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Characterization of colistin-resistant Acinetobacter baumannii and Klebsiella pneumoniae isolates from a tertiary-level hospital in Johannesburg, South Africa

Mathew, Anjali Susan

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Characterization of colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa

Anjali Susan Mathew
Student number: 1604479

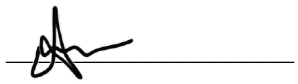
A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of

Master of Science in Medicine in
Clinical Microbiology and Infectious Diseases

Johannesburg,
October 2025

DECLARATION

I, Anjali Susan Mathew, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master in Science in Medicine in Clinical Microbiology and Infectious Diseases at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signed: 

13th day of October 2025

DEDICATION

"***Commit to the Lord whatever you do, and he will establish your plans."***
Proverbs 16:3

I dedicate my Masters dissertation to my beloved family.

To my dearest husband, Eby Silby, thank you for being my anchor through every storm and my cheerleader in every triumph. Your unwavering love, quiet strength and constant encouragement has carried me through this journey. I am endlessly grateful to you, my love.

To my beloved family, Annakkil Philip Mathew, Baby Mathew, Jose Mathew, Maya Mathew, Deepak Mathew and Asish Mathew. Thank you for all your love and prayers during this time. You have shaped the woman I am today and for that I am deeply grateful

.

ABSTRACT

Colistin is a last-line antibiotic for multidrug-resistant (MDR) gram-negative infections and has become less effective due to the emergence of colistin resistance. In South Africa, colistin resistance in *Acinetobacter baumannii* and *Klebsiella pneumoniae* have been reported, but the data is limited.

This study characterised colistin-resistant *A. baumannii* and *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg from January 2022 to June 2024 through epidemiological and molecular analysis. From 2900 *A. baumannii* and *K. pneumoniae* isolates, 830 underwent colistin broth microdilution (BMD) testing, with 22% (186/830) confirmed as colistin-resistant and 62% (116/186) were extensively drug-resistant (XDR). *Klebsiella pneumoniae* accounted for 63% (117/186) of all infections. Most colistin-resistant infections were from the adult intensive care units (ICUs) (45%, 84/186). Whole-genome sequencing of 60 isolates revealed no mobile colistin resistance (*mcr*) genes but identified chromosomal mutations linked to colistin resistance, including *pmr*A M12I in *A. baumannii* sequence type (ST)231 and *pmr*B R256G in five *K. pneumoniae* ST2497, with one also carrying *pho*Q G385S and an unidentified *K. pneumoniae* ST harbouring *pmr*B R256G mutation. Novel chromosomal mutations were also discovered, with four *A. baumannii* isolates harbouring a novel non-synonymous *pmr*C I131V mutation.

This study provides new molecular and epidemiological insights into colistin resistance in a South African healthcare setting. The findings highlight the predominance of chromosomal-mediated colistin resistance mechanisms and the identification of novel variants. These insights advance our understanding of resistance evolution and emphasise the need for strengthened genomic surveillance.

Keywords: *Acinetobacter baumannii*, *Klebsiella pneumoniae*, colistin resistance, *pmr*B R256G, *pmr*A M12I; *pho*Q G385S, South Africa

Word count: 238

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LIST OF ABBREVIATIONS AND SYMBOLS

A:	
AMR	Antimicrobial resistance
AWaRe	Access, Watch and Reserve
B	
BMD	Broth microdilution
C	
cgMLST	Core genome Multilocus Sequence Typing
D	
DNA	Deoxyribonucleic acid
E	
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species
ESBL	Extended-spectrum β-lactamase
H	
HAIs	Hospital-acquired infections
HGT	Horizontal gene transfer
I	
ICU	Intensive care units
Inc	Incompatibility
IS	Insertion sequences
L	
L-Ara4N	4-amino-4-deoxy-l-arabinose
LOS	Lipooligosaccharides
LPS	Lipopolysaccharides
M	
mcr	Mobilized colistin resistant gene
MDR	Multidrug-resistant
MGEs	Mobile genetic elements
MICs	Minimum inhibitory concentration
MLST	Multilocus sequence typing

N

NHLS	National Health Laboratory Service
NGS	Next-generation sequencing

O

OMP	Outer membrane proteins
ONT	Oxford Nanopore Technology

P

PBPs	Penicillin-binding proteins
PacBio	Pacific Biosciences
PEtN	Phosphoethanolanime
PCR	Polymerase chain reaction
PDR	Pan-drug resistant

Q

QC	Quality control

R

RNA	Ribonucleotide acid

S

SOLiD	Sequencing by Oligonucleotide Ligation and Detection
SMRT	Single-molecule real time sequencing
SNPs	Single-nucleotide polymorphisms
ST	Sequence type

U

USB	Universal Serial Bus

W

WES	Whole-exome sequencing
WGS	Whole genome sequencing
WHO	World Health Organization

X

XDR	Extensively drug-resistant

CHAPTER 1: INTRODUCTION

Globally, multidrug-resistant (MDR), extensively-drug-resistant (XDR) and pan-drug-resistant (PDR) strains of bacteria like *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli* and *Pseudomonas aeruginosa* are becoming increasingly common and harbour multiple mechanisms of resistance, making infections caused by these strains extremely difficult to treat (Ahmed *et al.*, 2020). The slow rate at which new antibiotics are produced to combat these "superbugs" have brought forth a renewed interest in older antibiotics that were once considered too toxic for clinical use (Muteeb *et al.*, 2023). One such example is colistin, which is now considered a last resort option for treating highly resistant infections (Bialvaei and Kafil, 2015).

Colistin, a polymyxin antibiotic, disrupts the bacterial cell membrane by targeting lipid A, a key component of lipopolysaccharide (LPS) (Poirel *et al.*, 2017; Andrade *et al.*, 2020). Although colistin plays a crucial role in treatment, its use is linked to serious side effects, such as nephrotoxicity and neuropathy (Arrayasillapatorn *et al.*, 2021). Resistance can be driven by mutations in both chromosomal and plasmid-borne genes (Li *et al.*, 2022; Zelendova *et al.*, 2023). Chromosomal mutations often affect two-component regulatory systems such as PhoPQ and PmrAB, which are involved in LPS modification or occur in genes responsible for lipid A biosynthesis such as the *lpx*ACD (Gogry *et al.*, 2021). Plasmid-mediated colistin resistance is through the presence of mobilised colistin resistance (*mcr*) genes, of which currently 10 of the genes (*mcr*-1-10) have been identified (Xavier *et al.*, 2016; AbuOun *et al.*, 2017; Carattoli *et al.*, 2017; Yin *et al.*, 2017; Yang *et al.*, 2018; Wang *et al.*, 2019b; Wang *et al.*, 2020).

In South Africa, colistin is an unregistered drug that is a last-resort treatment for resistant infections (Sekyere, 2016; Majavie *et al.*, 2021; Lowe *et al.*, 2022). Locally, colistin resistance has been reported in both clinical and environmental settings, highlighting a growing concern in antimicrobial resistance (Jayol *et al.*, 2014; Coetzee *et al.*, 2016; Newton-Foot *et al.*, 2017; Founou *et al.*, 2018; Igwaran *et al.*, 2018; Mbelle *et al.*, 2020; Snyman *et al.*, 2020; Snyman *et al.*, 2021a; Lowe *et al.*, 2022; Nogbou *et al.*, 2022). In two separate studies on *K. pneumoniae*, mutations in the *pmr* and *pho* regulatory genes and mutation in the PmrB protein were reported, respectively (Jayol *et al.*, 2014; Mbelle *et al.*, 2020). In *A. baumannii*, colistin resistance has also been linked to mutations in *lps*B, a gene involved in LPS biosynthesis (Lowe *et al.*, 2022; Nogbou *et al.*, 2022). The *lps*B gene encodes the glycosyltransferase involved in LPS synthesis

(Ahmed *et al.*, 2020). Mutations in *lps*B causes disruptions in the LPS biosynthesis reducing colistin binding and leading to resistance (Novović and Jovčić, 2023). Plasmid-mediated resistance (*mcr*-1; *mcr*-3; *mcr*-5) has been reported in *E. coli* from broiler chickens, pigs, and wastewater effluents, as well as in clinical isolates from various South African hospitals (Coetzee *et al.*, 2016; Newton-Foot *et al.*, 2017; Founou *et al.*, 2018; Igwaran *et al.*, 2018; Snyman *et al.*, 2021a)

Several molecular techniques have been developed to identify resistance mechanisms, with polymerase chain reaction (PCR) being the most widely used method in clinical laboratories (Bester *et al.*, 2016). However, recent advancements have brought on the rise of next-generation sequencing (NGS) technologies which have transformed how we study antibiotic resistance (Satam *et al.*, 2024). Sequencing methods, including whole genome sequencing (WGS), provide a comprehensive view of bacterial genomes, enabling the detection of mutations and the identification of novel resistance mechanisms (Bardet and Rolain, 2018). While various NGS platforms exist, including Illumina, Helicos, etc, however this study will focus on Oxford Nanopore Technology (ONT) (Satam *et al.*, 2024). Oxford Nanopore sequencing allows for real-time sequencing of long deoxyribonucleic acid (DNA) fragments without the need for amplification (Lu *et al.*, 2016; Leggett and Clark, 2017).

There is limited published research in South Africa specifically focusing on colistin resistance and its underlying mechanisms (Lowe *et al.*, 2022). In this study, the epidemiology of colistin resistance genes and genetic relatedness of the isolates of *A. baumannii* and *K. pneumoniae* were investigated using ONT.

Aim:

This study aims to characterise colistin-resistant *A. baumannii* and *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa.

Objectives:

- To describe the epidemiology of colistin resistant isolates of *A. baumannii* and *K. pneumoniae* at a tertiary-level hospital in Johannesburg.
- To identify and characterise the colistin resistance genes in these bacterial isolates using ONT.

- To determine the genetic relatedness of the colistin-resistant *A. baumannii* and *K. pneumoniae* isolates.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Antimicrobial resistance (AMR) has become one of the most threatening global health challenges, complicating the treatment of infectious diseases, especially those caused by MDR bacteria (Gajdács and Albericio, 2019; Mondal *et al.*, 2024). According to a 2019 World Health Organization (WHO) report, AMR is responsible for approximately 700 000 deaths annually and it is estimated that by 2050, this number could rise to 10 million (Uddin *et al.*, 2021).

Among the most concerning MDR pathogens are *A. baumannii* and *K. pneumoniae*, which have increasingly developed resistance to carbapenems, a class of antibiotics that have long been considered a vital treatment option for healthcare associated bacterial infections due to their broad-spectrum activity (Nguyen and Joshi, 2021). In response to the rising carbapenem resistance, colistin, a polymyxin antibiotic discovered in the mid-20th century, has re-emerged as a last-resort treatment for MDR gram-negative infections (Ahmed *et al.*, 2020; Mondal *et al.*, 2024). Unfortunately, the increased reliance on colistin has led to colistin-resistant strains (Mondal *et al.*, 2024). Colistin resistance develops either via chromosomal mutations or through the horizontal transfer of plasmid-mediated resistance *mcr* genes (Gogry *et al.*, 2021; Novović and Jovčić, 2023).

Whole genome sequencing (WGS) is a powerful tool for identifying STs, virulence factors, bacterial genetic relationships, genome mutations, antibiotic resistance genes and mobile genetic elements, such as transposable elements and plasmids (Deurenberg *et al.*, 2017). Despite the global prevalence of colistin resistance, there is limited data available in South Africa on the epidemiology and characteristics of colistin-resistant *A. baumannii* and *K. pneumoniae* and their associated plasmids (if present) in South Africa. Although data on colistin resistance in South Africa remain limited, existing studies have identified both chromosomal mutations and plasmid-mediated mechanisms in various bacterial species isolated from clinical and environmental samples, including broiler chickens and wastewater treatment plant effluents (Jayol *et al.*, 2014; Coetzee *et al.*, 2016; Poirel *et al.*, 2016; Newton-Foot *et al.*, 2017; Founou *et al.*, 2018; Snyman *et al.*, 2020; Snyman *et al.*, 2021a; Nogbou *et al.*, 2022). Enhanced surveillance and molecular characterisation of colistin-resistant isolates, as well as strict infection prevention and control measure and antimicrobial stewardship are essential particularly in regions with limited data like South Africa.

2.2 Overview of *Acinetobacter baumannii*

Acinetobacter baumannii is a gram-negative, non-motile coccobacillus within the *Acinetobacter calcoaceticus–baumannii* complex, a group of closely related species which includes *Acinetobacter calcoaceticus*, *Acinetobacter nosocomialis* and *Acinetobacter pittii* (Nurjadi and Boutin, 2022). Members of this complex are all clinically relevant, contributing to healthcare-associated infections (HAIs), with *A. baumannii* being the most common (Nurjadi and Boutin, 2022). Unlike other *Acinetobacter* species, *A. baumannii* is not considered ubiquitous and is rarely found as part of the normal flora of the human skin (Vrancianu *et al.*, 2020; Maslova *et al.*, 2022). *Acinetobacter baumannii* is considered to be a 'hardy' healthcare-associated pathogen due to its ability to survive exposure to common disinfectants in hospital environments (Vrancianu *et al.*, 2020). While *A. baumannii* primarily causes HAIs, studies have reported infections occurring outside healthcare facilities (community-acquired infection) (Chusri *et al.*, 2019). Key virulence factors in *A. baumannii* is its outer membrane proteins (OMP), particularly OmpA, which serves multiple functions, including adhesion to host epithelial cells, resistance against complement killing and biofilm formation (Morris *et al.*, 2019).

2.3 Overview of *Klebsiella pneumoniae*

Klebsiella pneumoniae is a gram-negative, rod-shaped bacterium belonging to the order Enterobacterales (Ashurst and Dawson, 2023). It is the most clinically relevant species within the *Klebsiella* genus and forms part of the normal flora of the gastrointestinal and respiratory tracts (Wang *et al.*, 2019a; Dong *et al.*, 2022). Due to its colonisation capacity and environmental resilience, it serves as a major reservoir for both HAIs and community-acquired infections (Chang *et al.*, 2021). *Klebsiella pneumoniae* comprises of two main pathotypes: classical *K. pneumoniae*, which often harbours multidrug resistance and are significant contributors to HAIs, and hypervirulent *K. pneumoniae*, which remains largely susceptible to antibiotics but causes severe infections, primarily severe community-acquired infections (Russo and Marr, 2019). Major virulence factors include capsule polysaccharides, which enable immune evasion, and LPS, which can trigger severe inflammatory responses and septic shock (Ochońska and Brzychczy-Włoch, 2024).

2.4 Multidrug-resistance and treatment options against multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* infections

Over the last decade, infections caused by MDR *A. baumannii* and *K. pneumoniae* have become more common and a growing concern in healthcare settings as fewer treatment options are available (Paczosa and Mecsas, 2016). As part of the "ESKAPE" (*Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), *A. baumannii* and *K. pneumoniae* are associated with life-threatening HAIs and high levels of resistance (Elias *et al.*, 2021).

