iran, the pooled *Enterobacter* spp. resistance against different antimicrobials was as follows: meropenem (16,2%), Imipenem (16,6%), levofloxacin (48%),

aztreonam (40,9%), norfloxacin (31%), ciprofloxacin (35,3%) and tobramycin [(37,2%) (Khademi *et al*., 2022)]. In Iran, there were 32,8% and 63,1% prevalence rates of *Enterobacter* species that produce ESBLs and MDRs, respectively (Khademi *et al*., 2022). Between the years 2015–2021 in China, *E. cloacae* was found to be resistant to most cephalosporins, such as ceftazidime (32,8%) and cefotaxime (41,6%), according to antimicrobial susceptibility testing. However, the prevalence of resistance to cefepime was relatively low, at about 15,0% (Yan *et al*., 2024). With sensitivity rates exceeding 90%, amikacin, polymyxin B, meropenem, and imipenem

demonstrated the most potent antibacterial activity (Yan *et al*., 2024). In 2020 in Ethiopia, the overall *Enterobacter* spp. resistance rates to cefuroxime and cefepime were 63,2% and 47,5%, respectively (Bitew and Tsige, 2020). Cefoxitin had superior activity among the nine cephalosporins examined, with a resistance rate of 22,7% (Bitew and Tsige, 2020). A rise in resistance patterns of *Enterobacter* spp. Causes longer hospitalization of patients, which exposes them to nosocomial infections.

2.3.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen frequently associated with severe respiratory infections in patients with impaired immunity (Gellatly and Hancock, 2013). It is prevalent in aquatic environments (Williams *et al*., 2010). While *P. aeruginosa* is primarily to blame for 10% of all nosocomial infections, its role in community acquired infections is increasingly recognized.

2.3.4.1 Pseudomonas aeruginosa epidemiology

In 2021, the incidence of multidrug-resistant *P. aeruginosa* was 8,9% in the United States of America and 12% in Alabama (CDC, 2021). Antimicrobial susceptibility percentages for a set of *P. aeruginosa* isolates from Middle Eastern nations (Israel, Jordan, Kuwait and Saudi Arabia) varied from 62,8% for levofloxacin to >90% for amikacin and colistin in 2021 (Alatoom, 2024). Furthermore, in 2021, amikacin resistance was greater in Kuwait (10,7%) and Oman (20,0%) but less than 5% in Qatar, Saudi Arabia and the United Arab Emirates (UAE), comparable to Southern Europe, North America and Southeast Asia (Alatoom, 2024). In the year 2021 in Northwest

Ethiopia, *P. aeruginosa* showed resistance to amikacin at 29,7%, imipenem at 28,4%, ciprofloxacin at 14,9%, ceftazidime at 51,4%, cefepime at 50%, and gentamicin at 62,2%. 45,9% of patients had multi-drug resistance (MDR), and 9,5% had questionable extreme-drug resistance [(XDR) (Asamenew, 2023)].

Pseudomonas aeruginosa's ability to persist chronically in the host and evade antibiotic treatment is mainly due to the pathogen's repertoire of regulatory genes, which account for 8,4% of the 6,3-Mb genome's plasticity and adaptability (Battle *et al*., 2008). *P. aeruginosa* is currently resistant to multiple classes of antimicrobial agents despite being intrinsically resistant to a wide range of antimicrobials (Wu and Li, 2015). Even though AMR rates are still high in the United States, surveillance shows that resistance rates are decreasing (Wu and Li, 2015). *P. aeruginosa* AMR patterns vary significantly across the globe. The regions of North, Central and South America, Western and Central Europe, China, India and Southeast Asia currently have the highest AMR rates for *P. aeruginosa*. *Pseudomonas aeruginosa* lineages ST235 and ST175 have emerged as high-risk, globally dispersed clones with an enhanced capacity to acquire and maintain foreign antibiotic resistance elements (Treepong *et al*., 2018). They continue to be a significant contributor to infections acquired in hospitals. In addition, it is well known that *P. aeruginosa* nosocomial isolates that are resistant to antibiotics of the carbapenem and polymyxin classes are prevalent. Chronic or inherited lung diseases like bronchiectasis and cystic fibrosis (CF) put patients at high risk for MDR selection and persistent pulmonary infection, with occasional exacerbations that necessitate hospitalization and intravenous antibiotics (Williams *et al*., 2010; Reynolds and Kollef, 2021). It has been demonstrated that CF patients' lungs harbor *P. aeruginosa* for over a decade. *P. aeruginosa* thrives in moist environments, so it can be found in many healthcare settings, particularly in chronic wounds, urinary tract devices, and respiratory support, where the formation of biofilms increases the likelihood of persistence, immune evasion, and antimicrobial resistance (Gellatly and Hancock, 2013).

CHAPTER 3: RESEARCH METHODOLOGY

3.1 RESEARCH DESIGN

The COVID-19 pandemic has directly or indirectly impacted all sectors of the economy, but it has been worse for the already overburdened health systems in many nations (Haileamlak, 2021). Therefore, there is a considerable difference when comparing studies done before March 2020

(before COVID-19) and those conducted during and after that period. This study analyzes antibiotic resistance trends in the PMB state hospitals over five years, focusing on four key pathogens*: K. pneumoniae*, *Enterobacter* spp., *P. aeruginosa*, and *A. baumannii* complex. The study will provide insights into the trending of these ESKAPE organisms and their sensitivity profiles over five years, and this may lead to recommendations based on policy reviews and future studies.

3.1.1 Data source

Retrospective data retrieved from the microbiological laboratory result from patient samples obtained from Northdale, Grey's, and Harry Gwala Regional Hospitals in PMB, South Africa. The data spans five years of specimens registered from February 2017 to January 2022.

3.2 LOCATION AND TYPE OF STUDY

This is a prevalence study in South Africa within the KwaZulu-Natal PMB area in the National Health Laboratory Services (NHLS) practice (Grey's, Harry Gwala and Northdale state hospitals). This location was chosen because PMB is the capital city of the KZN Province and hence, one of the tourist destinations. Therefore, antimicrobial resistance of such infections should be known to try and control the actual condition and resistance of these organisms by reviewing their antimicrobial profile, which is the aim of this research. When an area controls infections, tourists and residents feel safe, consequently boosting the economy's revenue.

These three out of many hospitals in the PMB city were chosen because they are state hospitals,

and many residents rely on them for hospitalization and immediate medical care since their services are cheap to almost free of charge. Knowing the prevalence of nosocomial infections causative agents would make the patients feel safe and be aware of what they are putting themselves through.

3.3 DATA COLLECTION AND TOOLS

The data for this research included retrospective data from 1 February 2017 to 31 January 2022. After receiving approval from the NHLS Academic Affairs and Research Management System [(AARMS) (Appendix A5)] and Health Sciences Research Ethics Committee [(HSREC) (Appendix A2)] for data use, data were requested from the CDW. Data for retrospective, consecutive, non-duplicate Microbiology isolates from 1 February 2017 to 31 January 2022 of the Gram-negative ESKAPE pathogens were requested from various clinical samples, namely wound swabs, blood, sputum, urine, Central Venous Catheter (CVC) tips, soft tissue and other body fluids (ascitic and synovial fluid, Cerebro-spinal fluid (CSF). These samples were previously sent to the laboratory for routine diagnostic workups from patients in the hospital's wards, ICU and outpatient departments according to standard methods for isolation, identification and susceptibility testing or microscopy, culture and susceptibility testing following Good Laboratory Practice. The results of the test were then recorded on the TrakCare database of the NHLS and stored in the CDW. The requested data were for patients with *K. pneumoniae*, A.*baumannii* complex, *P. aeruginosa* and *Enterobacter* species infection identified during the mentioned time. Documented NHLS laboratory results recorded for *K. pneumoniae*, *A. baumannii* complex, *P. aeruginosa*, and *Enterobacter* species, microscopy, culture and sensitivity to antibiotics and biochemical tests, where specimens were processed were also received. Data was safely stored in the Figshare system.

3.3.1 Inclusion criteria

The data set includes data on the Gram-negative ESKAPE organisms *K. pneumoniae*, *A. baumannii* complex, *P. aeruginosa* and *Enterobacter* species, isolated from patients of all ages from the three hospitals mentioned in chapter 2 and chapter 3.

3.3.2 Exclusion criteria

All data on infections other than those of Gram-negative ESKAPE pathogens were excluded.

The data collected before 1 February 2017 or after 29 Feb 2022 were excluded.

3.4 DATA STORAGE AND DISPOSAL

The protection of personal information Act (POPIA) governs data collection, management and privacy in this study (POPIA, 2013). An electronic system, Figshare, was used to save the original data. This data is stored confidentially on Figshare and protected by a unique security password created for each research group member upon receipt of data. Data is stored for five years and discarded using an IT specialist to ensure metadata is also deleted.

3.5 STATISTICAL ANALYSIS

After deidentification and pseudo-anonymization, data in Excel format was moved to a safe University of Free State (UFS) OneDrive file, and a link was shared with only the study team members who were able to word it. The data could only be downloaded by the co-supervisor, the owner of the folder, and the principal investigator. The data set was filtered and pre-processed by removing duplicates, handling missing values, and standardizing formats. Descriptive statistics and visualizations were generated, and graphs were used to compare organisms present in different wards and hospitals. The distribution of organisms and susceptibility profiles included descriptive statistics using tabulation and various tables and graphs.

CHAPTER 4: RESULTS

This chapter presents a short overview of the demographic information of the patients for whom the results were analyzed. The patterns of antibiotic resistance were examined in detail for each organism group and the relevant antimicrobials of importance. Attention was given to how these pathogens' antimicrobial resistance increased over the years, and scientific inputs are offered based on the research findings.