Carbapenems have long been considered the most active and potent agents against MDR gram-negative bacteria (Chang *et al.*, 2021; Ngbede *et al.*, 2021; Jiang *et al.*, 2022). Carbapenems are a widely used class of broad-spectrum β-lactam antibiotics and a last line of agents used against gram-negative bacteria (Zahra and Naqvi, 2021). Carbapenems fall under the 'reserve' category in the WHO 'Access, Watch and Reserve' (AWaRe) classification of antibiotics (World Health Organization, 2022). However, the extensive use of carbapenems in clinical practice has resulted in carbapenem resistance, often driven by carbapenemases such as OXA-type enzymes, KPC, NDM, VIM and IMP, limiting the use of these agents (Majavie *et al.*, 2021).

2.4.1 Colistin

Colistin, in recent years, has been re-introduced and considered a last resort drug for infections caused by MDR gram-negative bacteria (Andrade *et al.*, 2020; Sharma *et al.*, 2022). Colistin is a broad-spectrum antimicrobial that belongs to the polymyxin class of antibiotics (Andrade *et al.*, 2020). Polymyxin B and E (also called colistin) are used in clinical settings (Khuntayaporn *et al.*, 2022). The antimicrobial yields activity against most species in the order Enterobacterales as well as in non-fermentative gram-negative bacteria (Aghapour *et al.*, 2019; Doi, 2019). Colistin disrupts the outer membrane of gram-negative bacteria by binding to lipid A in LPS, displacing divalent cations, and compromising membrane integrity (Poirel *et al.*, 2017; Aghapour *et al.*, 2019; Elias *et al.*, 2021).

2.5 Mechanisms of resistance to colistin in *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Bacterial resistance arises through both intrinsic and acquired mechanisms (Mancuso *et al.*, 2021). Intrinsic resistance is the inherent ability to resist the action of an antibiotic due to existing structural modifications (Mancuso *et al.*, 2021). Certain bacteria exhibit intrinsic

colistin resistance due to LPS modifications, such as the addition of L-Ara4N, which reduces colistin binding (Gogry *et al.*, 2021). *Acinetobacter baumannii* and *K. pneumoniae* are not considered intrinsically resistant to colistin (Gogry *et al.*, 2021).

Acquired resistance develops through chromosomal mutations or the acquisition of resistance genes by horizontal gene transfer (HGT) driven by mobile genetic elements (MGEs) such as plasmids, integrons or phages (Rizi *et al.*, 2018; Christaki *et al.*, 2020). Chromosomal mutations occur at a low level every time the DNA is copied during growth and causes differences in how the organism interacts with the environment (Jian *et al.*, 2021). Plasmid-mediated resistance occurs when bacteria acquire resistance genes located on plasmids, which are small, extrachromosomal DNA molecules capable of autonomous replication (Carattoli, 2013). These plasmids can carry genes that confer resistance to various antibiotics and the spread of these plasmids are facilitated through HGT mechanisms, primarily through conjugation, but also through transduction and transformation (Rodríguez-Beltrán *et al.*, 2021). Additionally, plasmids may carry MGEs like transposons and insertion sequences (ISs), which help mobilize and spread resistance genes (Carattoli, 2013). This ability of plasmids to transfer resistance genes across bacterial species and genera contributes to the rapid dissemination of antimicrobial resistance, making plasmid-mediated resistance a significant concern in bacterial infections (Carattoli, 2013; Rodríguez-Beltrán *et al.*, 2021).

2.5.1 Chromosomal-mediated resistance in *Acinetobacter baumannii*

In *A. baumannii*, two main mechanisms of colistin resistance have been described: complete loss of the target LPS and modification in the target LPS (Novović and Jovčić, 2023). The complete loss of LPS results from mutations within genes essential for the lipid A biosynthetic pathway (*lpx*A, *lpx*C, and *lpx*D), whereas modification of LPS occurs through the addition of PEtN moieties to lipid A by the PmrCAB operon-encoded enzymes or through alterations in the core oligosaccharide structure mediated by mutations in the *lpsB* gene (Table 2.1) (Moffatt *et al.*, 2010; Novović and Jovčić, 2023). Efflux pumps (adeABC, HlyD family, emrA and emrB) also contribute to chromosomal-mediated resistance in *A. baumannii* (Zhang et al., 2020).

2.5.2 Chromosomal-mediated resistance in *Klebsiella pneumoniae*

Chromosomal resistance to colistin in *K. pneumoniae* is largely mediated by two-component systems and involves the modification of LPS, primarily through the PhoPQ and PmrAB systems, which modify lipid A by adding PEN and L-Ara4N (Table 2.3) (Ahmed *et al.*, 2020;

Gogry *et al.*, 2021; Shein, 2021). Mutations in *mgr*B disrupt its ability to regulate the PhoPQ system, leading to its overactivation and increased modification of lipid A, which reduces colistin's effectiveness (Shein, 2021). Similarly, mutations in *crr*B trigger the activation of PmrAB, further enhancing resistance (Cheng *et al.*, 2016). Other less common resistance mechanisms include lipid A hydroxylation and palmitoylation (Elias *et al.*, 2021). Efflux pumps (acrB and KpnEF) also play a role in colistin resistance in *K. pneumoniae* by exporting colistin out of the bacterial cell (Aghapour *et al.*, 2019; Ahmed *et al.*, 2020)

2.5.3 Plasmid-mediated colistin resistance in *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Following its initial discovery in China, a large number of studies reported various variants of *mcr* genes in various gram-negative bacteria from across the world (Aghapour *et al.*, 2019). To date, ten different *mcr* genes have been reported (*mcr*-1 to *mcr*-10) with more than twenty-five variants of *mcr*-1 gene (Novović and Jovčić, 2023; Mondal *et al.*, 2024; Singh *et al.*, 2025). The major driver of *mcr* gene dissemination is by HGT (Aghapour *et al.*, 2019). In *A. baumannii*, the *mcr*-1 and *mcr*-4.3 variants are the most frequently detected (Novović and Jovčić, 2023). Although *mcr*-1 and *mcr*-4.3 are the most prevalent, other *mcr* variants, including *mcr*-2 and *mcr*-3, have also been reported in clinical and environmental *A. baumannii* isolates (Novović and Jovčić, 2023). In *K. pneumoniae* isolates, *mcr*-1 and *mcr-9* have been reported to be the most widely disseminated, however, other *mcr* variants have also been reported (Elias *et al.*, 2021; Liu *et al.*, 2024). Table 2.3 lists the currently known colistin resistance genes found in *A. baumannii* and *K. pneumoniae* and the associated resistance mechanisms.

Table 2.1: Common chromosomal and plasmid-mediated colistin-resistant genes reported in *A. baumannii* and *K. pneumoniae* isolates

Pathogen	Mechanism type	Genes/ determinants	Resistance mechanisms	References
Acinetobacter baumannii	Chromosomal-mediated	*pmr*F	Modifications of the LPS moiety by L-Ara4N biosynthesis	Zhang *et al.*, 2020
		*lpx*A, *lpx*C and *lpx*D	Inactivation of lipid A biosynthesis abolishing LPS synthesis	
		*pmr*AB	Modification of lipid A by arnBCADTEF operon, *pmr*C and *pmr*E genes	
		*lps*B	Alterations or complete loss of LOS structure	
		*emr*A, *emr*B	Genes encoding components of the EmrAB efflux pump	
		adeABC, HlyD family,	Efflux pump	
	Plasmid-mediated	*mcr*-1; *mcr*-2; *mcr*-3; *mcr*-4.3	Phosphoethanolamine transferase enzyme catalyses the addition of phosphoethanolamine to lipid A	
Klebsiella pneumoniae	Chromosomal-mediated	*pmr*A/*pmr*B	Modification of lipid A by arn*BCADTEF* operon, *pmr*C and *pmr*E genes	Aghapour *et al.*, 2019
		*pho*P/*pho*Q	Modification of lipid A by activation of the pmrHFIJKLM operon/activation of pmrAB by pmrD	
		*arn*BCADTEF	Modification of lipid A by pEtN and I-4AraN	
		*mgr*B mutation	Overexpression of phoPQ and activation of pmrHFIKKLM	
		*crr*B mutation	Modification of lipid A by upregulation on pmrAB/activation of the glycosyltransferase	
		*ram*A	Modulates lipid A biosynthesis	
		*acr*B	Efflux pump	
		KpnEF	Efflux pump	
	Plasmid-mediated	*mcr*-1; *mcr*-9	Phosphoethanolamine transferase enzyme catalyses the addition of phosphoethanolamine to lipid A	

L-Ara4N = 4-amino-4-deoxy-L-arabinose; LPS = Lipopolysaccharide; LOS = Lipooligosaccharide; PEN = phosphoethanolamine;

2.6 Evolution of DNA sequencing technologies

Over the past two decades, DNA sequencing technologies have rapidly evolved, leading to the emergence of three generations of sequencing technologies (Figure 2.1) (Satam *et al.*, 2024). First generation sequencing began with the Sanger method in the 1970s (Sanger *et al.*, 1977; Satam *et al.*, 2024).

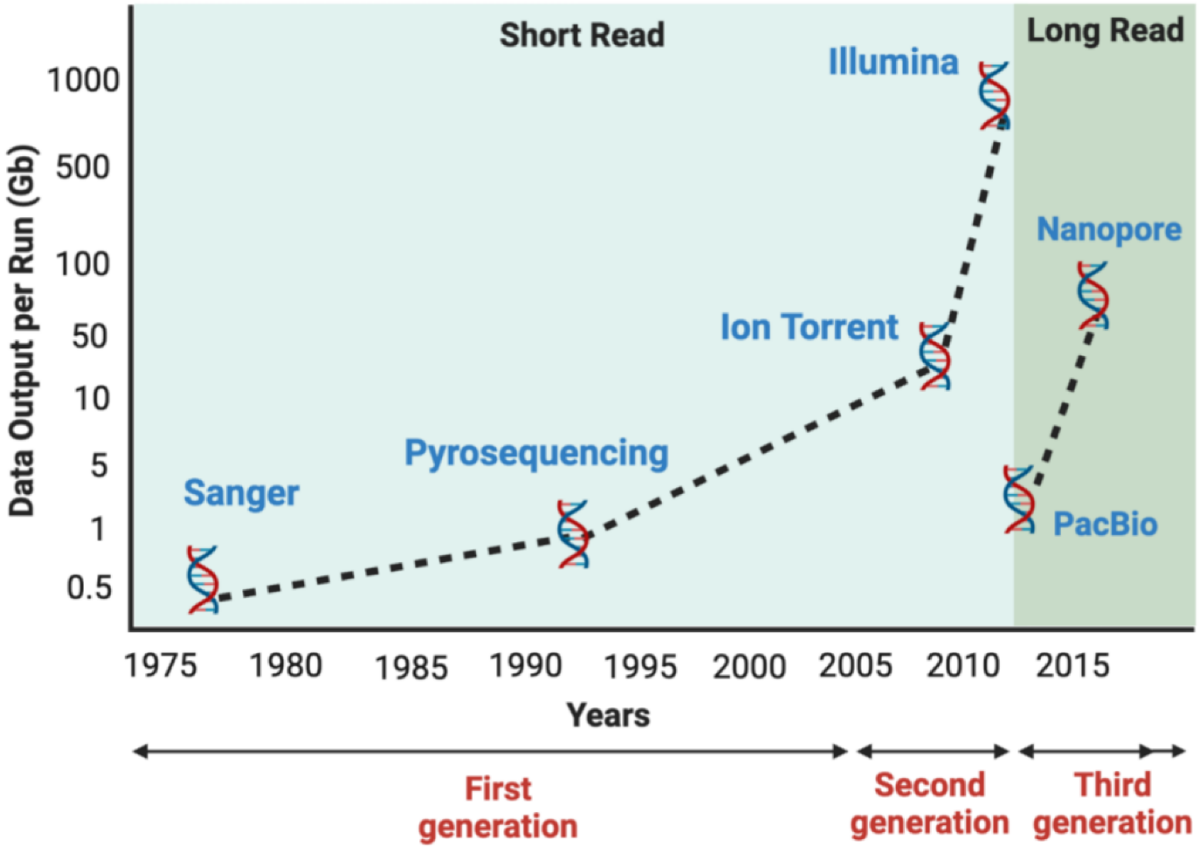


Figure 2.1: Evolution of sequencing technologies categorized into three generations (Satam *et al.*, 2024).

The next advancement of sequencing technology after the traditional first-generation Sanger sequencing is second-generation sequencing also known as short-read NGS (Hu *et al.*, 2021). Key platforms include Illumina and Ion Torrent (Hu *et al.*, 2021; Zhong *et al.*, 2021; Athanasopoulou *et al.*, 2022; Satam *et al.*, 2024). The latest advancements of DNA sequencing are third-generation sequencing technologies also called long-read technologies that use single-molecule real time sequencing (SMRT) without the need for PCR amplification (Hu *et al.*, 2021; Athanasopoulou *et al.*, 2022; Satam *et al.*, 2024).

2.6.1 Oxford Nanopore Sequencing

Oxford Nanopore Technology is a third-generation sequencing platform that makes use of SMRT nanopore sequencing (Kchouk *et al.*, 2017). The first ONT device, the MinION device, was released in 2014 and remains the smallest portable sequencer available (Figure 2.2) (Kchouk *et al.*, 2017; Athanasopoulou *et al.*, 2022). Other ONT platforms include the GridION, PromethION and Flongle with the key differences between each device shown in Table 2.2.

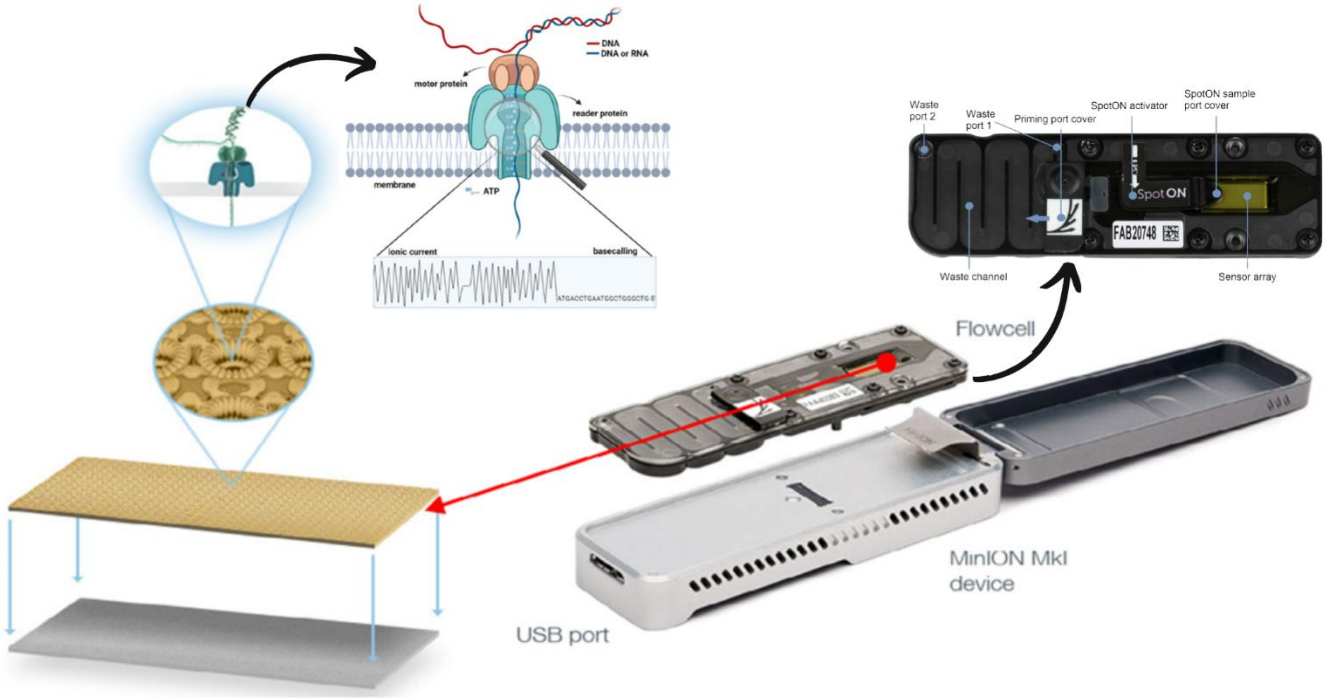


Figure 2.2: The MinION sequencing device (Lu *et al.*, 2016 ; Oxford Nanopore Technologies, 2025). Available at: https://nanoporetech.com/document/hardware (Accessed: 25 March 2025).

Nanopore sequencing involves passing single-stranded DNA through a nanopore (a nanoscale protein or synthetic pore embedded in a membrane) using a motor protein (Figure 2.2) (Lu *et al.*, 2016; Kchouk *et al.*, 2017; Athanasopoulou *et al.*, 2022). As each nucleotide moves through the pore, it disrupts the ionic current in a unique way which are detected by a sensor protein known as a reader and translated into a DNA sequence in a process known as basecalling (Kchouk *et al.*, 2017; Athanasopoulou *et al.*, 2022).

Table 2.2 Differences between sequencing devices offered by Oxford Nanopore Technology (Oxford Nanopore Technologies, 2025). Available at: https://nanoporetech.com/platform/technology/flow-cells-and-nanopores (Accessed: 25 March 2025).

Device	Throughput	Portability	Best use Case
MinION	Up to 50 Gb	High (USB-powered)	Small-scale projects, fieldwork
Flongle	Up to 2.8 Gb	High	Small, quick experiments
GridION	Up to 250 Gb	Medium	Medium-scale research labs
PromethION	Up to 14 Tb	Low	Large-scale genomic research

Oxford Nanopore Technology offers several advantages: (1) generates long-read sequences; (2) supports real-time data analysis; (3) inexpensive, compact and portable; (4) rapid identification of genes in comparison to other NGS approaches; (5) not requiring DNA amplification and (6) direct Ribonucleotide acid (RNA) sequencing (Lu *et al.*, 2016; Kchouk *et al.*, 2017; Leggett and Clark, 2017). A key disadvantage of Oxford Nanopore Technologies (ONT) is its higher nucleotide error rate compared to short-read platforms like Illumina, although improvements in base-calling and chemistry have reduced this from 10–20% to ~1% (Hallgren, 2024)

2.7 Genomics

Genomic studies using NGS enables thorough DNA analysis through various methods such as WGS, whole-exome sequencing and targeted sequencing (Satam *et al.*, 2024). Whole genome sequencing enables comprehensive genomic analysis, capturing an organism's complete DNA sequence to reveal the entirety of its genetic makeup such as genes, regulatory regions and non-coding elements present within the genome (Costain *et al.*, 2021; Satam *et al.*, 2024). This technique enables the detailed identification of genetic variations, spanning from single-nucleotide polymorphisms (SNPs) to larger structural modifications, including insertions, deletions, and rearrangements (Satam *et al.*, 2024).

The pan genome of a particular species represents its whole gene repertoire, made up of the core and dispensable genomes (Imperi *et al.*, 2011). The core genome includes core genes that are shared in all strains of a given species, while the dispensable genome includes accessory genes that are not essential for bacterial growth, but which may confer selective advantages, such as adaptation to specific environments, stress resistance and pathogenicity (Imperi *et al.*,

2011). Insertion sequences and incompatibility (Inc) plasmids are key MGEs in the spread of antimicrobial resistance in bacteria (Carattoli, 2013; Siguier *et al.*, 2014). Insertion sequence elements are small mobile DNA sequences that can 'jump' between locations in the genome, sometimes activating or spreading resistance genes (Mahillon and Chandler, 1998; Siguier *et al.*, 2014). Incompatibility plasmids act as carriers by transporting multiple resistance determinants between bacteria through HGT, enabling bacteria to survive exposure to different antibiotics (Carattoli, 2013).

2.7.1 Genomic insights into colistin resistance in *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Acinetobacter baumannii has a genome size of 3.5–4.3 Mb, the pan-genome is estimated to be around 19 000 genes, with the core genome being approximately 2 200 genes while the accessory genome to be roughly 16 000 genes (Rodrigues *et al.*, 2021). Insertion sequences like ISAba1 and ISAba11 have been reported to inactivate genes like *lpx*C and *lpx*A (Moffatt. *et al.*, 2011; Boinett *et al.*, 2019; Novović and Jovčić, 2023). Majority of *mcr*-1 genes that have been detected are located on plasmids, IncX4 IncHl1, IncHl2, IncP, Incl2 and IncY of which Incl2 and IncX4 are the most prevalent (Mondal *et al.*, 2024).

Klebsiella pneumoniae genomes typically contain between 5 000 to 6 000 genes, however, the species pangenome is estimated to be greater than 100 000 different genes (Gonzalez-Ferrer *et al.*, 2021). In *K. pneumoniae*, colistin resistance has been associated with several ISs, such as IS1-like, IS3-like, IS5-like, ISKpn14, ISKpn26, ISKpn28, IS1R, and ISL3 (Cannatelli *et al.*, 2013; Shankar *et al.*, 2019). Global studies have reported that plasmids carrying *mcr* genes were of IncFII, IncI2 or IncX4 types (Pilato *et al.*, 2016; Wang *et al.*, 2018; Yang *et al.*, 2018; Ahmed *et al.*, 2019; Hadjadj *et al.*, 2019).

2.8 Epidemiology of colistin resistance in South Africa

Since 2014, colistin-resistant bacteria have been frequently identified in South Africa from reservoirs including livestock, healthcare settings and the environment (Hassen *et al.*, 2022). The first clinical colistin-resistant *K. pneumoniae* isolate in South Africa was reported in 2014, with resistance attributed to a *pmr*B mutation (Table 2.3) (Jayol *et al.*, 2014). A year later, Jayol *et al.* (2015) reported another clinical colistin-resistant MDR *K. pneumoniae* isolate attributed to a single amino-acid change (Asp191Tyr) in protein PhoP (Table 2.3) (Jayol *et al.*, 2015). In the Gauteng province, Mbelle *et al.* (2020) reported chromosomal colistin-resistance in nine

out of 11 clinical extended-spectrum-β-lactamase (ESBL)-producing *K. pneumoniae* isolates with elevated colistin minimum inhibitory concentrations (MICs) were associated with mutations in the *pmr*, *pho* and *kpn* genes (Table 2.3). In the Western Cape province, Snyman *et al.* (2020) found no known *mcr* genes but the I527N mutation in the tripartite ATP-independent periplasmic transporter family protein was detected however this protein has not previously been associated with colistin resistance.

The first detection of *mcr*-1 in South Africa occurred in *E. coli* from numerous reservoirs including hospital and livestock sources (Coetzee *et al.*, 2016). Similarly, studies identified *mcr-1* in *E. coli* isolates from hospital patients and community outpatients in Johannesburg and Pretoria, distributed across six different STs (ST10, ST1007, ST624, ST57, ST101 and ST226) and carried on IncI2, IncHI2, and IncX4 plasmids (Poirel *et al.*, 2016). Not long after, the *mcr*-1 was detected in clinical *K. pneumoniae* isolates from the Western Cape (Table 2.3) (Newton-Foot *et al.*, 2017). Additionally, the *mcr*-9.1 gene, a variant of the *mcr*-like gene was detected in South Africa for the first time in a clinical *Enterobacter hormaechei* strain (Sekyere, 2016). Notably, while plasmid-mediated colistin resistance has been widely reported in *E. coli* and *K. pneumoniae*, it has yet to be detected in *A. baumannii* in South Africa (Snyman *et al.*, 2020).

Table 2.3: Dissemination of colistin resistance genes detected in clinical *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates in South Africa

Province	Nature of Isolate	Organism	Mechanism	Identified gene/variant	ST	References
Unknown	Clinical isolate (specimen type not mentioned)	*Klebsiella pneumoniae*	Mutations in the regulator MgrB	*mgr*B	ST14	Jayol *et al.*, 2014
Unknown	Clinical isolate (specimen type not mentioned)	*Klebsiella pneumoniae*	Mutation in the transcriptional regulator PhoP; colistin heteroresistance	*pho*PQ	ST101	Jayol *et al.*, 2015
Western Cape	Clinical isolate (urine specimen)	*Klebsiella pneumoniae*	Plasmid-mediated colistin resistance	*mcr*-1 gene	unknown	Newton-Foot *et al.*, 2017
Western Cape	Clinical isolate (specimen type not mentioned)	*Acinetobacter baumannii*	Undescribed mechanism of colistin resistance	unknown	ST1	Snyman *et al.*, 2020
Gauteng	Clinical isolates (blood, urine or sputum)	*Klebsiella pneumoniae*	Mutations in the histidine kinase PhoQ; Mutations in the response regulator Pmr; Mutations in the histidine kinase PmrB; Overproduction of efflux pumps mediated by *kpn*E/*kpn*F	*pmr*AB, *pho*PQ *kpn*EF	ST152; ST1414; ST39; ST234; ST101; ST323; ST1552	Mbelle *et al.*, 2020
Western Cape	Clinical isolates (specimen types not mentioned)	*Klebsiella pneumoniae*	Mutations in the regulator MgrB	*mgr*B	ST1	Snyman *et al.*, 2021b
Gauteng	Clinical isolate (specimen type not mentioned)	*Acinetobacter baumannii*	Mutations in *lps*B require more investigation to determine their implication in colistin resistance	*lps*B	ST1; ST2	Lowe *et al.*, 2022
Gauteng	Clinical isolate (tracheal aspirate)	*Acinetobacter baumannii*	Mutations in *lps*B require more investigation to determine their implication in colistin resistance	*lps*B	ST2	Nogbou *et al.*, 2022

ST = sequence type

2.9 Summary

Colistin resistance in *A. baumannii.* and *K. pneumoniae* has been reported in South Africa, contributing to treatment failure and increased mortality (Jayol *et al.*, 2014; Lowe *et al.*, 2022; Nogbou *et al.*, 2022). Antibiotic resistance spreads mainly through MGEs like plasmids, transposons and integrons, which facilitate gene transfer between bacteria (Mbelle *et al.*, 2020).

In *A. baumannii*, colistin resistance occurs through chromosomal mutations affecting LPS production (e.g., *lpx*A, *lpx*C, *lpx*D) or modifications regulated by the PmrCAB operon (Novović and Jovčić, 2023). *Klebsiella pneumoniae* primarily relies on two-component regulatory systems (PhoPQ, PmrAB, CrrAB), with *mgr*B mutations deregulating PhoPQ (Ahmed *et al.*, 2020; Shein, 2021). Both species can acquire plasmid-mediated resistance through *mcr* genes, with *mcr*-1 and *mcr*-4.3 common in *A. baumannii* and *mcr*-1 and *mcr*-9 in *K. pneumoniae* (Novović and Jovčić, 2023; Liu *et al.*, 2024).

Oxford Nanopore Technologies' MinION device uses nanopores to detect nucleotide-specific disruptions in electric currents, enabling portable and real-time third-generation sequencing (Lu *et al.*, 2016). These advancements have significantly impacted genomics, enabling detailed DNA analysis through methods like WGS which identify genetic variations from SNPs to large structural modifications (Satam *et al.*, 2024). The purpose of this study was to characterise clinical colistin-resistant *A. baumannii* and *K. pneumoniae* isolates obtained from a tertiary-level hospital in South Africa using epidemiological and genomic analysis. Information on the underlying molecular mechanisms and epidemiology associated with *A. baumannii* and *K. pneumoniae* harbouring colistin-resistant mechanisms can lead to better infection control in our local healthcare settings.

CHAPTER 3: METHODS

3.1 Epidemiological analysis

This retrospective cross-sectional study was conducted at a tertiary-level hospital in Johannesburg, South Africa. The epidemiological data of isolates was reviewed from 1 January 2022 to 30 June 2024. Patient demographic and sample information such as age, sex, ward, specimen type, organism identification, antibiotic susceptibility profile and colistin broth microdilution (BMD) results for each isolate were obtained from Corporate Data Warehouse of the National Health Laboratory Services (NHLS).

3.1.1 Statistical analysis

Data deduplication was performed in Microsoft Excel v2502 (Microsoft, USA) to remove repeat entries and ensure dataset accuracy. When multiple records shared the same unique identifier, a structured selection process was followed. If all duplicates referred to the same organism, the first sterile specimen was chosen. However, if both *A. baumannii* and *K. pneumoniae* were present under the same identifier, the most sterile specimen of each was selected. For colistin BMD results, if >1 result existed, the result corresponding to the sterile specimen used for demographic information was taken.

Epidemiology data was entered as binary code and imported into Stata 18 with the help from a statistician from the University of Witwatersrand and analysed (StataCorp, Texas, USA). Variables such as sex, age, ward and specimen type were taken as categorical, therefore proportions and frequency distributions were used to describe and characterise *A. baumannii* and *K. pneumoniae* isolates. Bar charts were used to graphically display the categorical data. The chi-square test was used to assess categorical outcomes between ward and colistin resistance. P-values (two-tailed) <0.05 was considered to be statistically significant.

a) Categorising of wards

Wards were categorised to allow for discipline-specific analysis (Table 3.1).

Table 3.1: Table showing wards allocated into relevant categories

ADULT MEDICAL Medical endo/cardiac 584 Neurology 586 Renal & nuclear medicine 597 Renal transplant 561	**PAEDIATRIC MEDICAL** Paediatric medical ward 285 Paediatric medical ward 287
	PAEDIATRIC SURGERY Paediatric surgical 277
	TRAUMA UNIT Trauma ICU 366 Trauma high-care unit 377
ADULT MULTIDISCIPLINARY ICUs Multidisciplinary ICU 567 Medical cardiology ICU 566 Multidisciplinary ICU 576	**ADULT ONCOLOGY** Gastro/haematology/oncology 595 Medical oncology 594 Radiation oncology 585
GENERAL SURGERY Maxillo-facial 384 Surgical general 396 Surgical head & neck 394 Surgical vascular 395	**GYNAECOLOGY** Gynaecology ward 196 Post-natal 187
NEONATAL UNIT Neonatal ICU 276 Paediatric ICU 278 Paediatrics (premature unit) 177	**OTHER SURGICAL** Cardio-thoracic 466 Neurosurgery ICU 565 Orthopaedic (trauma) 374 Orthopaedic cold cases 375 Plastic surgery 494 Septic orthopaedic 369 Surgical high care 579 Surgical special urology 386

b) Categorising multidrug and extensively-drug resistant organisms

The 24-hour on-site NHLS, Microbiology Laboratory routinely tested eight antibiotic classes (polymyxins, carbapenems, aminoglycosides, cephalosporins, β-lactamase inhibitor combinations, antifolates, fluoroquinolones and tetracycline) (Table 3.2). Samples were processed using standard microbiological techniques. The identification of isolates within the institution was performed using Vitek®2 (BioMerieux Inc., Marcy l'Étoile, France) or Vitek MS (BioMerieux Inc., Marcy l'Étoile, France). Antibiotic susceptibility testing of isolates was performed using the Vitek®2 AST N255 panel (BioMerieux Inc., Marcy l'Étoile, France). All XDR and some MDR isolates were referred from the on-site Microbiology laboratory to the Infection Control Services Laboratory, NHLS for colistin BMD testing. Of 2 900 *A. baumannii* and *K. pneumoniae* isolates, 830 isolates had colistin BMD testing done. Colistin MIC results were interpreted as ≤2 µg/ml as intermediately susceptible and ≥4 µg/ml as resistant according to CLSI M100 V32 to V34 for each year of study (Clinical and Laboratory Standards Institute, 2022 to 2024). There are no susceptible breakpoints for colistin for *A. baumannii* and *K. pneumoniae* (Clinical and Laboratory Standards Institute, 2022 to 2024).