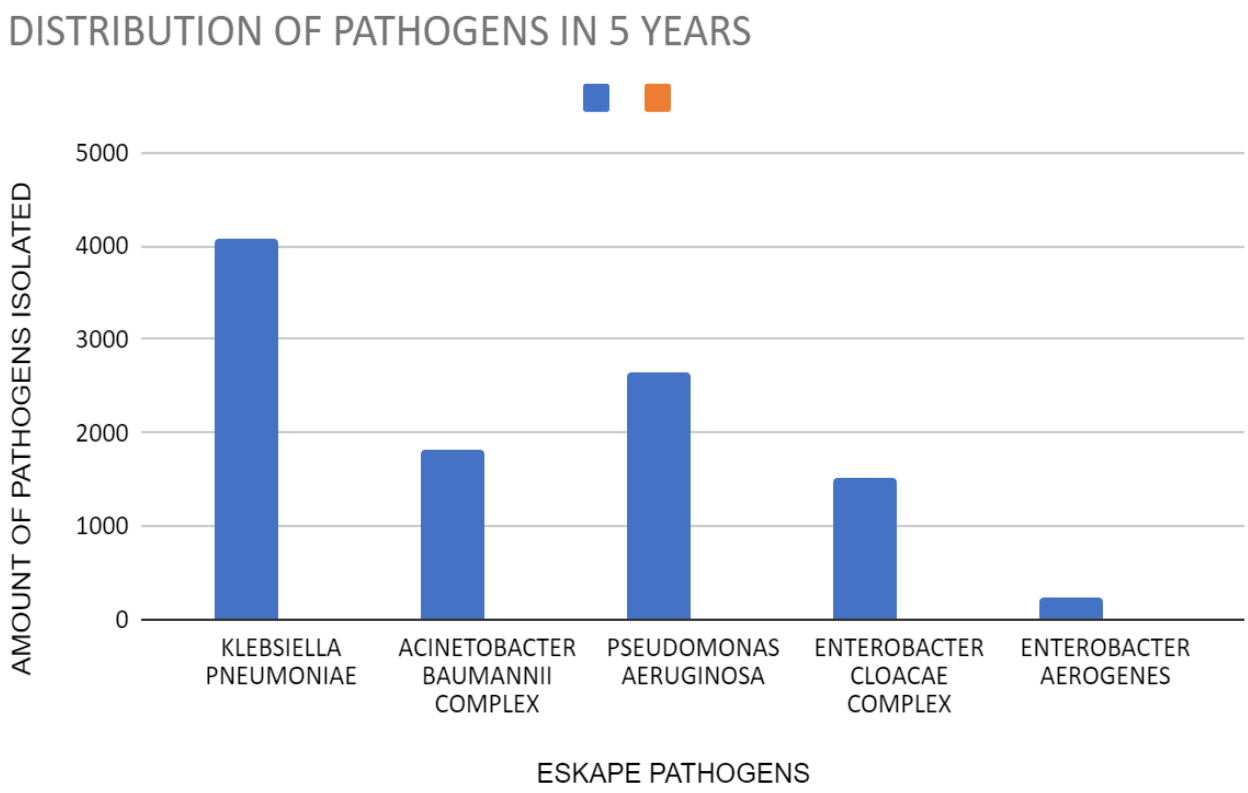
4.1 SEX DEMOGRAPHIC PROFILE

During the study period, a total of 11072 samples were received from the three hospitals, namely Harry Gwala, Grey's, and Northdale were then cleaned to avoid duplication. For patients with more than one sample during their hospital stay and consistent results, only one was included; patients with samples from different sites that had different organisms detected from the sites were kept as two cases. Out of the total 10 303 non-duplicate data entries remaining, 5 006 (48,6%) females, 4 769 (46,3%) males, while the remaining 528 (5,1%) is unknown.

4.2 DISTRIBUTION OF BACTERIAL ISOLATES

The data from 10 303 isolates were examined for the trends of organisms, with particular emphasis on four Gram-negative ESKAPE pathogens: *A. baumannii* complex, *P. aeruginosa, K. pneumoniae*, and *Enterobacter* spp., as shown in Figure 3. The *Klebsiella* genus was predominant among all ESKAPE isolates, accounting for 4088 (39,7%), followed by *P. aeruginosa* at 2656 (25,8%). The *A. baumannii* complex is the second to last least isolated genus with a total of 1813 (17,6%). *Enterobacter* genus played a limited role, with *E. cloacae* complex being the most frequent in the genus with a total of 1 506 (14,6%) and *E. aerogenes* with the least share within the ESKAPE pathogen group at 240 (2,3%).

Figure 3: Distribution of Gram-negative ESKAPE pathogens, *A. baumannii* complex; *P. aeruginosa*; *K. pneumoniae*; and *Enterobacter* spp. in Pietermaritzburg state hospitals from February 2017 to



January 2022.

It is important to know and understand the specific sites where the samples were collected for diagnosis because that makes analysis of the results easy by distinguishing the normal flora from the possible pathogen. Samples were from various clinical sites, as shown in Figure 4, such as endotracheal tubes, wound swabs, and different body fluids, and they were identified for their relevance in the development of resistance.

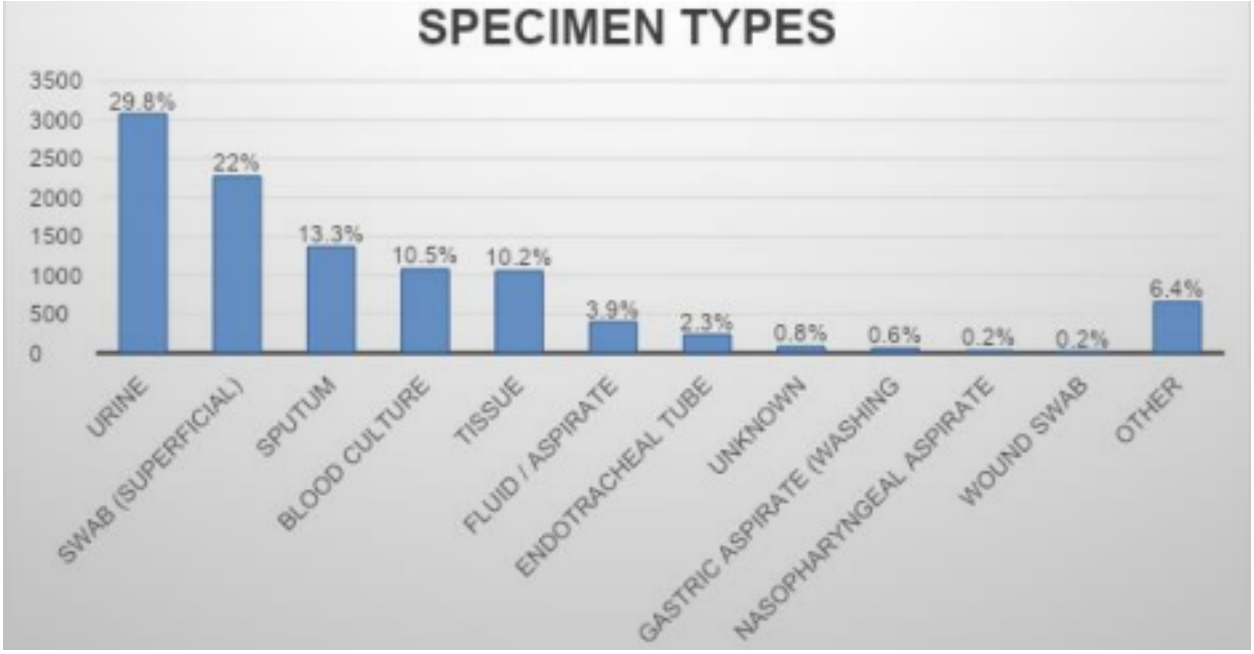


Figure 4: Graphic presentation of various clinical microbiology samples tested for *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Enterobacter* species over the five years, February 2017 to January 2022

The most frequent clinical specimens were urines at 3073 (29,8%), followed by superficial swabs; sputum and blood cultures at 2268 (22%); 1366 (13,3%); and 1077 (10,5%), respectively. Wound swabs were rarely taken at a total of 16, mainly from surgical ward patients. The dominance of *K. pneumoniae* isolates in urine 2013 (19,5%) and blood culture 537 (5,2%*)* samples was observed (Table 1). In urine samples, *E. cloacae* complex was the second-most frequent isolate at 4,5%, whereas in blood cultures, the *A. baumannii* complex was the second most frequent pathogen isolated (2,7%). In tissue specimens, *P. aeruginosa* was isolated the most often (4,9%), followed by *K. pneumoniae* spp. (2,1%).

Table 1: Distribution of ESKAPE pathogens as in the table headings, isolated from clinical samples collected in public sector hospitals in Pietermaritzburg from February 2017–January 2022

SPECIMEN TYPE	P. AERUGINOSA	A. BAUMANNII COMPLEX	E. CLOACAE COMPLEX	E. AEROGENES	K. PNEUMONIAE	GRAND TOTAL
URINE	181	296	464	119	2013	3073
SWAB (SUPERFICIAL)	1127	332	361	37	411	2268
SPUTUM	405	323	151	18	469	1366
BLOOD CULTURE	111	276	138	15	537	1077
TISSUE	507	150	162	15	220	1054
FLUID/ASPIRATE	97	94	102	13	93	399
INTRAVENOUS CATHETER TIP	66	153	51	11	106	387
ENDOTRACHEAL TUBE	52	88	16	3	77	236
TRACHEAL ASPIRATE	32	53	14	4	29	132
UNKNOWN	24	12	10	4	37	87
ABCESS (SUPERFICIAL) ASPIRATE	32	5	8		22	67
GASTRIC ASPIRATE (WASHING) (>DAY 1)	8	9	9		32	58
ARTERIAL CATHETER TIP	4	11	8	1	6	30
NASOPHARYNGEAL ASPIRATE	5	4	2		13	24
CSF	2	2	4		12	20
WOUND SWAB	8	2	1		4	15
PERITONEAL DIALYSIS FLUID	1		1		1	3
STOOL; ABSCESS (SUPERFICIAL) SWAB	1	1				2
SKIN TISSUE	2					2
CORNEAL SCRAPING; RESPIRATORY TRACT ASPIRATE; ASCETIC AND AMNIOTIC FLUID					1	1
DRAIN FLUID (STERILE CAVITY)		1				1
PLEURAL FLUID; BONE; CATHETER URINE AND SWAB (DEEP)			1			1
GRAND TOTAL	2665	1812	1503	240	4083	10303

P. aeruginosa = *Pseudomonas aeruginosa*; *A. baumannii* complex = *Acinetobacter baumannii* complex; *E. cloacae* complex = *Enterobacter cloacae* complex; *E. aerogenes* = *Enterobacter aerogenes*; *K. pneumoniae* = *Klebsiella pneumoniae*.