Table 3.2: Antibiotics of significance tested at the Microbiology Laboratory at the tertiary-level hospital for *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Class	Antibiotic
Polymyxin	Colistin
Carbapenem	Ertapenem
	Imipenem
	Meropenem
Aminoglycoside	Amikacin
	Gentamicin
	Tobramycin
Cephalosporins	Cefuroxime (2nd generation)
	Cefotaxime (3rd generation)
	Ceftazidime (3rd generation)
	Cefepime (4th generation)
β-lactamase inhibitor combination	Piperacillin-tazobactam Amoxicillin-clavulanate
Antifolate	Trimethoprim-sulfamethoxazole
Fluoroquinolone	Ciprofloxacin
Tetracycline	Tigecycline

In this study, MDR was defined as "non-susceptibility to at least one agent in three or more antimicrobial categories" and XDR was defined as "non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories)" (Magiorakos *et al.*, 2012).

3.2 Molecular analysis

Ninety-six colistin-resistant isolates were collected retrospectively. Selected isolates were previously identified as either *A. baumannii* or *K. pneumoniae* using Vitek®2 (BioMerieux Inc., Marcy l'Étoile, France) or Vitek MS (BioMerieux Inc., Marcy l'Étoile, France). Colistin-resistant isolates were sub-cultured from microbanks (Pro-Lab Diagnostics, Bromborough, Wirral, UK) in storage onto MacConkey agar (Laboratorios CONDA, South Africa) to assess purity. High genomic DNA was extracted using the Rapid Automated Processing Instrument–X 96 (RAPI-X™ 96) system (Omega Biotek, Norcross, GA, United States) with the MagNXT DNA extraction kit (Gurgaon, India). Qubit 4.0 (Thermo Scientific, Waltham, MA, United States) was used to quantify DNA concentration. The Rapid Barcoding Sequencing Kit (SQK-RBK114-96, Oxford Nanopore Technologies, United Kingdom) was used to prepare sequencing libraries, following the manufacturer's protocol with minor modifications. A detailed step-by-step description of the method, including these modifications, is provided in

Annexure A. Sequencing was performed on the MinION platform using R9.4.1 flow cells (FLO-MIN114, Oxford Nanopore Technologies, United Kingdom).

Real-time sequencing data was collected using MinKNOW software (v23.11.5, Oxford Nanopore Technologies, United Kingdom) and real-time basecalling using Guppy (v6.3.8). The EPI2ME bacterial genomes (v1.4.1; https://github.com/epi2me-labs/wf-bacterial-genomes; Accessed: 25 January 2025) workflow was used to process raw sequencing reads. The workflow incorporates the following tools: Fastcat/bamstats (v0.20.0; https://github.com/epi2me-labs/fastcat; Accessed: 25 January 2025) tool to concatenate multifile samples; Flye (v2.9.5; https://github.com/mikolmogorov/Flye; Accessed: 25 January 2025) constructed a draft genome assembly, which was polished using Medaka (v2.0.1; https://github.com/nanoporetech/medaka; Accessed: 25 January 2025) and the assembled genome was annotated using Prokka (v1.14.5; https://github.com/tseemann/prokka; Accessed: 25 January 2025).

Bioinformatic analysis was done in Galaxy (v24.2.1. dev0; https://usegalaxy.org/; Accessed: 25 January 2025). Post-sequencing quality control was done by FastQC tool (v0.74; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/devteam/fastqc/fastqc/0.74+galaxy1; Accessed: 2 February 2025). Genome assembly quality (N50), the GC content and number of contigs were calculated using Quality Assessment Tool (v5.3.0; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/quast/quast/5.3.0+galaxy0; Accessed: 25 January 2025). Multi-Locus Sequence Typing (MLST) (v2.22.0; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/mlst/mlst/2.22.0; Accessed: 2 February 2025) tool on the Galaxy platform was used to determine ST (Pasteur scheme). Antibiotic resistance genes, plasmids and virulence factor genes were identified in the draft genomes using ABRicate (v1.0.1; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/abricate/abricate/1.0.1; Accessed: 2 February 2025). Point mutations associated with colistin resistance were identified using AMRFinderPlus (v3.12.8; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/amrfinderplus/amrfinderplus/3.12.8+galaxy0; Accessed: 2 February 2025) and Pathogenwatch (v23.1.5; https://pathogen.watch/; Accessed: 2 February 2025) was utilized to determine the isolates' core genome (cg) MLST classification, core genome clustering (threshold at 5) and surface polysaccharide typing.

3.3 Ethical considerations

Ethical approval was granted by the University of Witwatersrand Human Research Ethics Committee (Ethics number: M231162).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Epidemiological analysis

During the 2-year 5-month period, 2 900 clinical isolates of *A. baumannii* (*n* =724) and *K. pneumoniae* (*n* =2 176) were identified. Of these, 830 isolates underwent colistin BMD testing with 22% (186/830) confirmed as colistin-resistant. Resistance was highest in 2022 for both *A. baumannii* and *K. pneumoniae* at 29% (40/140 and 42/143, respectively), followed by a decline in 2023 to 19% (24/125) and 20% (32/163). In 2024, *A. baumannii* resistance further declined to 11% (5/47), while *K. pneumoniae* remained unchanged at 20% (43/212) (Figure 4.1). The downward trend in *A. baumannii* was statistically significant (ρ = 0.022), whereas in *K. pneumoniae* it was not (ρ= 0.073). It is to be noted that the year 2024 data only reflect six months of data, therefore the results are not a full representation for the year.

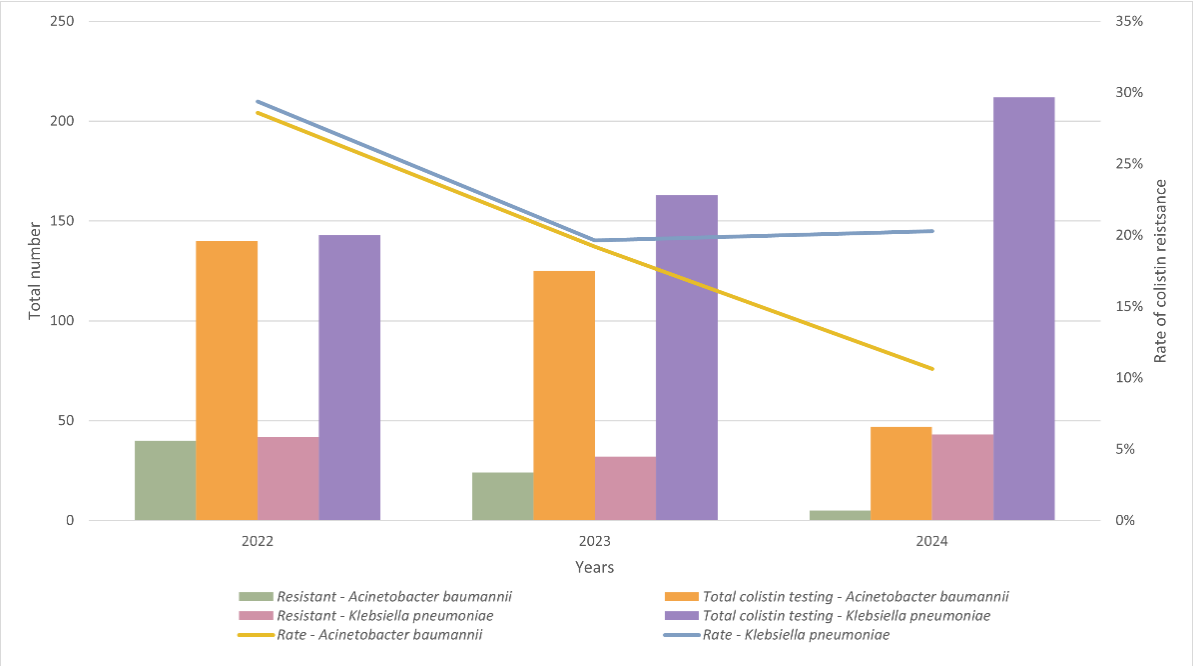


Figure 4.1: Number of *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates for which colistin broth microdilution testing was done (*n* = 830) and proportion of isolates that tested colistin resistant.

4.1.1 Demographic distribution of colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates

Of the 186 colistin-resistant isolates, 40% (75/186) were from female and 60% (111/186) from male patients (Table 4.1). Age distribution analysis showed that the highest resistance rate was in patients aged 30–39 years with 26% (48/186) of tested isolates being colistin-resistant,

followed by ≥60 years with 18% (33/186) (Table 4.1). Colistin resistance rate within each age group was calculated based on the number of isolates tested in that specific age group.

Table 4.1: Demographic data of colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* from a tertiary-level hospital.

Characteristics		**Colistin-resistant *Acinetobacter baumannii*** *n* = 69 (%)	**Colistin-resistant *Klebsiella pneumoniae*** *n* = 117 (%)	**Total colistin-resistant isolates** *n* = 186 (%)	**Total isolates that underwent colistin broth microdilution testing** *n* = 830 (%)
Sex					
	Female	25 (36%)	50 (43%)	**75 (40%)**	
	Male	44 (64%)	67 (57%)	**111 (60%)**	
Age					
	≤ 28 days	3 (4%)	2 (2%)	**5 (3%)**	**82 (10%)**
	29 days – <1 year	2 (3%)	6 (5%)	**8 (4%)**	**44 (5%)**
	1 years – 9 years old	3 (4%)	2 (2%)	**5 (3%)**	**29 (3%)**
	10 years – 19 years old	4 (6 %)	3 (3%)	**7 (4%)**	**32 (4%)**
	20 years – 29 years old	9 (13%)	20 (17%)	**29 (16%)**	**120 (14%)**
	30 years – 39 years old	22 (32%)	26 (22%)	**48 (26%)**	**161 (19%)**
	40 years – 49 years old	8 (12%)	17 (15%)	**25 (13%)**	**125 (15%)**
	50 years – 59 years old	10 (14%)	16 (14%)	**26 (14%)**	**110 (13%)**
	≥ 60 years old	8 (12%)	25 (21%)	**33 (18%)**	**127 (15%)**

4.1.2 Sample characteristics of colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Bloodstream isolates were the most prevalent, accounting for 44% (82/186) of all colistin-resistant isolates (Table 4.2). Overall, 76% (141/186) of all colistin-resistant isolates were from sterile sites and likely reflect invasive infection (rather than just colonization). This highlights the clinical burden of colistin-resistant pathogens, which is associated with higher mortality rates and prolonged hospital stays (Arjun *et al.*, 2016).

Table 4.2: Distribution of specimen types among colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*

Specimen category	**Samples**	**Sterility Classification**	**Colistin-resistant *Acinetobacter baumannii*** *n* = 69 (%)	**Colistin-resistant *Klebsiella pneumoniae*** *n* = 117 (%)	**Total colistin-resistant isolates** *n* = 186 (%)
Blood	Blood	Sterile	26 (38%)	56 (48%)	**82 (44%)**
Cerebrospinal fluid (CSF)	CSF	Sterile	-	2 (2%)	**2 (1%)**
Respiratory tract sample	Sputum, tracheal aspirate	Non-sterile	10 (14%)	11 (9%)	**21 (11%)**
Fluid aspirate	Fluid aspirate, tissue, wound aspirate, abscess aspirate	Sterile	25 (36%)	32 (27%)	**57 (31%**
Swab	Swab	Non-sterile	3 (4%)	4 (3%)	**7 (6%)**
Urine	Urine	Non-sterile	5 (7%)	12 (10%)	**17 (9%)**

- = no result

Overall, colistin resistance was most prevalent in intensive care unit (ICU) settings, with 45% (84/186) in the adult multidisciplinary ICUs, followed by 12% (23/186) in the trauma unit. The paediatric oncology department reported no colistin-resistant isolates. A statistically significant difference ($\rho = 0.017$) was observed in the distribution of colistin-resistant isolates between the adult multidisciplinary ICUs *vs* the combined adult medical and general surgery wards, emphasizing the critical role of ICU environments in the spread of resistant strains.

Among the 186 colistin-resistant isolates, 30% (55/186) were MDR, 62% (116/186) were XDR and eight percent (15/186) were non-MDR (Figure 4.2). Multidrug resistance was observed in 40% (22/55) of *A. baumannii* isolates and 60% (33/55) of *K. pneumoniae* isolates. Extensively drug-resistance rates were notably high in both organisms, 40% (46/116) of *A. baumannii* isolates and 60% (70/116) of *K. pneumoniae* isolates. The adult multidisciplinary ICUs accounted for 46% (79/171) of all MDR and XDR cases, reinforcing the need for targeted infection prevention and control and antimicrobial stewardship programmes in this area (Figure 4.2).

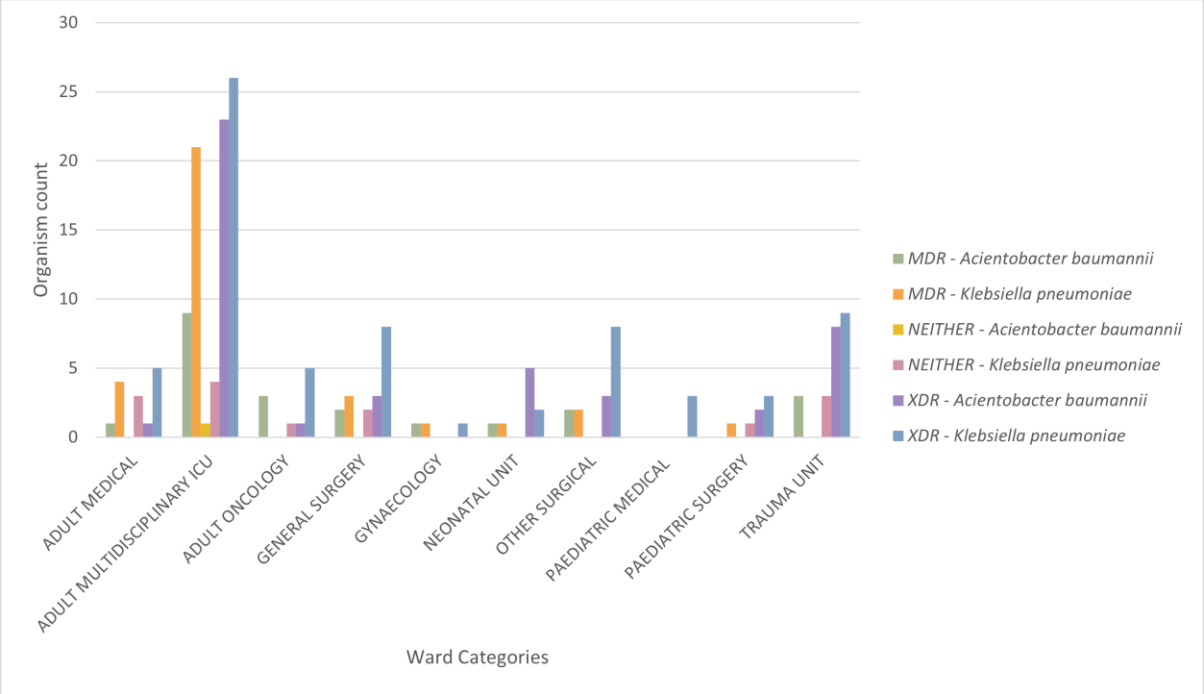


Figure 4.2: Distribution of multidrug-resistance and extensively drug-resistance in *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates among wards in a tertiary-level hospital in Johannesburg, South Africa.

A high proportion of *A. baumannii* and *K. pneumoniae* isolates exhibited resistance to majority of antibiotics tested. Resistance patterns showed high resistance to carbapenems, with 82% (152/186) for meropenem and 78% (146/186) for imipenem. The colistin MIC distribution of colistin-resistant *A. baumannii* and *K. pneumoniae* isolates varied. Colistin resistance MIC of ≥16 µg/ml was present in 75% (139/186) of isolates, while 25% (47/186) showed resistance at MIC of <16 µg/ml. Among *A. baumannii* isolates, 71% (49/69) had MICs ≥16 µg/ml and 29% (20/69) had MICs <16 µg/ml. Similarly, 77% (90/117) of *K. pneumoniae* isolates had MICs of ≥16 µg/ml and 23% (27/117) had MICs <16 µg/ml. These findings highlight the predominance of isolates with colistin MICs ≥16 µg/ml.

4.2 Molecular analysis

A total of 96 *A. baumannii* (20/96) and *K. pneumoniae* (76/96) isolates were collected during the study period. Oxford Nanopore sequencing was performed on all 96 colistin-resistant *A. baumannii* and *K. pneumoniae* isolates. However, 36 isolates were excluded from the study (isolates did not pass the post-sequencing quality control done by FastQC tool (v0.74; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/devteam/fastqc/fastqc/0.74+galaxy1; Accessed: 2 February 2025).

4.2.1 Molecular typing and phylogenetic analysis

The ST could only be determined for 13 of the 60 isolates. Surface polysaccharide typing and cgMLST analysis were conducted on 13 colistin-resistant isolates (5 *Acinetobacter baumannii* and 8 *Klebsiella pneumoniae*) (Table 4.3). Among the *A. baumannii* isolates, four different STs were identified using the Pasteur scheme, with ST2 as predominant. Capsule KL17/KL18 types and OCL1–OCL5 lipooligosaccharide loci were predominant. The isolates were grouped into three distinct cgMLST clusters, indicating genetic diversity. In *K. pneumoniae*, five STs were observed, notably, ST2497 was the most frequently encountered (4/8, 50%) and clustered consistently in cgMLST cluster 138. Capsule KL64, O1/O2v1 and O-locus types, respectively, were common among these isolates. A novel ST (*e1fe) grouped in a distinct cgMLST cluster (1287).

Table 4.3: Molecular typing of colistin-resistant isolates

Lab No	Organism	ST (Pasteur scheme)	K-locus (capsule type)	OC-locus (O type)	cgMLST cluster
A7	*Acinetobacter baumannii*	2	KL4 (K4)	OCL1 (OC1)	1
A8	*Acinetobacter baumannii*	2	KL18 (K18)	OCL5 (OC5)	2
A19	*Acinetobacter baumannii*	*05b4	KL17 (K17)	OCL1 (OC1)	3
A20	*Acinetobacter baumannii*	*788f	KL17 (K17)	OCL1 (OC1)	3
A21	*Acinetobacter baumannii*	*830d	KL17 (K17)	OCL1 (OC1)	3
K8	*Klebsiella pneumoniae*	*a162	KL148 (K148)	O12 (OC12)	2
K48	*Klebsiella pneumoniae*	*69bd	KL103 (K103)	O12 (OC12)	2
K56	*Klebsiella pneumoniae*	*ef1e	KL64 (K64)	O1/O2v2/O1a	2
K71	*Klebsiella pneumoniae*	2497	KL64 (K64)	O1/O2v1/O1ab	138
K72	*Klebsiella pneumoniae*	2499	KL102 (K102)	O1/O2v2	1 287
K73	*Klebsiella pneumoniae*	2497	KL64 (K64)	O1/O2v1/O1ab	138
K83	*Klebsiella pneumoniae*	2497	KL64 (K64)	O1/O2v1/O1ab	138
K84	*Klebsiella pneumoniae*	2497	KL64 (K64)	O1/O2v1/O1ab	138

*= novel

Since only one *A. baumannii* isolate contained a known point mutation, no phylogenetic tree could be produced. The phylogenetic tree of *K. pneumoniae* only included six of seven known chromosomal point mutations detected. Isolate K1 was excluded from the phylogenetic tree because it had too many missing SNPs or alignment inconsistencies, preventing the tree from being successfully constructed. The *K. pneumoniae* phylogenetic tree indicates a potential clonal relationship between K71 and K73 as they are clustered closely (Figure 4.3). Isolate K71 from a 23-year-old female in the other surgical ward (from respiratory tract sample) and K73 from a 74-year-old male in the general surgical ward (from urine), both share the KL64/K62 capsule type and O1/O2v1 outer core loci, supporting a potential clonal relationship. Interestingly, K48 isolated from blood culture in the ICU ward from a male patient was 59 years old, also clustered closely with K71 and K73 despite differing in capsule and outer core loci. The distribution of these genetically related isolates across different wards suggests possible inter-ward transmission or the presence of a persistent hospital associated clone circulating within the hospital.

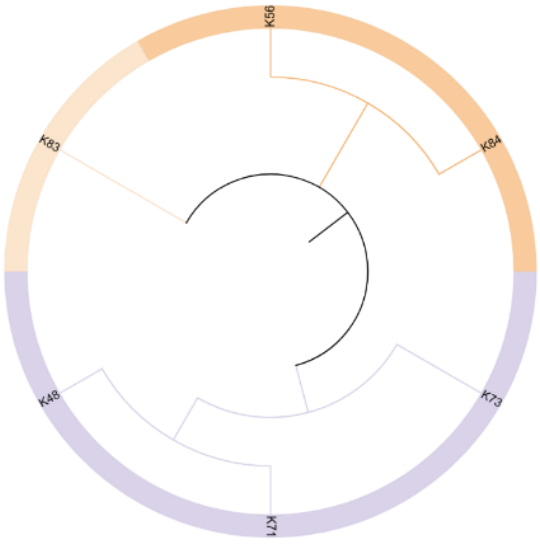


Figure 4.3 Phylogenetic tree of six chromosomal-mediated colistin-resistant *Klebsiella pneumoniae*.

Similarly, K56, K83 and K84 also clustered closely in the phylogenetic tree and shared the KL64 7/K62 capsule type and either the O1/O2v1 or O1a outer core loci. K56 was isolated from a 45-year-old male from ICU (blood), K84 was from a 45-year-old male in the orthopaedic ward (fluid aspirate) and K83 from a 51-year-old female in the cardiology ward (respiratory tract sample).

4.2.2 Known and novel colistin-resistant mutations

Among 60 isolates, no plasmid-mediated colistin (*mcr*) gene was detected in any isolate, whereas 13% (8/60) were observed to have chromosomal mutations that conferred resistance to colistin. AMRFinderPlus (v3.12.8; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/amrfinderplus/amrfinderplus/3.12.8+galaxy0; Accessed: 2 February 2025) confirmed known point mutations in genes such as *pmr*A, *pmr*B and *pho*Q, while 11 isolates exhibited unknown point mutations (Table 4.5 and Table 4.6). Mutations were in the *pmr*A gene in *A. baumannii* (1/4) (Table 4.5) while, *Klebsiella pneumoniae* had mutations in the *pmr*B gene in 77% (7/9) of isolates with one isolate having a co-mutation also in the *pho*P gene (Table 4.6). The most common mutation was R256G amino acid substitution of *pmr*B in five ST2497 *K. pneumoniae* isolates (Table 4.6). The absence of known plasmid-mediated colistin resistance genes, such as *mcr* variants is consistent with findings from previous studies conducted in clinical settings in South Africa (Lowe *et al.*, 2022; Nogbou *et al.*, 2022).

The AMRFinderPlus genomic tool (v3.12.8; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/amrfinderplus/amrfinderplus/3.12.8+galaxy0; Accessed: 2 February 2025) detected a few unknown point mutations in a total of 11 isolates (four *A. baumannii* and seven *K. pneumoniae* isolates) (Table 4.4, Table 4.5 and Table 4.6). These mutations may represent novel or less-characterised genetic variations that could potentially contribute to colistin resistance. Some unknown point mutations were found in previous studies (Table 4.4). Novel mutations in this study were *pmr*C A304H, T246M, HS543SQ, I535V, PQ537NK, A54G, AI28LF, AI49SV, F45N, FM16LN, IIA21LLS, IL70LI, QIS12DVT, T40Q, T63A, V47I, YAVL65NLIF, YHQV35FKKI, H390Y; *pmr*B M1V, HKTYHEED66SNLPQKN, L56F, TVSSKFD58NCKQVR, GQ359G*T, R349C, W351R; *pmr*A A41T; *lap*B N212S; *lpx*A D233D, G68G, H159H, Q72Q.

Table 4.4: Unknown point mutations detected or described in previous studies

Novel colistin associated point mutation detected	Literature reference
*pmr*C A370S; *pmr*C F166L; *pmr*C K531T; *pmr*C V58I; *pmr*C I131V; *pmr*C R230Q; *pmr*C H499R; *pmr*C V151A; *pmr*C V118F	Goic-Barisic *et al.*, 2023; Zafer *et al.*, 2023; Vijayakumar *et al.*, 2024
*pmr*C V47I	Gerson *et al.*, 2020
*pmr*C V61I	Wang *et al.*, 2022
*pmr*B P360Q	Arroyo *et al.*, 2011; Vijayakumar *et al.*, 2024
*pmr*B T246A	Rodrigues *et al.*, 2019
*pmr*B G207V	Benito, 2023
*arn*C S30T	Pragasam *et al.*, 2016
*lap*B N212T	Hatrongjit *et al.*, 2024
*pmr*B L213M; *mgr*B M1V	Li *et al.*, 2023

4.2.3 Acquired antimicrobial resistant genes

A sub-analysis of sequencing data was conducted to detect/describe AMR genes in *A. baumannii* (n = 8) and *K. pneumoniae* (n = 52) isolates (Tables 4.5 and 4.6). Two *A. baumannii* isolates (A4 and A5) and seven *K. pneumoniae* isolates showed no detectable AMR genes.

Among the *A. baumannii* isolates, β-lactam resistance genes were prevalent with $bla_{\text{OXA-23}}$ gene detected in 63% (5/8) of isolates, and both $bla_{\text{NDM-1}}$ and bla_{ADC} genes in 50% (4/8) of isolates. Notably, one isolate (A3) harboured the ESBL gene $bla_{\text{PER-7}}$. Several aminoglycoside resistance genes were also identified, including *arm*A, *aad*A1, *aac(3)-Ia*, *aph(6)-Id* and *ant (3'')-Iia*, showing resistance to amikacin and gentamicin. Quinolone resistance-associated mutations (*gyr*A and *par*C), efflux pump-associated genes (*qac*E, *msr*E) and rifampicin resistance determinant (*arr*-2) were also present in isolates.

In *K. pneumoniae*, the most common carbapenemase gene was $bla_{\text{OXA-232}}$ (56%; 29/52), followed by $bla_{\text{OXA-181}}$ (10%; 5/52) and $bla_{\text{NDM-1}}$ (8%; 4/52), indicating the circulation of both OXA-type and metallo-β-lactamases. Extended-spectrum β-lactamase genes were identified: $bla_{\text{CTX-M-15}}$ with 19% (10/52), $bla_{\text{TEM-1}}$ with 15% (8/52) and $bla_{\text{SHV-11}}$ 8% (4/52), often co-occurring with plasmid-mediated quinolone resistance determinants such as *qnr*B and *qnr*S. Chromosomal mutations in *gyr*A and *par*C were also observed in some isolates.

Table 4.5: Demographic and molecular characteristics of *Acinetobacter baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa.

Lab No.	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	AMR genes
A3	Trauma Unit	M	29	Blood	>64	Not detected	Not detected	Not detected	*sul*2; *cml*A1; *aad*A1; *sat*2; *dfr*A1; *aph*(6)-Id; bla_{NDM-1}; *ble*; *sul*1; *qac*E; bla_{PER-7}
A7	Adult multidisciplinary ICU	F	52	Respiratory tract sample	4	2	Not detected	*pmr*C A304H; *pmr*C A370S; *pmr*C F166L; *pmr*C K531T; *pmr*C T246M; *pmr*C V58I; *pmr*B_M1V; *pmr*C_HS543SQ; *pmr*C_I131V; *pmr*C I535V; *pmr*C PQ537NK; *pmr*C R230Q; *pmr*C V151A	*ant*(3")-Iia; *par*C_S84L; bla_{OXA-23}; armA; *msr*E; *mph*E; sul1; *qac*E; *cml*A5; *arr*-2; *sul*2; *tet*B; *aph*(6)-Id; *aph*(3")-Ib; bla_{OXA}; *aac*(3)-Ia; *aad*A1; *gyr*A S81L; bla_{ADC}
A8	Paediatric medical	M	40 days	Blood culture	4	2	Not detected	*pmr*C_H499R; *pmr*C_I131V; *pmr*B_M1V; *pmr*B P360Q; *pmr*C A54G; *pmr*C AI28LF; *pmr*C AI49SV; *pmr*C F45N; *pmr*C FM16LN; *pmr*C HS543SQ; *pmr*C I131V; *pmr*C I535V; *pmr*C_IIA21LLS; *pmr*C_IL70LI; *pmr*C PQ537NK; *pmr*C QIS12DVT; *pmr*C R230Q; *pmr*C T40Q; *pmr*C T63A; *pmr*C V118F; *pmr*C V151A; *pmr*C_V47I; *pmr*C V61I; *pmr*C YAVL65NLIF; *pmr*C YHQV35FKKI; *lpx*A D233D; *lpx*A G68G; *lpx*A H159H; *lpx*A Q72Q	*sul*1; *qac*E; *aad*A1; *aac*(3)-Ia; *aph*(3')-Ia; *cat*A1; *tet*A; *par*C S84L; *ant*(3")-Iia; *sul*2; *tet*B; *mph*E; *msr*E; *arm*A; *qac*E; *cml*A5; *arr*-2; *sul*2; *aph*(6)-Id; *dfr*A1; *sat*2; *aad*A1; *gyr*A S81L; *aph*(3')-Via; bla_{OXA-69}; bla_{ADC}; bla_{OXA-23}
A19	Neonatal unit	M	43 days	Sputum	4	*05b4	*pmr*A M12I	*pmr*C_H390Y; *pmr*C_H499R; *pmr*C_I131V; *pmr*B M1V; *pmr*B P360Q; *lpx*A G68G	*aad*A1; *sat*2; *dfr*A1; aph(6)-Id; bla_{OXA-69}; ant(3")-Iia; *sul*2; *tet*(B); bla_{NDM-1}; bla_{ADC}; *sul*1; *qac*E; *aad*A1; aac(3)-Ia; aph(3')-Ia; bla_{OXA-23}; *mph*(E); *msr*(E); *arm*A; *cml*A5; *arr*-2; *gyr*A_S81L; aph(3")-Ib; aph(6)-Id; *ble*
A20	Trauma unit	M	38	Urine	>64	*788f	Not detected	*pmr*C_H390Y; *pmr*C_H499R; *pmr*C_I131V; *pmr*B HKTYHEED66S*NLPQKN; *pmr*B L56F; *pmr*B_P360Q; *pmr*B TVSSKFD58NCKQ*VR	bla_{ADC}; bla_{OXA-23}; *sul*1; *qac*E; aph(3')-Ia; *par*C S84L; ant(3")-Iia; *sul*2; *ble*; bla_{NDM-1}; aph(6)-Id; aph(3")-Ib
A21	Adult Oncology	F	47	Fluid	>64	*830d	Not detected	Not detected	*gyr*A_S81L; *sul*2; ant(3")-Iia; aph(6)-Id; *arr*-2; *cml*A5; *qac*E; *sul*1; bla_{OXA-23}; bla_{OXA-23}; *par*C_S84L; aph(3')-Ia; bla_{OXA-69}; bla_{NDM-1}; *ble*; aph(6)-Id; aph(3")-Ib