Corneal scraping, respiratory tract aspirate, ascitic fluid and amniotic fluid all had one *K. pneumoniae* isolated in their respective specimen. Stool and abscess (superficial) swabs had only one *P. aeruginosa* and *A. baumannii* complex isolated, while pleural fluid, bone, catheter urine, and swab (deep) had one *E. cloacae* complex isolated in each specimen.

After determining the most frequent specimens at these hospitals, one needs to understand the type of wards where patients were hosted. Understanding the areas dominated by the Gram negative ESKAPE may assist in infection control measures and treatment regimes in those specific wards. Figure 5 shows a summary analysis of all wards of the three hospitals without being specific at each hospital.

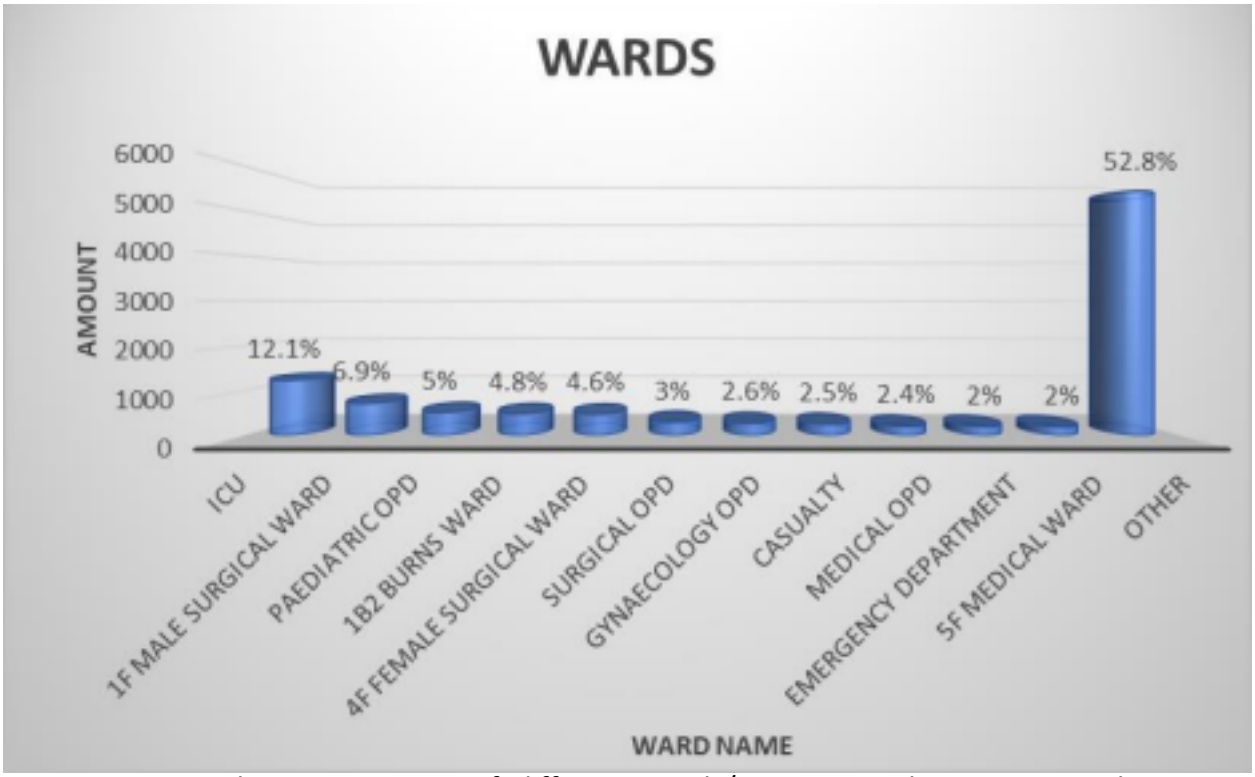


Figure 5: A graphic presentation of different wards/sections in the Pietermaritzburg state hospitals in which various clinical specimens were collected to isolate the ESKAPE pathogens between 2017 and 2022.

In this study, patients admitted to the ICU had the highest number of ESKAPE pathogens isolated from them at 1232 (12,1%), followed by the male surgical ward and pediatric ward at 715 (6,9%) and 516 (5%), respectively. The emergency department and medical ward had the least number of Gram-negative ESKAPE pathogens isolated from it at 2% each. A total of 71 wards had very few samples isolated from each, which was also of no significance to this study. These were added together and totaled 5442 and put under the “other” section of Figure 5.

4.2.1 Comprehensive report on antimicrobial resistance trends of the Gream-negative ESKAPE organisms in the PMB state hospitals

This section presents the main objective, including Objectives 1 and 3 of this study, indicating the resistance profiles and trends for each pathogen over the five-year period. The surveillance antimicrobial activity of *K. pneumoniae*, *P. aeruginosa*, *Enterobacter* spp. and *A. baumannii* complex towards various antimicrobial agents is visualized**,** including the relevant agents for indicating MDR organisms when resistant to three or more drugs, namely amikacin, cefepime, piperacillin-tazobactam, and meropenem in the plots of Table 2.

The resistance profile of the Gram-negative ESKAPE pathogens tabulated in Table 2 is resistance percentages excluding those found sensitive, null (samples not tested for that antimicrobial) and intermediate. It is important to mention that this study focuses mainly on establishing resistance trends of Gram-negative ESKAPE isolates. For a comprehensive analysis containing the entire antimicrobial profile and trends that speak to objective 3 of this study, please refer to Figure 7A-E**.** High resistance levels were found in the ESKAPE pathogens for five years. Overall, most isolates were resistant to third generation cephalosporins, including ceftazidime and ceftriaxone, indicative of ESBL production. Colistin, tobramycin, and ertapenem had the least resistance. The least isolated species, *Enterobacter* species, had the highest resistance to amoxicillin clavulanic acid and cefotaxime with over 98%, but were still highly susceptible to the currently preferred antimicrobials such as tobramycin (0,6%), colistin (0,3%) and ertapenem (3,3%).

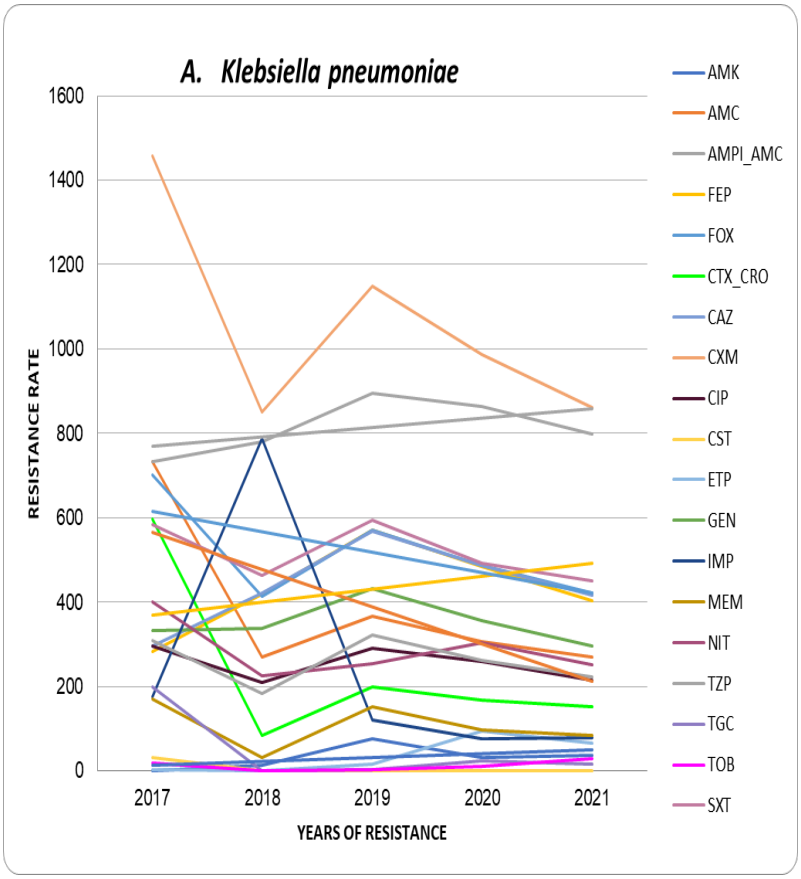
A. baumannii had more than 50% resistance to half of the antimicrobials tested, with cefotaxime_ceftriaxone leading the trend at 88,9%, followed by piperacillin_tazobactam and trimethroprim_sulfamethoxazole at 77,7% and 77,4%, respectively. Higher MDR rates were observed, with isolates showing more than 70% resistance to carbapenems, meropenem and imipenem (74% and 74,5%). This group of bacteria had an overall resistance rate of 42,6% and 32% to the aminoglycosides, tobramycin and gentamicin. Overall, the Gram-negative ESKAPE pathogens exhibited a significant resistance rate of 34,2% to a fluoroquinolone tested, ciprofloxacin. Besides colistin, which exhibited an overall resistance rate of 2,5%, the two antimicrobials that were more effective against this type of bacteria were a piperacillin_tazobactam combination (29,4%) and nitrofurantoin (9,5%).