*:novel; ST: Sequence type; MIC: Minimum inhibitory concentration

Table 4.6: Demographic and molecular characteristics of *Klebsiella pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	AMR genes
K1	General surgery	F	72	Blood Culture	8	Not detected	*pmr*B R256G	*pmr*B T246A	$bla_{OXA-232}$
K2	Adult multidisciplinary ICUs	F	28	Fluid aspirate	>64	Not detected	Not detected	Not detected	$bla_{OXA-232}$; *sul*2; aph(3")-Ib; aph(6)-Id; bla_{TEM}; *dfr*A
K3	Other surgical	M	45	Urine	>64	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K4	General surgery	M	45	Fluid aspirate	>64	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K5	Adult Medical	M	57	Blood Culture	>64	Not detected	Not detected	Not detected	$bla_{OXA-181}$; *fos*A3; *qnr*S
K8	Adult multidisciplinary ICUs	F	37	Fluid aspirate	16	*a162	Not detected	Not detected	aac(6')-Ib3; *arr*-3; *sul*1; *qac*E; *aad*A16; *dfr*A27; aph(6)-Id; aph(3")-Ib; *sul*2; aac(6')-Ib-cr5; bla_{TEM-1}; $bla_{CTX-M-15}$; *oqx*B25; *oqx*A; *fos*A; *qnr*S; $bla_{OXA-181}$
K11	Adult multidisciplinary ICUs	F	29	Sputum	64	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K12	Paediatric Surgery	F	8	Urine	32	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K13	Trauma unit	M	67	Blood	64	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K15	General Surgery	M	74	Blood Culture	16	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K17	Other surgical	F	73	Blood	4	Not detected	Not detected	Not detected	$bla_{OXA-232}$

*:novel; ST: Sequence type; MIC: Minimum inhibitory concentration

Table 4.6: Demographic and molecular characteristics of *Klebsiella pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	AMR genes
K18	Trauma unit	M	37	Fluid aspirate	64	Not detected	Not detected	Not detected	$bla_{\text{CTX-M-15}}$; aph(3")-Ib; *sul*1; aph(6)-Id; *sul*2; aac(6')-Ib-cr; aac(6')-Ib3; *aad*A16; *arr*-3; *dfr*A27; *qac*E
K19	Adult multidisciplinary ICUs	F	36	Blood Culture	64	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K21	Other surgical	F	58	Blood Culture	16	Not detected	Not detected	Not detected	$bla_{\text{OXA-181}}$
K22	Other surgical	M	37	Swab	64	Not detected	Not detected	Not detected	aac(3)-Iid; aph(3")-Ib; aph(6)-Id; aac(6')-Ib3; *arr*-3; *dfr*A27; *aad*A16; *qac*E; *sul*1; $bla_{\text{CTX-M-15}}$; bla_{OXA}
K23	General surgery	F	29	Fluid aspirate	8	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K25	Adult multidisciplinary ICUs	M	33	Respiratory tract sample	8	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K27	Adult multidisciplinary ICUs	M	22	Blood Culture	4	Not detected	Not detected	Not detected	*qnr*S1; $bla_{\text{OXA-181}}$
K28	Trauma unit	M	35	Respiratory tract sample	4	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K30	Adult multidisciplinary ICUs	M	49	Blood Culture	64	Not detected	Not detected	Not detected	aac(6')-Ib; $bla_{\text{OXA-232}}$
K34	Neonatal unit	M	218 days	Respiratory tract sample	8	Not detected	Not detected	Not detected	bla_{OXA}
K36	Other surgical	M	46	Blood Culture	16	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K37	General surgery	F	62	Urine	64	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K39	Other Surgical	M	47	Fluid aspirate	8	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$
K42	Other Surgical	F	39	Blood Culture	16	Not detected	Not detected	Not detected	$bla_{\text{CTX-M-15}}$; $bla_{\text{OXA-232}}$
K45	Trauma unit	M	67	Respiratory tract sample	32	Not detected	Not detected	Not detected	$bla_{\text{OXA-232}}$

ST = Sequence type; MIC = Minimum inhibitory concentration

Table 4.6: Demographic and molecular characteristics of *Klebsiella pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	AMR genes
K46	Other Surgical	F	44	Urine	32	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K48	Adult multidisciplinary ICUs	M	59	Blood culture	16	69bd	Not detected	Not detected	*qnr*B; *qac*E; *sul*1; *bla*$_{SHV-11}$; *bla*$_{OXA-232}$
K51	Gynaecology	F	36	Fluid aspirate	64	Not detected	Not detected	Not detected	*bla*$_{OXA}$
K53	Other Surgical	F	59	Urine	4	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K55	General Surgery	M	74	Blood Culture	16	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K56	Adult multidisciplinary ICUs	M	45	Blood Culture	>64	2497	*pmr*B R256G	*arn*C S30T; *lap*B N212T	*fos*A; *bla*$_{SHV}$; *par*C_S80I; *gyr*A_S83I; *bla*$_{OXA-232}$; *oqx*A; *oqx*B; *bla*$_{TEM}$; aac(6')-Ib; *qnr*B; *dfr*A14
K68	Adult multidisciplinary ICUs	M	35	Fluid aspirate	>64	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K70	General surgery	M	36	Fluid aspirate	32	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K71	Other Surgical	F	23	Respiratory tract sample	32	2497	*pmr*B R256G	*pmr*B G207V; *pmr*B T246A	*fos*A; *bla*$_{CTX-M-15}$; *cat*B3; *bla*$_{OXA-1}$; aac(6')-Ib-cr5; *cat*A1; *bla*$_{SHV-11}$; *gyr*A_S83I; *oqx*A; *oqx*B; *par*C_S80I; *qnr*B1; *dfr*A14; *tet*(D); *bla*$_{TEM-1}$; aac(6')-Ib; arr-2; *ere*(A); *aad*A1; *cml*A5; *ble*; *bla*$_{NDM-1}$; *mph(*E); *msr*(E); *arm*A; *sul*1; *qac*Edelta1; *dfr*A5; aph(6)-Id; aph(3")-Ib; *sul*2; *bla*$_{OXA-232}$
K72	Adult multidisciplinary ICUs	F	23	Respiratory tract sample	32	2499	*pmr*B R256R	*pmr*B L213M; *pmr*B T246A; *pmr*A A41T; *lap*B N212S; *arn*C S30T	*fos*A; *bla*$_{SHV-28}$; *omp*K36_D135DGD; *gyr*A_S83I; *oqx*A; *oqx*B19; *par*C_S80I; *tet*(A); *qnr*B1; *sul*2; aph(3")-Ib; aph(6)-Id; *sul*1; *qac*E; *aad*A2; *dfr*A12; *cat*B3; aac(6')-Ib-cr5; *bla*$_{OXA-1}$; aac(3)-Iie; *bla*$_{CTX-M-15}$; *bla*$_{TEM-1}$; *mph*(A); *qnr*S1; *bla*$_{OXA-181}$

ST = Sequence type; MIC = Minimum inhibitory concentration

Table 4.6: Demographic and molecular characteristics of *Klebsiella pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	AMR genes
K73	General surgery	M	74	Urine	16	2497	*pmr*B R256G	*pmr*B G207V; *pmr*B T246A; *lap*B N212T; *mgr*B M1V; *lpx*M S253G; *arn*C S30T	*fos*A; *cat*B3; bla_{OXA-1}; aac(6')-Ib-cr5; *cat*A1; bla_{SHV-11}; *gyr*A_S83I; *oqx*A; *oqx*B; *cml*A5; *aad*A1; ere(A); arr-2; $bla_{CTX-M-15}$; aac(6')-Ib; bla_{TEM-1}; tet(D); *dfr*A14; *qnr*B; *qac*E; *sul*1; *arm*A; *msr*E; *mph*E; bla_{NDM-1}; *ble*; $bla_{OXA-232}$; *dfr*A5; aph(6)-Id; aph(3")-Ib; *sul*2
K74	Adult multidisciplinary ICUs	M	78	Blood culture	>64	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K78	General Surgery	M	36	Respiratory tract sample	32	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K80	General Surgery	M	36	Blood Culture	32	Not detected	Not detected	Not detected	$bla_{OXA-232}$
K83	Adult multidisciplinary ICUs	F	51	Respiratory tract sample	16	2497	*pho*Q G385S; *pmr*B R256G	*mgr*B M1V; *lap*B N212T	gyrA_S83I; $bla_{CTX-M-15}$; bla_{OXA-1}; aac(6')-Ib-cr5; *cat*A1; bla_{SHV-11}; *fos*A; *par*C_S80I; *oqx*B; *oqx*A; *qnr*B1; *dfr*A14; *tet*(D); bla_{TEM-1}; aac(6')-Ib; *arr*-2; *ere*(A); *aad*A1; *cml*A5; aac(6')-Ib-cr5; *bla*OXA-1; *cat*B3; *ble*; *bla*NDM-1; *mph*E; *msr*E; *arm*A; *sul*1; *qac*E; $bla_{OXA-232}$
K84	Other surgical	M	45	Fluid aspirate	65	2497	*pmr*B R256G	*lpx*M S253G; *arn*C S30T; *pmr*B T246A; *lap*B N212T	bla_{SHV-11}; *cat*A1; aac(6')-Ib-cr5; bla_{OXA-1}; *cat*B3; $bla_{CTX-M-15}$; *gyr*A_S83I; *oqx*A; *oqx*B; *par*C_S80I; *fos*A; *cat*B3; bla_{OXA-1}; aac(6')-Ib-cr5; *cml*A5; *aad*A1; *ere*(A); *arr*-2; bla_{TEM-1}; *tet*(D); *dfr*A14; *qnr*B; *qac*E; *sul*1; *arm*A; *msr*E; *mph*E; bla_{NDM-1}; *ble*; bla_{OXA}; $bla_{OXA-232}$

ST = Sequence type; MIC = Minimum inhibitory concentration

This study highlights a concerning burden of colistin resistance in *A. baumannii* and *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa. In this study, colistin resistance was observed in 22% (69/312) of *A. baumannii* and 23% (117/518) of *K. pneumoniae* tested by colistin BMD. The interpretation of these resistance rates must take into consideration the fact that only MDR/XDR isolates were included and that these comprised 29% (830/2900) of all clinical *A. baumannii* and *K. pneumoniae* isolates for the period analysed. A South African study done by Lowe *et al.* (2022) reported a 9.4% colistin resistance rate in clinical *A. baumannii* isolates, while Salvador-Oke *et al.* (2024) found a 7% colistin resistance rate in clinical *K. pneumoniae* isolates.

In this study, a high resistance rate to colistin in *K. pneumoniae* was observed. The high prevalence of *K. pneumoniae* in this study could be due to the pathogen being part of the normal flora of the human gut in immunocompromised and immune-competent patients and also due to its presence in healthcare setting environments, where frequent antibiotic use and prolonged hospital stays drive resistance and increase its spread. (Wang *et al.*, 2019a; Zhong *et al.*, 2020; Chang *et al.*, 2021). South African studies have reported *A. baumannii* and *K. pneumoniae* to be the most common pathogens acquiring colistin resistance (Messina *et al.*, 2017; Majavie *et al.*, 2021; Matshediso *et al.*, 2024).

Males (60%; 111/186) exhibited higher infection rates with colistin resistant isolates than females, aligning with previous findings conducted in South Africa (Messina *et al.*, 2017; Majavie *et al.*, 2021; Lowe *et al.*, 2022; Ngoma *et al.*, 2024). Males are generally more susceptible to infections than females due to the difference in immune system function between the sexes (Dias *et al.*, 2022). Oestrogen enhances immune responses in females, while testosterone can suppress certain immune responses, making males more vulnerable to infections (Dias *et al.*, 2022). Majority of colistin resistance infections were found to be in 30 to 39-year age range and ≥60-year age range. Studies done on colistin resistance in South Africa have reported the median age to be between or close to 30 to 39-year age range (Majavie *et al.*, 2021; Ngoma *et al.*, 2024). This suggests that younger adults in South Africa may also be at significant risk, potentially due to factors such as prolonged hospital stays, ICU admissions or prior exposure to broad-spectrum antibiotics, leading to acquisition of resistance mechanisms, which confer resistance to various agents by colonizing bacteria.

The major specimen type was blood cultures which is consistent with a South African study done by Majavie *et al.* (2021); however this study reports a lower proportion (44%; 82/186)

than the cited study (65%). Despite this, it still highlights bloodstream infections (BSIs) as a concern due to their association with high mortality rates, particularly when caused by carbapenem and colistin-resistant pathogens (Uzairue *et al.*, 2022). In the South African public healthcare sector, BSIs caused by *A. baumannii* have been increasing and now rank third in prevalence after *K. pneumoniae* and *S. aureus* (Finlayson *et al.*, 2024). This study only included *A. baumannii* and *K. pneumoniae* isolates and therefore cannot comment on the contribution of these two pathogens to the overall BSI burden at the tertiary-level hospital.

A significant proportion of isolates were observed to be from the adult multidisciplinary ICUs (45%; 84/186) and the trauma unit (12%; 23/186). The significant association between ICU admission and colistin-resistant infections (p = 0.017) emphasizes the need for enhanced infection control measures. Among the total 186 isolates of *A. baumannii* and *K. pneumoniae* analysed, more than half (62%; 116/186) were XDR. The adult multidisciplinary ICUs accounted for 46% (79/171) of all MDR and XDR cases. This result is not surprising as studies have found that extended durations of admission in ICU is linked to a higher incidence of infections (Masih *et al.*, 2016; Wilson *et al.*, 2024). A study found that an ICU stay of eight days or more was significantly associated with healthcare-associated infections (Masih *et al.*, 2016). Furthermore, patients admitted to ICUs often have multiple factors that predispose them to infections: invasive devices in-situ, undergo invasive procedures, have multiple underlying comorbidities like diabetes mellitus and chronic renal disease (Zhang *et al.*, 2025).