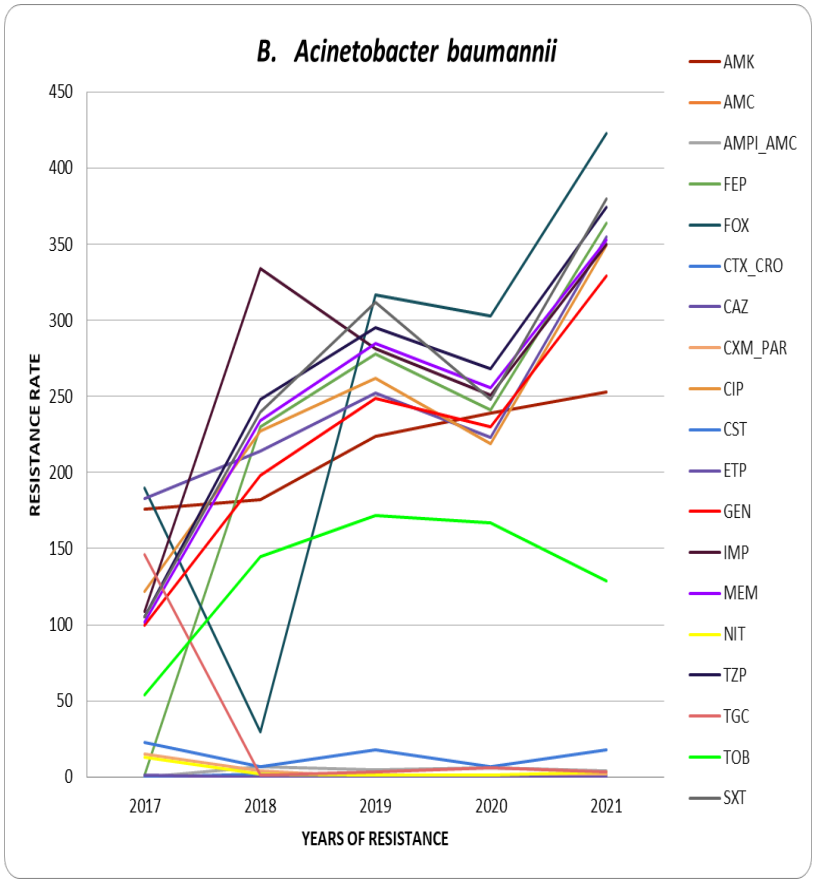
Table 2: Resistance profile of the Gram-negative ESKAPE pathogens (*Klebsiella pneumoniae; Acinetobacter baumannii; Pseudomonas aeruginosa;* and *Enterobacter* species in Pietermaritzburg for 19 antimicrobial agents over 5 years.

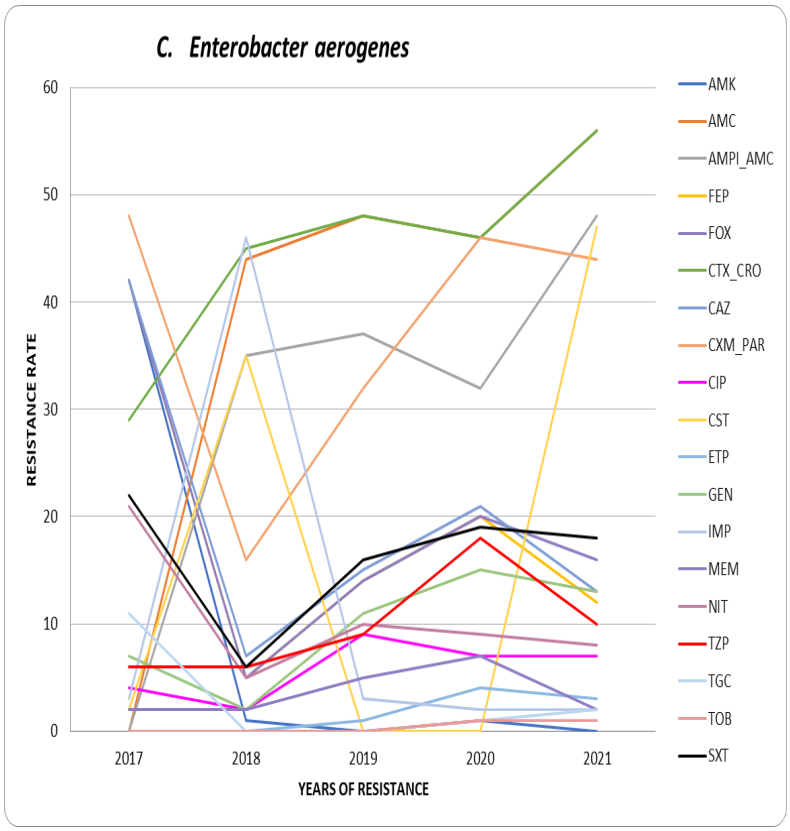
PATHOGENS	AMK	TGC	AMC	AMPI_AMOX	FEP	CTX_CAZ	FOX	CAZ	CXM	CIP	CST	ETP	GEN	IMP	MEM	NIT	TZP	TOB	SXT
P. AERUGINOSA	5,70%	98,80%	11,40%	11,40%	5,90%	96,40%	11,30%	10,50%	11,40%	13,50%	8,20%	0%	5,00%	14,20%	10%	11,50%	12,80%	0,10%	11,80%
	151	2625	304	302	158	2561	299	279	604	360	217	0	234	378	265	306	341	2	314
A. B COMPLEX	58,50%	0,80%	10,10%	10,70%	73,80%	88,90%	10,10%	69,80%	10,20%	70,10%	3,40%	0%	66,30%	74%	74,50%	10,10%	77,70%	40,30%	77,40%
	1061	14	183	194	1339	1612	183	1267	183	1272	62	0	1202	1342	1351	184	1410	731	1405
E. CLOA COMPLEX	0,70%	1,30%	98,50%	77,10%	31,30%	36,60%	98,60%	37,30%	99,20%	19,90%	0,50%	3,20%	26%	4,80%	5,40%	9,30%	25,80%	0,30%	38,30%
	11	19	1483	1161	471	551	1485	561	1466	300	8	48	391	72	82	140	388	5	577
E. AEROGENES	0,80%	1,25%	98,30%	78,80%	23,30%	25,80%	98,70%	26,70%	33,80%	12,10%	0%	3,30%	19,20%	3,70%	7,10%	15,80%	20,40%	0,80%	27,10%
	2	3	236	189	56	62	237	64	81	29	0	8	46	9	17	38	49	2	65
K. PNEUMONIAE	6,10%	11%	36,70%	99%	52,30%	56,10%	17,60%	55,60%	60,50%	55,20%	0,20%	41,20%	43,40%	9,90%	11,10%	0,70%	10,10%	1,10%	60%
	250	448	1498	4044	2135	2293	718	2274	2343	2256	1659	1684	12	404	453	28	413	43	2452

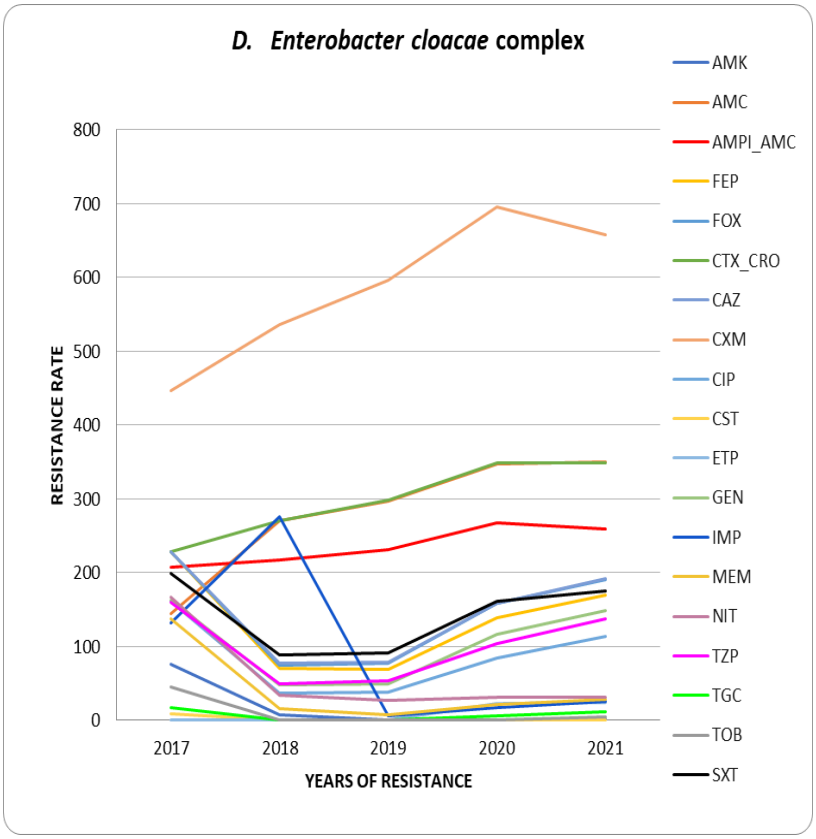
P. aeruginosa = Pseudomonas aeruginosa. A. b complex = *Acinetobacter baumannii* complex. *E. cloacae* complex = *Enterobacter cloacae* complex. *E. aerogenes = Enterobacter aerogenes. K. pneumoniae = Klebsiella pneumoniae.* AMK = amikacin. TGC = tigecycline. AMC = Amoxicillin_clavulanic acid. AMPI_AMC = Ampicillin_amoxicillin. FEP = cefepime. CTX_CRO = cefotaxime_ceftriaxone. FOX = cefotaxime. CAZ = ceftazidime. CXM = cefuroxime (oral and parenteral). CIP = ciprofloxacin. CST = colistin. ETP = ertapenem. GEN = gentamicin. IMP = imipenem. MEM = meropenem. NIT = nitrofurantoin. TZP = piperacillin/tazobactam; TOB = tobramycin. SXT - trimethoprim/sulfamethoxazole.

In addition to Table 2, Figure 6a-e elaborates more on the main objective of this study and shows PMB antimicrobial agent use and resistance trends. Overall, there is a notable and significant incline in resistance in the Gram-negative ESKAPE pathogens during the study period, especially in *A. baumannii* complex.