Previous South African studies have reported on colistin-resistance mechanisms in *A. baumannii* and *K. pneumoniae* in clinical samples (Jayol *et al.*, 2014; Jayol *et al.*, 2015; Snyman *et al.*, 2020; Lowe *et al.*, 2022; Nogbou *et al.*, 2022). In this study, we report on substitutions in *pmr*A, *pmr*B and *pho*Q.

A single substitution M12I was detected in the *pmr*A gene in *A. baumannii* in this study. The *pmr*A gene encodes for the response regulator of the PmrCAB two-component system. According to Arroyo *et al.* (2011), who was the first to identify the M12I mutation in the *pmr*A gene, the substitution is known to be associated with the *pmr*A G54E mutation characterising colistin heteroresistance. However, in this study, no other known mutations were present and among the novel mutations, substitutions were found in the *pmr*B, *lpx*A and *pmr*C genes. In all four *A. baumannii* isolates, the novel mutation *pmr*C I131V was identified. Literature has found this mutation to be previously linked to colistin resistance, however the specific role of I131V in mediating colistin resistance remains unclear (Arroyo *et al.*, 2011;

Misic *et al.*, 2018; Zafer *et al.*, 2023). Colistin MICs of isolates ranged between MIC of 4 µg/ml and MIC of >64 µg/ml. The mutation likely forms part of a broader set of mutations within the PmrCAB regulatory system. Future research should include determining and verifying the significance of these mutations on colistin resistance. Interestingly, isolate A3, despite its high colistin MIC (>64 µg/ml), lacked any detectable colistin-resistance-associated mutations or genes.

Sub-genomic analysis to determine AMR gene profiling revealed the presence of multiple β-lactamases (*bla*$_{\text{OXA-23}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{ADC,}}$ *bla*$_{\text{TEM}}$), aminoglycoside resistance genes (*aph(6)-Id*, *arm*A, *aad*A1, *aac(3)-Ia*), plasmid-associated efflux and resistance elements (*qac*E, *sul*1, *sul*2, *arr*-2). The predominance of chromosomal mutations and the wide-ranging co-resistance patterns further complicate treatment options, increasing the need for ongoing genomic surveillance and antimicrobial stewardship especially in ICUs and surgical wards.

In *K. pneumoniae*, five isolates exhibited the *pmr*B R256G substitution. Among them, one isolate also harboured the *pho*Q G385S substitution. The *pmr*B R256G mutation found is similar to reports from other studies done in Brazil (Aires *et al.*, 2016; Pitt *et al.*, 2018; Rodrigues *et al.*, 2019). However, one study found the same mutation (*pmr*B R256G) in both colistin-susceptible and colistin-resistant *K. pneumoniae*, which could suggest that the R256G mutation alone is not a definitive cause of colistin resistance and that other factors or mutations may contribute to resistance in these bacteria (Pitt *et al.*, 2018; Rodrigues *et al.*, 2019). The *pho*Q G385S substitution has previously been described in a colistin-resistant clinical *K. pneumoniae* strain (Olaitan *et al.*, 2016; Janssen *et al.*, 2020; van der Lans *et al.*, 2023). Mutations in this gene have been linked to colistin resistance, primarily due to modifications in lipid A that reduce the negative charge of the outer membrane, which decreases colistin binding, thereby contributing to resistance (van der Lans *et al.*, 2023).

A novel mutation in *pmr*B T246A was identified in five *K. pneumoniae* isolates. The mutation may lead to L-Ara4N modifications of lipid A, regulated by the PmrAB system. However, its precise impact on resistance levels remains unclear. It is likely that this modification interferes with target binding and membrane disruption, which reduces the activity and synergistic potential of colistin (Olsson *et al.*, 2023). The mutation in *pmr*B T246A was co-harboured with mutations in *pmr*B R256G by two isolates.

Moreover, the *mgr*B gene was found as an unknown point mutation in two *K. p*neumoniae isolates. The mutation found in this study *mgr*B M1V, was found in another study as a missense

mutation (Li *et al.*, 2023). The *mgr*B gene is significant as it is a major mechanism of colistin resistance in *K. pneumoniae* (Cannatelli *et al.*, 2015). Other novel mutations found in majority of *K. pneumoniae* isolates included *lap*B N212T, *arn*C S30T and *pmr*B T246A.

Sub-genomic analysis revealed that *K. pneumoniae* isolates with colistin resistance mutations also frequently carried multiple resistance determinants. Among isolates harbouring chromosomal mutations such as *pmr*B R256G had a strong co-occurrence of key β-lactamase resistance genes including $bla_{\text{OXA-232}}$, $bla_{\text{OXA-181}}$, $bla_{\text{NDM-1}}$, $bla_{\text{SHV-11}}$, and $bla_{\text{CTX-M-15}}$. The strong correlation between colistin-resistant mutations and high-risk AMR determinants supports the notion that selective pressures in the hospital environment are favouring the emergence and persistence of hyper-resistant pathogens. These findings highlight the importance of genomic surveillance that can monitor both chromosomal and plasmid-mediated resistance mechanisms simultaneously to guide infection control and antimicrobial stewardship efforts effectively.

In contrast to many international reports where plasmid-mediated *mcr* genes have associated with the rapid dissemination of colistin resistance, none of the isolates in the present study harboured known *mcr* variants. Instead, colistin resistance in this sample pool appears to be driven by a combination of resistance genes and point mutations.

CHAPTER 5: CONCLUSION

5.1 Concluding remarks

This study set out to investigate the genomic and epidemiological characteristics of colistin resistance in *A. baumannii* and *K. pneumoniae.* These pathogens are of critical concern within South African healthcare settings. In this study, nearly a quarter of the isolates were resistant to colistin, with *K. pneumoniae* comprising the majority of these. Most resistant cases were identified in patients in ICUs. Whole-genome sequencing revealed that resistance was driven predominantly by chromosomal mutations. Known chromosomal mutation included *pmr*A M12I, *pmr*B R256G and *pho*Q G385S. In addition to these, several novel mutations were observed, notably *pmr*C I131V, which was present in all four *A. baumannii* isolates that passed quality control. The absence of *mcr* genes suggests a distinct chromosomal path of resistance in our clinical setting. The study also identified dominant sequence types such as ST2 and ST2497, which may represent high-risk clones in the hospital setting.

In addition to analysing colistin resistance mechanisms, a key finding was the strong correlation between chromosomal colistin resistance mutations and the presence of high-risk AMR genes. Isolates with colistin-resistant mutations such as *pmr*AB, *pho*Q and novel variants also carried an extensive array of resistance genes including $bla_{\text{OXA-232}}$, $bla_{\text{OXA-181}}$, and $bla_{\text{NDM-1}}$, $bla_{\text{OXA-23}}$, $bla_{\text{NDM-1}}$. The co-occurrence of colistin and carbapenem resistance poses significant challenge for clinical treatment and infection control.

The findings of this study add to the global understanding of antimicrobial resistance. While much attention has been given to the horizontal transfer of resistance genes, this research emphasises that chromosomally driven resistance remains an under-recognised mechanism, particularly in high-risk and high-antibiotic-pressure settings. In the context of South Africa, where genomic surveillance is still developing, this study contributes essential data and a localised perspective. Few published studies to date have explored the genomic basis of colistin resistance in South African *A. baumannii* and *K. pneumoniae* isolates, particularly using long-read sequencing technologies such as Oxford Nanopore. As a result, much of the national understanding of colistin resistance dynamics has been based on international trends, which may not fully reflect the South African genetic diversity, healthcare pressures or antibiotic use patterns.

This study fills a critical knowledge gap by showing that chromosomal mutations are likely playing a dominant role in colistin resistance in this setting. Clinically, the absence of mobile resistance elements may provide temporary reassurance regarding rapid spread between facilities. However, the adaptive potential of chromosomal resistance poses a challenge that is less visible, harder to contain and requires strict infection control measures, strengthened diagnostic infrastructure and antimicrobial stewardship. The relevance of these findings extends across multiple disciplines. Microbiologists, infectious disease clinicians and infection control teams can use this evidence to inform targeted interventions. Public health authorities may leverage the insights to refine national AMR strategies.

Limitations of this study include: (1) a small sample size; (2) a single-site study therefore the findings of this study is not a full reflection of the overall epidemiology of colistin-resistant *A. baumannii* and *K. pneumoniae* in South Africa; (3) only a subset of all *A. baumannii* and *K. pneumoniae* isolates had colistin BMD testing done; (4) inadequate sequencing data was produced causing limitations in analysis; (5) limited demographic data for 96 isolates that WGS was performed on.

Future research should aim to better understand whether these “unknown” point mutations directly cause colistin resistance and if so, how they contribute to it. Expanding the geographical scope of research to include more hospitals within the province would also provide insights into differences in resistance profiles and the spread of known point mutations that cause colistin resistance. Expanding the research to include other common hospital pathogens and studying how mobile genetic elements contribute to the spread of resistance would provide a broader view of resistance dynamics in hospital-associated bacteria.

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ANNEXURE A: EXPERIMENTAL AND DATA CAPTURING PROCEDURES

1. Patient and isolate information

Epidemiological data of clinical *Acinetobacter baumannii* and *Klebsiella pneumoniae* was received from Corporate Data Warehouse (CDW), National Health Laboratory Services (NHLS). The patient details were kept confidential by CDW, NHLS and no access was granted. However, the following isolates details were provided: (i) collection date; (ii) specimen type; (iii) hospital information; (iv) ward information; (v) Broth microdilution (BMD) minimum inhibitory concentration (MIC) results; (vi) antimicrobial susceptibility test results; (vii) demographic information such as age and gender and (ix) organism identification.

All clinical *A. baumannii* and *K. pneumoniae* isolates were received from Infection Control Services, NHLS. The patient details were kept confidential by Infection Control Services.

2. Culturing and storage of clinical colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates

a) Preparation of MacConkey Agar (1 L)

MacConkey agar powder	52 g
Distilled water	1 L

MacConkey agar powder (Laboratorios CONDA, South Africa) (52 g) was weighed out and dispensed into 1 L of distilled water. The mixture was brought to a boil ensuring the powder dissolved completely. The dissolved solution was sterilised in autoclave at 121°C for 15 minutes at 15 pound-force per square inch (psi). Once sterilised, the medium was allowed to cool to ~45 to 50°C before it was poured into sterile petri dishes. Plates were stored at 4°C for later use.

b) Bolton Broth

Bolton broth powder	13.8 g
Distilled water	500 mL

A total of 13.8 g of the Bolton broth powder (ThermoFisher, USA) was dissolved in 500 mL of distilled water, ensuring thorough mixing. Once fully dissolved, the solution was carefully distributed into sterile containers. The containers filled with the Bolton broth solution, were then sterilized by autoclaving at 121°C for 15 minutes.

Procedure:

The Microbank vial (Pro-Lab Diagnostics, Bromborough, Wirral, UK) was removed from -70°C freezer storage (New Brunswick, USA) and allowed to thaw briefly at room temperature. Using a sterile inoculation loop, a single bead was carefully picked from the vial and placed directly onto a MacConkey agar plate (Laboratorios CONDA, South Africa). The bead was gently rolled across the surface with the loop to distribute the bacteria evenly. A quadrant streak technique was then performed to ensure the isolation of single colonies. The plate was incubated in an aerobic incubator at 35 to 37°C for 18 to 24 hours, allowing bacterial growth to develop.

After 24 hours, bacterial growth on the MacConkey agar (Laboratorios CONDA, South Africa) plate was examined. Using a sterile inoculation loop, a single colony was carefully selected and inoculated into Bolton broth (ThermoFisher, USA). The inoculated broth was then placed in an incubator set at 35 to 37°C for 18 to 24 hours.

3. Automated Genomic Deoxyribonucleic acid (gDNA) extraction by RAPI-X-96

Bacterial broth culture	1.2 mL
Buffer DLB	400 µL
Lysozyme solution	20 µL
RNase A	5 µL
Proteinase K	20 µL
DBL-20	20 µL
Ethanol	220 µL

Procedure:

A total of 1.2 mL of an overnight bacterial broth culture was collected in a 1.5 mL microcentrifuge tube and centrifuged at 13 000×g for 1 minute at room temperature (15–25°C). The supernatant was discarded, and the process was repeated to increase the pellet yield if necessary. Buffer DLB (400 µL), 20 µL of lysozyme solution, and 5 µL of RNase A were added to the pellet, followed by incubation at room temperature for 20 minutes. After incubation, 20 µL of Proteinase K and 20 µL of DLB-20 were added, and the mixture was vortexed for 30 seconds. Then, 220 µL of ethanol (96–100%) was added, and the tube was inverted to mix

From the prepared sample, approximately 700 µL was aspirated and transferred to an empty 96 deep-well plate for automated processing. Three more 96 deep-well plates were used. In each well-plate: (1) wash buffer 1 and MagPure particles were added; (2) Wash buffer 2 and (3) Buffer EB (Table A1).

Table A1: Cartridge Workstation Positions and Corresponding Volumes for RAPI X-96

Workstation	**Cartridges**	**Volume**
Position 1	Sample plate	700 µL
Position 2	Wash Buffer 1 + MagPure particles (MPN)	500 µL + 20 µL
Position 3	Wash Buffer 2	500 µL
Position 4	Buffer EB	100 - 50 µL

The Microbial DNA program was initiated (Table A2) and the filled cartridges were placed at designated workstations. Once completed, the eluted product was then stored at -20°C for further analysis

Table A2: The Microbial DNA program in RAPI-X 96

Step	**Position**	**Step Name**	**Time (seconds)**	**Volume (µL)**	**Temperature (ºC)**
1	3	Get Beads	70	500	OFF
2	1	Binding	490	700	56
3	2	Washing	60	500	OFF
4	3	Washing	70	500	OFF
5	3	Incubate	30	500	OFF
6	4	Elution	360	100	56

4. Oxford Nanopore Sequencing of *Acinetobacter baumannii* and *Klebsiella pneumoniae*

a) Preparation of DNA Library

Template DNA	10 µL
Rapid barcodes	1.5 µL

AMPure XP Beads (AXP)	Depending on pooled barcoded sample volume
80% Ethanol	2 ml
Elution Buffer (EB)	15 µL per 24 barcodes
Rapid Adapter (RA)	1.5 µL
Adapter Buffer (ADB)	3.5 µL

The Automated Rapid Sequencing Protocol (SQK-RBK114.96) was carried out following the required preparation steps. Genomic DNA (gDNA) was quantified to ensure 200 ng per sample. If the concentration was below 200 ng, 10 µL of gDNA was transferred into a 0.2 mL thin-walled polymerase chain reaction (PCR) tube. If the concentration exceeded 200 ng, the DNA was adjusted to 10 µL using nuclease-free water. The sample was mixed by pipetting to minimize shearing before a brief spin-down. Barcoding was performed by adding 1.5 µL of Rapid Barcode (RB01-96) to each 10 µL DNA sample, mixing thoroughly, and incubating at 30°C for 2 minutes, followed by 80°C for 2 minutes using a thermocycler. The tubes were briefly placed on ice, spun down, and pooled into a 2 mL DNA LoBind tube, with the total volume recorded. An equal volume of AXP beads was added to the pooled sample (Table A3) and mixed by flicking the tube.