A. Klebsiella pneumoniae
RESISTANCE RATE
YEARS OF RESISTANCE
2017
2018
2019
2020
2021
AMK
AMC
AMPI_AMC
FEP
FOX
CTX_CRO
CAZ
CXM
CIP
CST
ETP
GEN
IMP
MEM
NIT
TZP
TGC
TOB
SXT


B. Acinetobacter baumannii
RESISTANCE RATE
YEARS OF RESISTANCE
2017
2018
2019
2020
2021
AMK
AMC
AMPI_AMC
FEP
FOX
CTX_CRO
CAZ
CXM_PAR
CIP
CST
ETP
GEN
IMP
MEM
NIT
TZP
TGC
TOB
SXT


C. Enterobacter aerogenes
RESISTANCE RATE
YEARS OF RESISTANCE
2017
2018
2019
2020
2021
AMK
AMC
AMPI_AMC
FEP
FOX
CTX_CRO
CAZ
CXM_PAR
CIP
CST
ETP
GEN
IMP
MEM
NIT
TZP
TGC
TOB
SXT


D. Enterobacter cloacae complex
RESISTANCE RATE
YEARS OF RESISTANCE
2017
2018
2019
2020
2021
AMK
AMC
AMPI_AMC
FEP
FOX
CTX_CRO
CAZ
CXM
CIP
CST
ETP
GEN
IMP
MEM
NIT
TZP
TGC
TOB
SXT

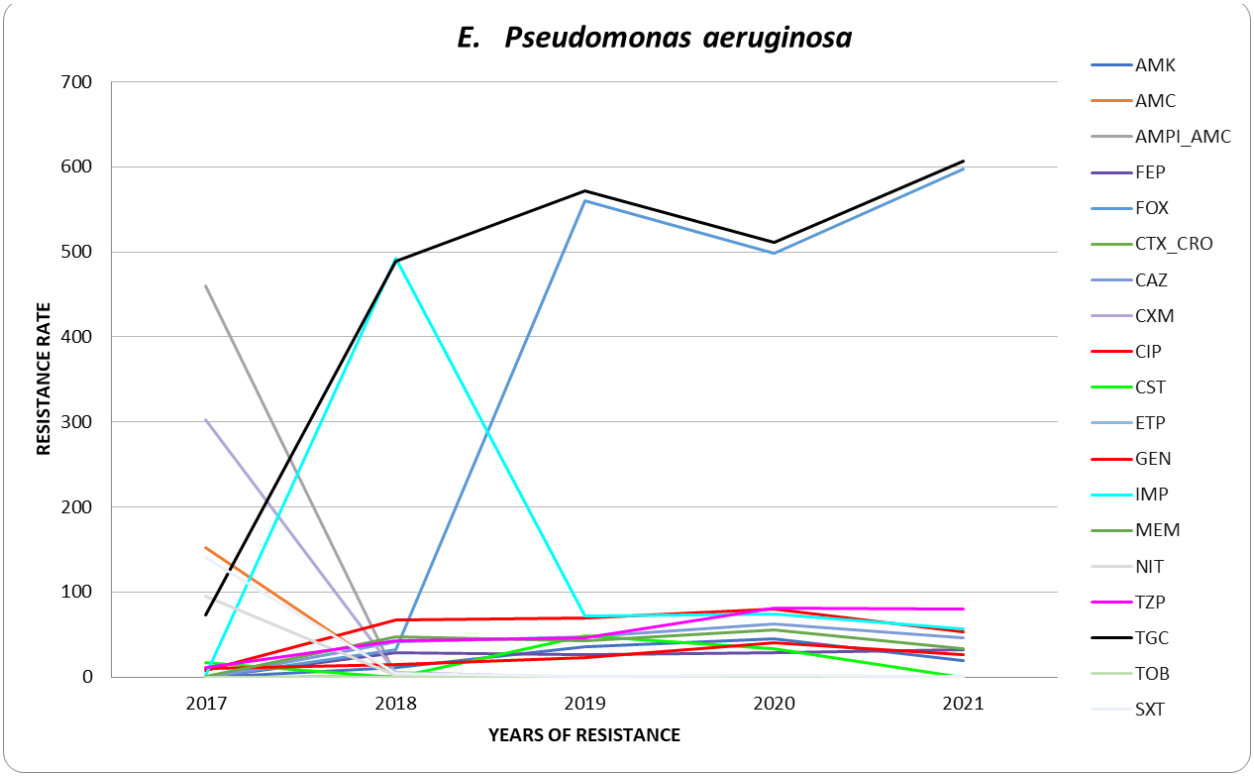
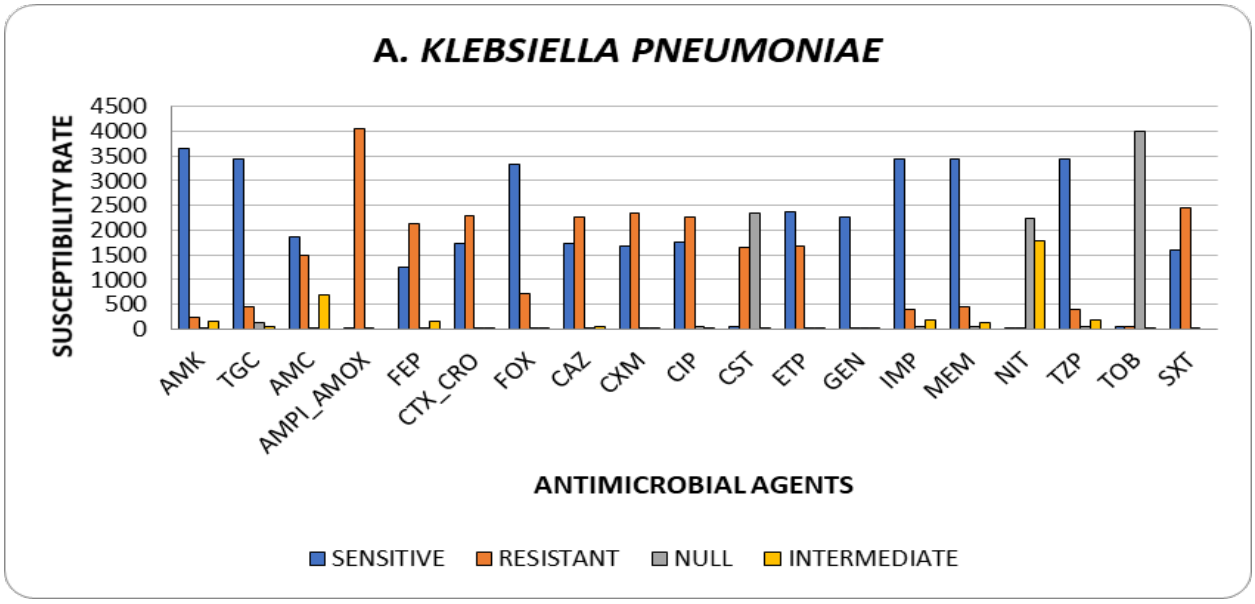
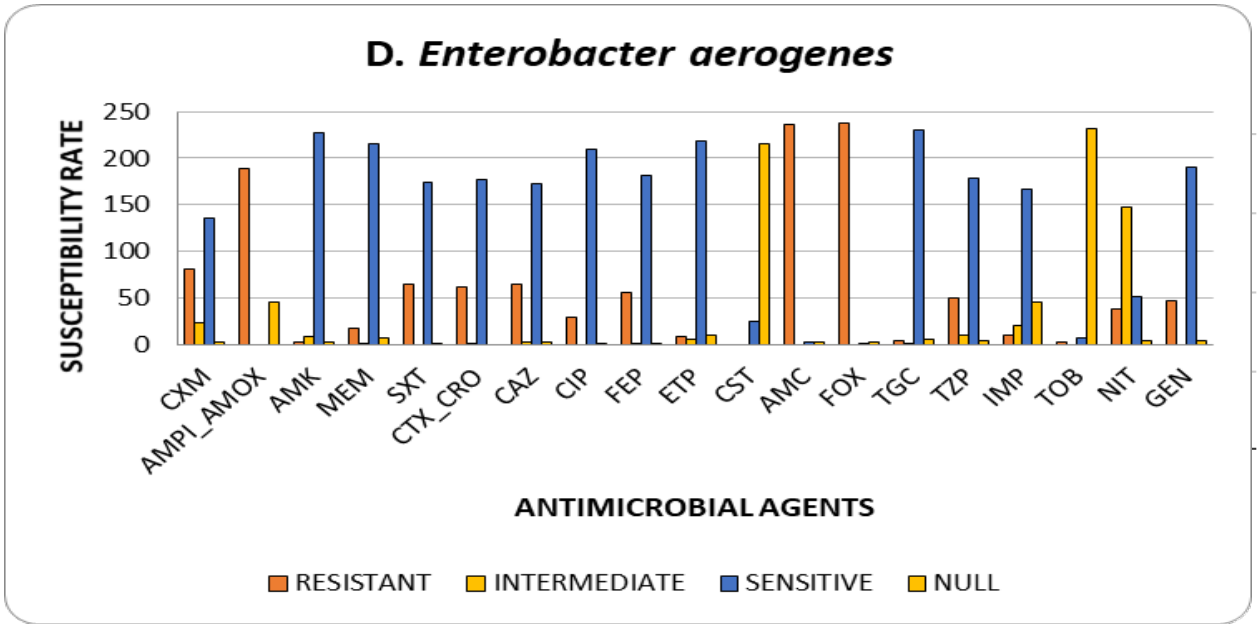


Figure 6A-E: Annual trends of 19 antimicrobial agents' resistance in PMB during the study period. **A**- *Klebsiella pneumoniae,* **B** *Acinetobacter baumannii* complex, **C**- *Enterobacter aerogenes,* **D**- *Enterobacter* cloacae *complex,* and **E**- *Pseudomonas aeruginosa*. Antimicrobial agents' abbreviations: AMK = amikacin. TGC = tigecycline. AMC = Amoxicillin_clavulanic acid. AMPI_AMC = Ampicillin_amoxicillin. FEP = cefepime. CTX_CRO = cefotaxime_ceftriaxone. FOX = cefotaxime. CAZ = ceftazidime. CXM = cefuroxime (oral and parenteral). CIP = ciprofloxacin. CST = colistin. ETP = ertapenem. GEN = gentamicin. IMP = imipenem. MEM = meropenem NIT = nitrofurantoin, TZP=piperacillin/tazobactam; TOB = tobramycin and SXT trimethoprim/sulfamethoxazole


A. KLEBSIELLA PNEUMONIAE
SUSCEPTIBILITY RATE
4500
4000
3500
3000
2500
2000
1500
1000
500
0
AMK
TGC
AMC
AMPI_AMOX
FEP
CTX_CRO
FOX
CAZ
CXM
CIP
CST
ETP
GEN
IMP
MEM
NIT
TZP
TOB
SXT
ANTIMICROBIAL AGENTS
SENSITIVE
RESISTANT
NULL
INTERMEDIATE


D. Enterobacter aerogenes
SUSCEPTIBILITY RATE
250
200
150
100
50
0
CXM
AMPI_AMOX
AMK
MEM
SXT
CTX_CRO
CAZ
CIP
FEP
ETP
CST
AMC
FOX
TGC
TZP
IMP
TOB
NIT
GEN
ANTIMICROBIAL AGENTS
RESISTANT
INTERMEDIATE
SENSITIVE
NULL

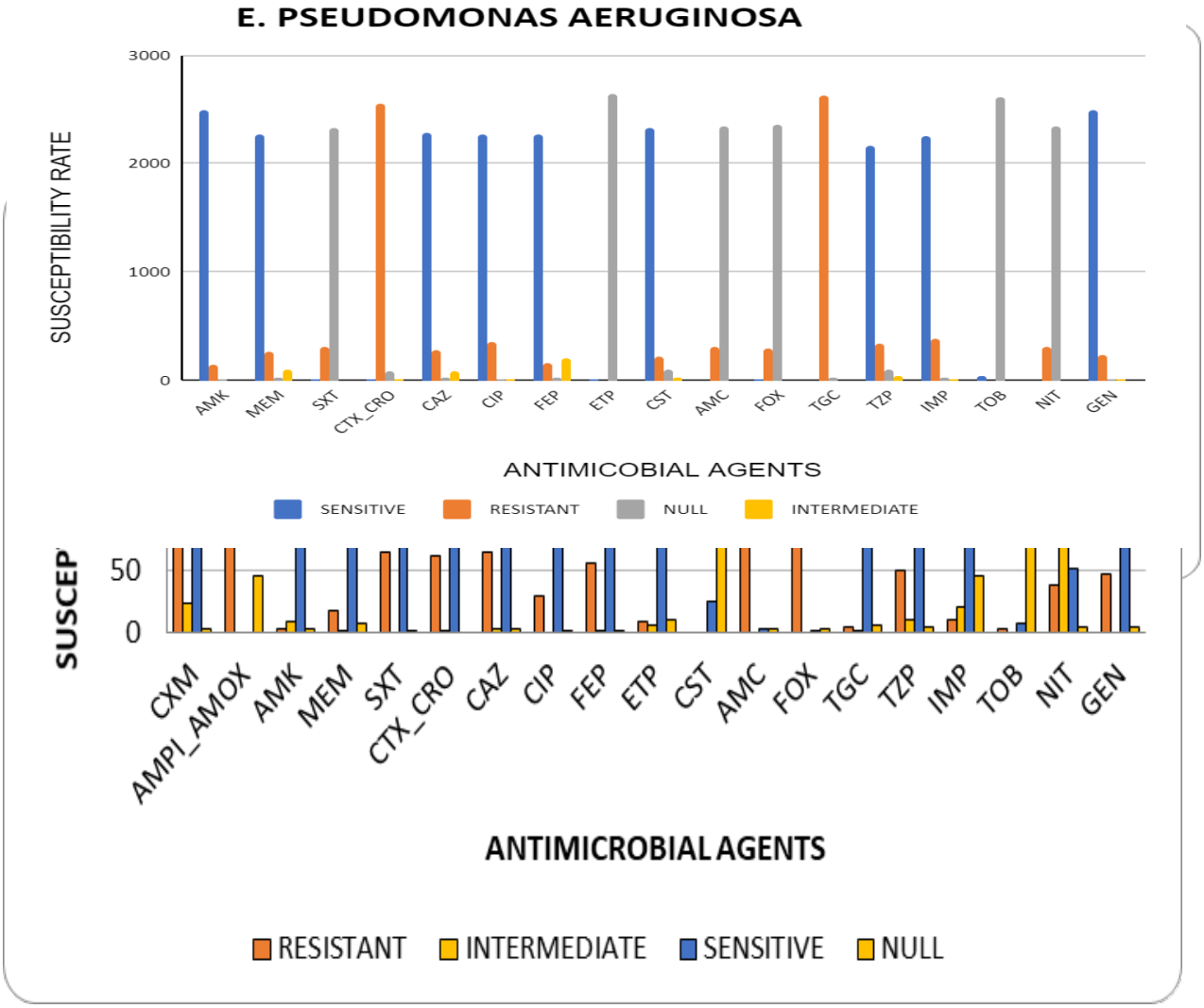

E. PSEUDOMONAS AERUGINOSA
SUSCEPTIBILITY RATE
3000
2000
1000
0
AMK
MEM
SXT
CTX_CRO
CAZ
CIP
FEP
ETP
CST
AMC
FOX
TGC
TZP
IMP
TOB
NIT
GEN
ANTIMICOBIAL AGENTS
SENSITIVE
RESISTANT
NULL
INTERMEDIATE
SUSCEP
50
0
CXM
AMPI_AMOX
AMK
MEM
SXT
CTX_CRO
CAZ
CIP
FEP
ETP
CST
AMC
FOX
TGC
TZP
IMP
TOB
NIT
GEN
ANTIMICROBIAL AGENTS
RESISTANT
INTERMEDIATE
SENSITIVE
NULL

Figure 7A-E: Comparative analysis showing the susceptibility profile of the Gram-negative ESKAPE pathogens' (a - *Klebsiella pneumoniae*; b - *Acinetobacter baumannii* complex; c - *Enterobacter cloacae* complex; d - *Enterobacter aerogenes*; and e - *Pseudomonas aeruginosa*) antimicrobial profile and trend against 19 antibacterial agents in Pietermaritzburg state hospitals over five years.

AMK = amikacin. TGC = tigecycline. AMC = Amoxicillin_clavulanic acid. AMPI_AMC = Ampicillin_amoxicillin. FEP = cefepime. CTX_CRO = cefotaxime_ceftriaxone. FOX = cefotaxime. CAZ = ceftazidime. CXM = cefuroxime (oral and parenteral). CIP = ciprofloxacin. CST = colistin. ETP = ertapenem. GEN = gentamicin. IMP = imipenem. MEM = meropenem. NIT = nitrofurantoin. TZP = piperacillin/tazobactam; TOB = tobramycin and SXT trimethoprim/sulfamethoxazole.

The overall susceptibility rate of *K. pneumoniae* was 47,8%. *A. baumannii* had an interestingly low susceptibility rate of 21,3% over five years, with only 28% of specimens not tested for any antimicrobials and less than 5% of the 19 antimicrobial agents showing an intermediate reaction

(Figure 7b). The most effective antimicrobial agent against *A. baumannii* was tigecycline, with a sensitivity rate of 95,5%. *E. cloacae*, as shown in (Figure 7c) had a very high antimicrobial susceptibility rate (52,1%), which qualifies it as the least problematic group of species in the Gram negative ESKAPE pathogens family. However, this pathogen's extremely low susceptibility rate against amoxicillin clavulanic acid (0,01%) cannot be ignored. Similar to the *E. cloacae* complex, *E.* aerogenes showed very little overall resistance to most antimicrobial agents tested for, with amikacin and tobramycin showing the least resistance (both at 0,8%), as shown in Figure 7d. This makes these two antimicrobial agents the most effective after colistin (0%) against Enterobacter species. A large portion (39,2%), as shown in (Figure 7e) of *P. aeruginosa* isolates was not tested for any of these 19 antimicrobials for the duration of this study (null), which makes the significance of the presented antimicrobial profile results unreliable/imprecise. During this study, *P. aeruginosa* (Figure 6e) showed the highest resistance to a last resort antimicrobial against MDR Gram-negative bacteria, colistin (8,2%). However, on the tested portion of *P. aeruginosa*, 41,4% of isolates were sensitive to the tested antimicrobial agents, and only 18,6% were resistant. The remaining 10% accounts for the intermediate reaction of *P. aeruginosa* against antimicrobial agents.

CHAPTER: 5: DISCUSSION

In this study, Gram-negative ESKAPE pathogens were tested against 19 antimicrobial agents. The results reveal high resistance trends that align with global concerns about rising antimicrobial resistance in healthcare settings. Additionally, this study provides some recent research on the impact of the global pandemic on antimicrobial resistance.

Colistin was widely used in the 1940s until it was discovered in the 1970s to be extremely detrimental to the kidneys and nervous system, which resulted in its discontinuation as a systemic antibacterial treatment (Karki *et al*., 2021). Simultaneously, medications with less detrimental side effects were widely available and effective; however, this was transient since resistance to those novel antibiotics emerged. The usage of polymyxins as a last-resort medication has increased due to the growth of Gram-negative pathogens resistant to carbapenem (Arteaga-Livias *et al*., 2020). Between 2006 and 2012, the use of colistin nearly tripled worldwide (Arteaga-Livias *et al*., 2020 and Karki *et al*., 2021). This study showed that colistin was the most effective antimicrobial agent, with an overall resistance of 2,5%, and this compares well with a 3,1% colistin resistance found in a recent study (Uzairue *et al*., 2022).

Among drugs of beta-lactam inhibitor combinations, beta-lactam/TZP was more active against the isolates with a resistance rate of 25,2%, which indicates that ESBLs were sensitive to TZP. Compared to other antibiotics, cephalosporins had a lower susceptibility to the isolates, apart from Cefotaxime, which had the lowest overall resistance rate of 28,4%. Above 45% of the isolates exhibited overall drug

resistance to cephalosporins, including extended beta-lactams. One potential explanation for the rise in drug resistance to this class of antibacterial agents could be the high prevalence rate of ESBL-producing isolates seen in this study. The primary cause of Enterobacteriaceae resistance to beta lactams is their synthesis of beta-lactamases, which can be encoded on plasmids or chromosomes (Bitew and Tsige, 2020).

Extended-spectrum beta-lactamases are enzymes that possess the capacity to hydrolyze and induce resistance to a range of contemporary beta-lactam antibiotics. These antibiotics include extended spectrum cephalosporins, such as ceftriaxone, ceftazidime, and cefotaxime, but not cephamycins such.

as cefoxitin (Ghafourian *et al*., 2015). In the current study, the isolates' overall drug resistance rates to other types of antimicrobial drugs, including the aminoglycoside (gentamicin) and fluoroquinolone (ciprofloxacin), which had an overall resistance rate of 34,4% and 41%, respectively. High percentages of antimicrobial resistance to these isolates correlate with the study of (Lord *et al*., 2021) which shows that ESBLs with plasmid-encoded or chromosomal linked to mobile genetic elements carrying gene encode resistance against additional antimicrobial agents (Lord *et al*., 2021). Also, high levels of drug resistance to fluoroquinolones are particularly concerning because female patients with complex cystitis are often prescribed these drugs. Because fluoroquinolones have significant advantages over co-amoxiclav, notably their pharmacokinetic features, they are also regarded as the primary choice for treating UTI in men (Mostafa and Miller, 2014).

Global public health is seriously threatened by the growth of MDR bacterial pathogens linked to nosocomial and community-acquired illnesses (Anonymous, 2013; Solomon and Oliver, 2014). The challenge is particularly significant with Enterobacteriaceae due to their widespread distribution in the environment and among human hosts, as well as the relative ease with which plasmids containing genes encoding for ESBL and other resistance genes conferring resistance to numerous other classes of antibiotics can be acquired (Brolund, 2014).

K. pneumoniae was the most often isolated Gram-negative ESKAPE bacteria during the study period at 39,7%, and it constituted 65,5% of all ESKAPE pathogens isolated from urine specimens. According to the 2015 Global Surveillance Report of the Healthcare Infections Surveillance Network and the US

National Health Safety Network, 8,7% and 9,9% of all HA infections were attributed to *K. pneumoniae* (Perez and Villegas, 2015). The steady rise in antimicrobial resistance to ampicillin, amoxicillin and cephalosporins reflects inhibitor resistance and ESBL production in *K. pneumoniae*. Similar proportions of ESBL-producing *K. pneumoniae*, at 63,3%, were reported from bloodstream infections over a four-year period in SA (Perovic *et al*., 2015). In another South African study, 78,3% and 75,7% of all *K. pneumoniae* isolates from HA bloodstream and paediatric CA were ESBL producers (Dramowski *et al*., 2015). *K. pneumoniae* resistance rates to third generation cephalosporins in Africa ranged from 8% to 77% (WHO, 2014). *K. pneumonia*e and *E. coli* have been shown to produce ESBL at varying rates in China (31,9% and 65%); Thailand (23,1% and 3,0%);

India (55,0% and 67,0%) (Brink *et al*., 2012). The WHO reports that resistance to third generation cephalosporins observed in K. pneumoniae was greater than 30% worldwide and greater than 60% in some countries (WHO, 2014).

Because most *P. aeruginosa* was isolated from non-sterile sites, this may have contributed to the notable decline in resistance to several anti-pseudomonal antimicrobial drugs in this study. Many clinicians choose not to give systemic antibiotics to a patient who is otherwise clinically healthy since *P. aeruginosa* is known to be a long-term colonizer and to survive for extended periods. Antibiotic resistant Gram-negative pathogens arise and spread due to selection pressure exerted by antibiotics (Donskey, 2006). As a result, the risk of developing multidrug-resistant *P. aeruginosa* is decreased when antimicrobials are not given to colonizing bacteria. In contrast to this low resistance, *P. aeruginosa* shows a 98,8% resistance to tigecycline. Tigecycline does not consistently suppress *P. aeruginosa*; however, in extremely ill and immunocompromised patients, it appears to be a very effective antimicrobial agent (Bassetti *et al*., 2014).

A resistance rate greater than 60% was observed for most *A. baumannii* tested antimicrobials, except for, for example, ertapenem, colistin and tigecycline at 0%, 3,4% and 0,8%, respectively.

The antimicrobial susceptibility testing revealed varying degrees of resistance among the pathogens. *K. pneumoniae* and *A. baumannii* complex showed significant resistance to multiple antibiotics, complicating treatment options. *P. aeruginosa* and Enterobacter spp. also demonstrated notable resistance patterns, though some antibiotics, like colistin for *A. baumannii,* retained efficacy. These

findings are in line with previous studies, such as those by (Boral *et al*., 2022 and Polemis *et al*., 2021), which report similar resistance profiles.

The E. cloacae complex showed minimal resistance to all tested carbapenems (ertapenem, imipenem, and meropenem) with a resistance of 3,2%, 4,8% and 5,4%, respectively. This low resistance picture towards carbapenems is similar to *E. aerogenes*. These results are consistent with a similar study done in KZN between 2011 and 2015, which states that *Enterobacter* spp. was shown to have an average accumulative resistance rate of 5% to available carbapenems (Ramsamy *et al*., 2018). In this study, *E. cloacae* showed a higher resistance of 16% to ciprofloxacin than *E. aerogenes*, with only 8%.

Further research also suggested that the most common resistance pattern noted in the Gram-negative ESKAPE pathogens was resistance to ceftriaxone and amoxicillin-clavulanate (Ramsamy *et al*., 2018). In this section, the third and main objective of this study is elaborated on, which is to determine the resistance profiles and trends for each pathogen over five years, helping to identify critical resistance patterns and potential areas for further investigation. The surveillance antimicrobial activity of *K. pneumoniae, P. aeruginosa, Enterobacter* spp. and *A. baumannii* towards various antimicrobial agents are visualized, including amikacin, cefepime, piperacillin-tazobactam, and meropenem in the plots of (Figure 4 and Table 1). Other study objectives included determining wards with the highest rates of resistance and analyzing the antimicrobial profile of the pathogens. The visualizations (Figure 3, A1) for each antibiotic show resistance trends over the years for various pathogens. These charts can help understand the resistance patterns and identify areas of concern. Figure 3 shows the trends for four sample antibiotics, with each antibiotic representing a different drug class: Ceftazidime representing Cephalosporins; Ciprofloxacin representing Fluoroquinolones; Imipenem; and Vancomycin representing Carbapenems and Glycopeptides, respectively.

The statistical analysis and visualizations indicate significant changes in antibiotic resistance trends for certain antibiotics pre- and post-COVID-19, particularly for the *E. cloacae* Complex (Table 2). These trends suggest increased resistance rates, highlighting the need for continued surveillance and intervention.

An overall picture of this study revealed a significant resistance pattern in *K. pneumoniae* to most

common antimicrobials such as cephalosporins (ceftazidime) and fluoroquinolones (ciprofloxacin) (Table 2). Specifically, resistance to ceftazidime showed a resistance of 55,6%, while ciprofloxacin resistance reached 55,2%. Resistance to carbapenems like imipenem and ertapenem showed varying degrees, with imipenem resistance at a low 9,9% and ertapenem at 41,2% (Table 2). This is in contrast with research by Langford *et al*. (2023), who state that Klebsiella spp. were found to be 88,3% resistant to Carbapenems and Colistin. A study done by Gaibani *et al*. (2022) and Gaibani *et al*. (2021) in Northern Italy showed that Carbapenem (Imipenem and Meropenem) resistance emerged due to gene rearrangement and mutation. In addition to this, Abdelaziz *et al*. (2024) state that several studies have proved that there has been an increase in AMR for different types of bacteria,

including *K. pneumoniae*. These results are consistent with those of Ramsamy *et al*. (2018), who report high levels of resistance to ceftriaxone and amoxicillin-clavulanate among Gram-negative ESKAPE pathogens. The observed resistance trends highlight the growing challenge of treating infections caused by *K. pneumoniae*, especially in the context of the increased empirical use of broad-spectrum antibiotics during the COVID-19 pandemic.

Resistance trends in *P. aeruginos*a varied across different antibiotics. The resistance to ceftazidime showed a gradual rise, while ciprofloxacin resistance remained high throughout the study period. These results correlate with Mesquita *et al*.'s study, showing similar trends, noting increased resistance to fluoroquinolones and piperacillin-tazobactam during and after the pandemic (Mesquita *et al*., 2023). A summary of antimicrobial use from a study conducted by Xia et al. (2023) shows an inclining trend in beta-lactams (such as Piperacillin-Tazobactam) prescription, which is consistent with a prior study that reported that *P. aeruginosa* was becoming more resistant to antibiotics because of improper antibiotic use (Botelho *et al*., 2019). When Mesquita *et al*. (2023) evaluated the antimicrobial susceptibility profile of *P. aeruginosa* in Northeast Brazil, they observed that both in the pre-pandemic and pandemic periods, the strains of *P. aeruginosa* were mainly resistant to fluoroquinolones (ciprofloxacin), which increased from 33,6% to 36,7%. The most significant increase of 13,6% between pre- and post-COVID-19 was noted on piperacillin-tazobactam (Mesquita *et al*., 2023). This PMB study proves ceftazidime resistance to *P. aeruginosa* infections was modest before and after COVID-19, with a gradual rise (Table 2). Piperacillin-tazobactam showed a rise over the previous years following COVID-19. Tobramycin, as an antibacterial drug against *P. aeruginosa*, exhibited low to moderate resistance, while colistin had low resistance overall (Figure 6e and Table 2). It has been observed that during the pandemic, co-infection

with the COVID-19 virus caused a multitude of complications that led to an overload in most hospitals. As an antibacterial drug against *P. aeruginosa*, tobramycin exhibited low to moderate resistance, while colistin had low overall resistance, especially in ICUs (Vrancianu *et al*., 2023). The variability in resistance highlights the adaptive nature of *P. aeruginosa*, necessitating continuous monitoring and tailored antibiotic stewardship programmes.

A. baumannii complex demonstrated high resistance to multiple antibiotic classes (Figure 6b and Table 2). Imipenem and meropenem resistance rates were

notably high, aligning with the findings of (Gaibani *et al*., 2021; Abdelaziz *et al*., 2024), who reported increased carbapenem resistance due to genetic mutations and horizontal gene transfer. The persistence of colistin susceptibility in some isolates is encouraging, but the overall resistance trend underscores the need for alternative therapeutic strategies, as emphasized by (Lim *et al*. 2018).

The drug-resistant *A. baumannii* complex and its susceptibility trends (Figure 2c and Table 1) have increased the complexity of therapeutic and empirical decision-making (Rangel *et al*., 2021; Bassetti *et al.,* 2014). An *A. baumannii* complex study conducted by (Boral *et al*., 2022) in Istanbul, Turkey, proved imipenem, meropenem, and ciprofloxacin resistance rates to be 90,12%; 91,36% and 90,12%, respectively. Colistin, on the other hand, showed complete susceptibility to all isolates. In contrast, a study in Greece conducted by (Polemis *et al*., 2021) showed a decline in Imipenem and meropenem resistance post-COVID-19, which supports this study. Imipenem, meropenem, and ciprofloxacin were used to treat *A. baumannii* complex-related infections (Figure 6b and Table 2), and some years have shown 100% resistance.

Enterobacter spp. exhibited intrinsic resistance to several antibiotics, including amoxicillin and cefoxitin. The resistance to carbapenems such as imipenem and meropenem was relatively low but showed an increasing trend post-COVID-19 (Figure 6c, d and Table 2). This aligns with global data suggesting that carbapenems remain effective against multidrug-resistant *Enterobacter* spp., as reported by (Ramirez and Giron, 2023; Langford *et al.,* 2023), who found 96,5% of Acinetobacter spp. to be multidrug-resistant and 95,9% was carbapenem resistant. However, the emergence of beta lactamase-producing strains poses a significant threat, as highlighted by (Bush and Bradford, 2020).

Enterobacter spp. exhibit a constitutive AmpC beta-lactamase. Therefore, they are intrinsically resistant to amoxicillin, cefoxitin, ampicillin, and first-generation cephalosporins (Bush and Bradford, 2020). An *Enterobacter* infections study done by (Ramirez and Giron, 2023) states that beta-lactams, carbapenems, aminoglycosides, beta-lactamase inhibitors, sulfamethoxazole/trimethoprim, and fluoroquinolones are possible treatments. Although ciprofloxacin resistance varied, it generally showed an upward trend. This trend may be because carbapenems were not really affected by ESBL previously, even though there has been a slight increase in resistance lately, which results from the fact that Enterobacter spp. have now developed beta-lactamases which hydrolyse the beta-lactam ring seen in

cephalosporins and penicillins (Ramirez and Giron, 2023; Table 2). This increased resistance may also be a result of multiple plasmid-mediated enzymes, leading to the development and accessible horizontal transfer of widespread resistance.

5.1 IDENTIFICATION OF THE MOST COMMON WARDS WHERE ESKAPE ORGANISMS WERE ISOLATED

The three PMB state hospitals have numerous wards/sections in total n = 82, as shown in Figure 5. This is mainly because this study period includes a COVID-19 era, and many subsections/temporary wards were created. Most of these wards have very few specimens because they were closed post-COVID-19 pandemic and are included under the ‘’other’’ section of demographics. Also, this number of wards is attributed to these hospitals being huge. Grey’s Hospital is categorized as a tertiary institution with a 510-bed capacity; Harry Gwala Regional Hospital is a regional hospital with the largest bed capacity of 897; and a District hospital, Northdale, has a bed capacity of 431 (KZN-DoH, 2024).

Patients in Intensive Care Units (ICUs) are always at risk from the global issue of antimicrobial agent resistance among Gram-negative bacteria (Strich and Kadri, 2019). Regarding the prevalence of these resistant organisms according to wards, our data indicated that the ICU and male surgical ward had the highest rates of resistant isolates. This is consistent with findings by (Abubakar and Salman, 2023) and (Greatorex and Oosthuizen, 2015), who note that nosocomial and invasive infections are prevalent in ICU settings due to the high use of invasive procedures and prolonged hospital stays. (Chakraborty *et al*., 2023) also conducted a study and obtained 695 and 556 specimens from adult and paediatric ICUs,

respectively.

A prospective cohort study from Nepal found that the prevalence of antibiotic-resistant Gram-negative bacterial infections in their ICUs was strikingly elevated and had a noteworthy correlation with infections related to healthcare and hospital mortality (Siwakoti *et al*., 2018). Among adults and paediatrics, the culture positivity rate was 37% and 40%, respectively, which is also consistent with this study and global findings. Empirical antibiotic treatment in septic patients is difficult due to the global rise in AMR in ICUs (Kalin *et al*., 2023). Intensive Care Units are special and unique because they provide an extremely common use of antimicrobials in a confined environment for seriously ill patients.

The increased presence of multidrug-resistant bacteria in these units underscores the need for stringent infection control practices and targeted antimicrobial stewardship interventions. Limiting an unnecessary overuse of antibiotics and boosting adherence to infection control procedures can be a very effective measure for preventing antimicrobial resistance in ICUs. When developing routine treatment plans for critically ill patients, clinicians should consider antibiotic resistance. Using a multidisciplinary strategy and paying close attention to this issue at the local ICU level will likely prevent the emergence and spread of antibiotic-resistant infections.

5.2 THE IMPACT OF COVID-19 ON ANTIMICROBIAL RESISTANCE

The COVID-19 pandemic has likely influenced antibiotic resistance trends due to increased use of broad spectrum antibiotics and changes in infection control practices. From March to October 2020, almost 80% of COVID-19 patients were prescribed an antibiotic (Vrancianu *et al*., 2023). Although the current study did not focus on the differences/increases in resistance to the antimicrobials in the five years, reports suggest a surge in resistance rates post-COVID-19 (Abubakar, 2020). The study by Abubakar (2020) highlights the urgent need for robust antibiotic stewardship and infection control measures to mitigate further resistance development. Studies by (Monnet and Harbarth, 2020) and (Vrancianu *et al*., 2023) support these observations, noting the pandemic's role in exacerbating antimicrobial resistance due to increased empirical antibiotic use.

Over 29 400 fatalities were reported due to antibiotic-resistant infections commonly linked to healthcare in the first year of the pandemic. Of these, about 40% of the patients contracted the virus while hospitalized (nosocomial infections) (U.S. Special Report, 2022). Some COVID-19 patients

developed a secondary bacterial infection, which necessitated antibiotic therapy, and these patients were often given empiric broad-spectrum antimicrobial therapy (Monnet and Harbarth, 2020).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

This study provides a comprehensive analysis of the resistance trends of Gram-negative ESKAPE pathogens in PMB state hospitals over five years. This analysis reveals significant changes in resistance rates for several antibiotics, particularly in the *A. baumannii* complex. These findings underscore the need for continued surveillance, effective antibiotic stewardship, and robust infection control measures to address the growing challenge of antibiotic resistance. By implementing these recommendations, PMB state hospitals can better manage and mitigate the impact of antibiotic-resistant infections, ultimately improving patient outcomes and public health. The alignment of these findings with existing literature further validates the global challenge posed by these resistant pathogens. It highlights the importance of collaborative efforts in addressing this public health crisis**.**

6.2 RECOMMENDATIONS

6.2.1 Antibiotic Stewardship Program

Building on the current study, enhancing Antimicrobial Stewardship Programmes across healthcare facilities is essential for optimizing antibiotic use and minimizing the development of resistance. Future efforts should focus on training healthcare professionals, implementing antibiotic prescribing guidelines, and monitoring antibiotic use.

6.2.2 Infection control measures

Future studies should incorporate Whole Genome sequencing to identify specific resistance genes and mutations responsible for AMR in the ESKAPE pathogens. This can provide insights into the genetic mechanisms driving resistance and help to develop targeted interventions. Conducting molecular epidemiological studies can help trace the transmission pathways of resistant strains within and between hospitals. Understanding these pathways can inform infection control measures and prevent the spread of resistant pathogens. Increasing awareness about AMR among healthcare providers, patients, and the public through education campaigns can promote responsible antibiotic use and

adherence to infection control practices.

6.2.3 Research and development

Global support for research on the surveillance, monitoring, and discovery of new antibiotics and alternative treatments to combat resistant infections effectively is of utmost importance. There is a critical need for the development of new antibiotics that are effective against multidrug-resistant Gram-negative bacteria. Future research should focus on discovering and developing novel antimicrobial agents with unique mechanisms of action. Exploring alternative therapies such as bacteriophage therapy, antimicrobial peptides, and immunotherapies can provide new treatment options for infections caused by MDR pathogens. These alternatives could complement existing antibiotic therapies and reduce reliance on traditional antibiotics.

Conducting longitudinal studies to assess the long-term impact of the COVID-19 pandemic on AMR trends will be important. These studies can help understand the changes in resistance patterns and guide future preparedness for similar global health crises. Investigating the impact of COVID-19-related treatments, such as the use of broad-spectrum antibiotics and other therapeutics on AMR development, can provide valuable insights for managing future pandemics.

6.2.4 Education and training

Providing ongoing education and training for healthcare workers on the importance of antimicrobial agent stewardship and infection control practices is paramount to prevent further increase in resistance. Future research should aim to expand surveillance networks to include more hospitals and regions within South Africa. This would provide a more comprehensive understanding of antimicrobial resistance patterns and facilitate timely interventions.

Implementing standardized reporting mechanisms for AMR data across healthcare facilities will ensure consistent and comparable data collection. This can be achieved through integrated health information systems that facilitate real-time data sharing and analysis.

ETHICS CONSIDERATION

Ethics clearance/approval was obtained from the AARMS and UFS HSREC. After that, the principal investigator obtained data from NHLS Central Data Warehouse (CDW). All data used in this study were treated with strict confidentiality and will not be traceable to any patient or individual. Before supplying the data, the CDW ensured that duplicates were excluded before removing all specific identifiers except for the episode number, which the principal investigator replaced with a unique study number. Confidentiality was ensured through unique reference numbers given to each episode instead of patients' real names, lab numbers or hospital numbers. Data was then entered into Figshare and analyzed. There was no interaction or conduction of surveys with patients or families, and this study respected patients' rights and dignity. None of the participants suffered harm, as this is a retrospective study. There was no invasion of privacy and space.

LIMITATIONS TO THE STUDY

There are several limitations of this study. First, although conclusions drawn from this study are for the PMB area, the private health sector was not included, and the results only reflect the public health sector in PMB, KwaZulu-Natal, South Africa. Secondly, because this was a retrospective study, it was not possible to access key con-founders, such as the variation in hospital stay duration, previously recorded factors influencing the resistance towards antimicrobial agents, and infection control procedures, all have a vital role to play in antimicrobial resistance rates.

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APPENDICES

TABLE A1: All PMB state hospital wards where Gram-negative ESKAPE pathogens were isolated between February 2017 and January 2022.

Appendix A1: Health Sciences Research Ethics Committee (HSREC) ethics clearance approval letter.

Appendix A2: Central University of Technology Faculty of Research and Innovation Committee letter.

Appendix A3: Title linguistic approval letter. **Appendix A4:**

National Health Laboratory Service data extraction approval letter.

Appendix A4:

Appendix A5a - b: Turnitin report, a title page; b- similarity result.