Table A3: Volume of AMPure XP Beads (AXP) added per sample

Number of samples	**Volume of AMPure XP Beads (AXP)**
1	11.5 µL
4	46 µL
12	138 µL
24	276 µL
48	552 µL
96	1 000 µL

If the specific sample number was not listed in the table, the volume corresponding to the next highest available sample number was used.

The mixture was incubated at room temperature for 10 minutes. Beads were pelleted using a magnetic rack, followed by two washes with 80% ethanol to remove contaminants. After the

ethanol was carefully removed, the pellet was air-dried for 30 seconds before resuspension in Elution Buffer (EB), with the volume determined by the number of barcodes used (Table A4).

Table A4: Volume of Elution Buffer needed per 24 barcodes used

Number of barcodes	Volume of Elution Buffer (EB)
24	15 µL
48	30 µL
72	45 µL
96	60 µL

If the specific barcode number was not listed in the table, the volume corresponding to the next highest available barcode number was used.

The sample was incubated at room temperature for 10 minutes, and once the eluate cleared, it was transferred into a 1.5 mL DNA LoBind tube. The purified sample was quantified using a Qubit fluorometer. For adapter ligation, 1.5 µL of Rapid Adapter (RA) was diluted with 3.5 µL of Adapter Buffer (ADB) in a new 1.5 mL tube, and 1 µL of the diluted RA (Rapid Adapter and Adapter Buffer solution) was added to the barcoded DNA library. The mixture was incubated at room temperature for 5 minutes, and the prepared library was stored on ice until loading.

b) Priming and loading the MinION Flow Cell

Flow Cell Flush (FCF)	1 170 µL
Flow Cell Tether (FCT)	30 µL
Final Volume	**1 205 µL**

Flow cell priming was initiated by thawing and mixing Flow Cell Tether (FCT) and Flow Cell Flush (FCF). A priming mix was prepared by combining 1,170 µL of FCF and 30 µL of FCT in a 1.5 mL DNA LoBind tube. The MinION device was opened and the flow cell (Figure A1) was inserted.

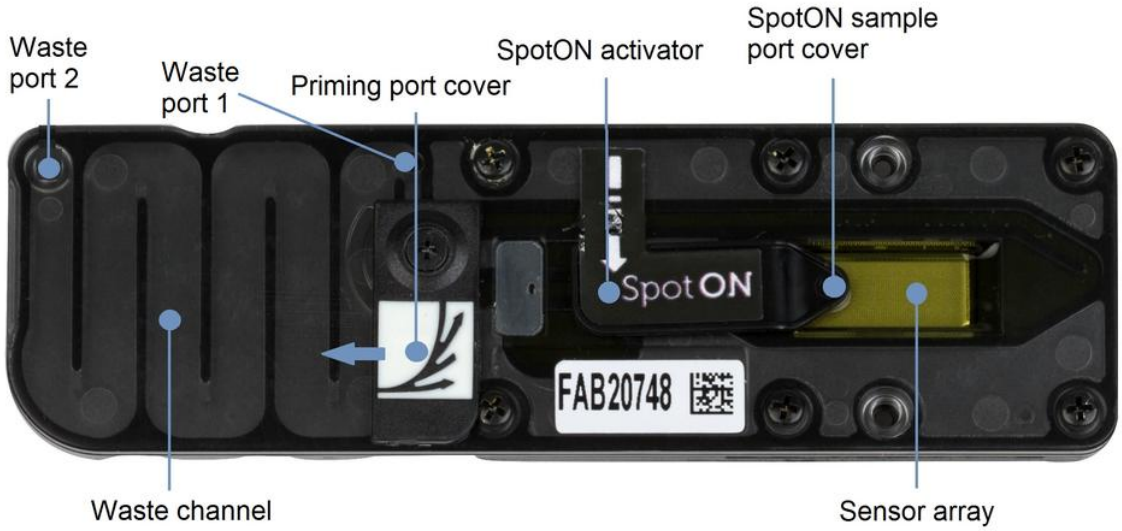


Figure A1: Oxford Nanopore Flow Cell

Priming mix (800 µL) was loaded into the priming port, followed by a 5-minute wait. The priming port was checked for air bubbles, which were removed if present by carefully extracting 20 to 30 µL of buffer by setting a P1000 pipette to 200 µL and inserting the tip into the priming port and turning the wheel until the dial shows 220 to 230 µL.

c) Preparing the DNA library for loading

Library Beads (LIB)	25.5 µL
Sequencing Biffer (SB)	37.5 µL
DNA library	12 µL

The Library Beads (LIB) were mixed immediately before use, and a sequencing mix was prepared using 37.5 µL SB, 25.5 µL LIB, and 12 µL of the DNA library. Priming mix (200 µL) was added to the priming port. The DNA library (75 µL) was added in a dropwise fasion into the SpotON sample port. The SpotON cover was secured, the light shield was installed, and the device lid was closed before the sequencing run was started using MinKNOW software.

d) Sequencing run using the MinKNOW software

In MinKNOW (v24.06.16), the Start page was accessed and the flow cell check was performed before starting the sequencing experiment to assess the number of active pores available in the flow cell. The Start page was accessed in MinKNOW, and “Start Sequencing” was selected. In

the Positions tab, the experiment name, sample ID and flow cell type were entered before proceeding to kit selection. The appropriate kit and any expansions used for library preparation were selected and the process continued to Run Options, where the sequencing run length and minimum read length were set. The Output section was then configured by specifying the output data location, format, and filtering options. A final review was conducted before clicking Start to begin the sequencing run.

e) Flushing / washing MinION Flow cell

For flow cell maintenance, if reuse was required, a Flow Cell Wash Mix was prepared by combining 2 µL Wash Mix (WMX) with 398 µL Wash Diluent (DIL). 200 µL of the wash mix was loaded into the priming port, incubated for 5 minutes, and then the remaining 200 µL was added. The wash solution was left for 1 hour before waste removal.

f) Storage of flow cell

Storage Buffer (S)	500 µL

For storage, the Storage Buffer (S) was thawed and mixed thoroughly before 500 µL was loaded into the priming port. After closing the priming port, all the waste buffer was removed through the waste port 1 port and the flow cell was stored at 4–8°C for future use.

5. Whole-genome sequencing analysis of *Acinetobacter baumannii* and *Klebsiella pneumoniae*

a) EPI2ME

Fastq files (bacterial reads) obtained after the sequencing run were used to assemble genomes and annotate regions of interest. The raw sequencing data was processed using the EPI2ME bacterial genomes (v1.4.1) (https://github.com/epi2me-labs/wf-bacterial-genomes) workflow. The Fastcat/bamstats (v0.20.0; https://github.com/epi2me-labs/fastcat; Accessed: 25 January 2025) tool was employed to concatenate multiple sample files for efficient processing. A draft genome assembly was generated using Flye (v2.9.5; https://github.com/mikolmogorov/Flye; Accessed: 25 January 2025), which was then refined through error correction with Medaka (v2.0.1; https://github.com/nanoporetech/medaka; Accessed: 25 January 2025) and the assembled genome underwent annotation via Prokka (v1.14.5; https://github.com/tseemann/prokka; Accessed: 25 January 2025).

b) Galaxy

For bioinformatic analyses, the web-based platform Galaxy (v24.2.1. dev0; https://usegalaxy.org/; Accessed: 25 January 2025) was utilized. Post-sequencing quality control was done by FastQC tool (v0.74; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/devteam/fastqc/fastqc/0.74+galaxy1; Accessed: 2 February 2025). Genome assembly quality, including N50, GC content and the number of contigs was assessed with Quality Assessment Tool (v5.3.0; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/quast/quast/5.3.0+galaxy0; Accessed: 25 January 2025). Sequence typing was carried out using the Multi-Locus Sequence Typing (MLST) tool (v2.22.0; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/mlst/mlst/2.22.0; Accessed: 2 February 2025), referencing the PubMLST database and the Center for genomic epidemiology MLST platform (https://cge.food.dtu.dk/services/MLST/).

The draft genomes were further screened for antibiotic resistance genes, plasmids, and virulence factor genes through ABRicate (v1.0.1; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/abricate/abricate/1.0.1; Accessed: 2 February 2025), which integrates databases like Resfinder, PlasmidFinder, CARD, VFDB, and NCBI. Colistin resistance-associated point mutations were detected using AMRFinderPlus (v3.12.8; https://usegalaxy.org/root?tool_id=toolshed.g2.bx.psu.edu/repos/iuc/amrfinderplus/amrfinderplus/3.12.8+galaxy0; Accessed: 2 February 2025). Pathogenwatch (v23.1.5; https://pathogen.watch/; Accessed: 2 February 2025) was used to determine the core genome (cg) MLST classification, capsular serotypes and plasmid replicons for each isolate. Finally, the sequencing reads of *K. pneumoniae* were mapped to the reference genomes *K. pneumoniae* MGH 78578 (Accession number: CP000647) to build a phylogenetic tree (https://cge.food.dtu.dk/services/CSIPhylogeny/).

ANNEXURE B: ISOLATE INFORMATION (RAW DATA)

Table B1: Demographic data of colistin-resistant *Acinetobacter baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa

No	Date	Ward	Gender	Age	Specimen type	Colistin MIC (µg/ml)
A19	2024/02/02	Adult multidisciplinary ICU	F	28	Blood	>64
A5	2024/01/27	Trauma unit	M	29	Blood	>64
A20	2024/01/22	Trauma unit	M	35	Sterile site aspirate	64
A6	2024/02/01	Other surgical	M	36	Sterile site aspirate	>64
A21	2023/08/03	General surgery	F	57	Blood	8
A22	2023/05/03	Neonatal unit	M	2	Blood	8
A23	2023/04/11	Trauma unit	M	35	Blood	>64
A24	2023/03/29	Neonatal unit	M	4 days	Blood	8
A25	2023/04/04	Paediatric surgery	M	1 month 9 days	Blood	>64
A26	2023/04/27	Other surgical	M	47	Sterile site aspirate	16
A27	2023/01/17	Adult multidisciplinary ICU	F	63	Urine	64
A28	2022/08/02	Adult multidisciplinary ICU	M	51	Blood	>64
A29	2022/10/26	Adult multidisciplinary ICU	M	33	Respiratory tract sample	>64
A30	2022/08/29	Other surgical	M	25	Sterile site aspirate	>64
A31	2022/08/29	Adult multidisciplinary ICU	M	15	Blood	32
A32	2023/03/28	Adult multidisciplinary ICU	F	36	Blood	>64
A33	2022/07/25	Neonatal unit	M	8 days	Blood	16
A34	2022/07/24	General surgery	M	33	Swab	>64
A35	2022/08/01	Adult multidisciplinary ICU	M	33	Blood	>64
A36	2023/04/11	Adult oncology	M	49	Blood	>64
A37	2022/06/25	Trauma unit	M	37	Sterile site aspirate	>64
A38	2022/06/17	Trauma unit	M	43	Blood	>64
A39	2022/05/21	General surgery	M	29	Sterile site aspirate	32
A40	2022/07/28	Adult multidisciplinary ICU	F	35	Respiratory tract sample	8

Table B1: Demographic data of colistin-resistant *Acinetobacter baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	Date	Ward	Gender	Age	Specimen type	Colistin MIC (µg/ml)
A41	2022/12/11	Adult multidisciplinary ICU	F	73	Respiratory tract sample	32
A42	2022/05/05	Adult multidisciplinary ICU	M	34	Respiratory tract sample	>64
A43	2022/02/27	Trauma unit	M	35	Blood	4
A44	2022/02/03	Adult multidisciplinary ICU	M	28	Respiratory tract sample	4
A45	2022/03/06	Neonatal unit	M	2 months 6 days	Sterile site aspirate	8
A46	2022/10/20	Adult multidisciplinary ICU	F	49	Respiratory tract sample	>64
A47	2022/06/24	Other surgical	M	53	Sterile site aspirate	>64
A48	2024/01/11	Trauma unit	M	39	Sterile site aspirate	4
A49	2022/08/24	Adult multidisciplinary ICU	M	39	Blood	>64
A50	2022/01/13	Adult multidisciplinary ICU	F	58	Swab	8
A51	2022/01/05	Adult oncology	F	53	Blood	>64
A52	2022/07/17	Adult medical	F	59	Respiratory tract sample	4
A53	2022/07/10	Adult multidisciplinary ICU	F	41	Blood	>64
A54	2023/01/31	Adult oncology	M	67	Sterile site aspirate	64
A55	2022/07/23	General surgery	M	40	Sterile site aspirate	64
A56	2022/06/24	Adult multidisciplinary ICU	M	19	Blood	>64
A57	2022/10/27	Adult multidisciplinary ICU	F	49	Sterile site aspirate	>64
A58	2022/01/03	Adult multidisciplinary ICU	M	68	Blood	4
A59	2022/07/13	Adult multidisciplinary ICU	F	20	Respiratory tract sample	>64
A60	2023/01/07	Trauma unit	M	43	Sterile site aspirate	4
A61	2022/10/30	Paediatric surgery	F	4	Sterile site aspirate	64
A62	2023/11/03	Adult multidisciplinary ICU	F	33	Blood	>64

Table B1: Demographic data of colistin-resistant *Acinetobacter baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	Date	Ward	Gender	Age	Specimen type	Colistin MIC (µg/ml)
A63	2022/01/17	Adult multidisciplinary ICU	M	15	Blood	4
A64	2022/09/01	Adult multidisciplinary ICU	F	28	Blood	64
A65	2023/03/29	Adult multidisciplinary ICU	M	30	Blood	8
A66	2022/06/10	Adult multidisciplinary ICU	M	31	Blood	>64
A67	2023/02/15	Trauma unit	F	39	Sterile site aspirate	64
A68	2023/02/05	Gynaecology	F	25	Urine	32
A69	2022/02/24	Adult multidisciplinary ICU	M	73	Urine	4
A70	2022/06/23	Trauma unit	F	62	Sterile site aspirate	>64
A71	2022/09/15	Neonatal unit	M	9	Respiratory tract sample	>64
A72	2022/09/05	Adult multidisciplinary ICU	M	37	Sterile site aspirate	64
A73	2023/05/02	Adult multidisciplinary ICU	M	67	Sterile site aspirate	>64
A1	2023/10/15	General surgery	M	57	Sterile site aspirate	>64
A74	2022/02/22	Adult multidisciplinary ICU	M	68	Sterile site aspirate	>64
A75	2023/01/31	Adult multidisciplinary ICU	F	32	Blood	4
A76	2023/02/13	Adult multidisciplinary ICU	M	31	Blood	4
A77	2022/12/15	Adult multidisciplinary ICU	F	51	Respiratory tract sample	4
A78	2023/04/04	Trauma unit	M	25	Sterile site aspirate	64
A79	2022/07/04	Adult oncology	M	31	Sterile site aspirate	>64
A80	2023/04/29	Neonatal unit	F	9 days	Blood	4
A81	2022/06/15	Adult medical	F	13	Sterile site aspirate	>64
A82	2023/05/04	Other surgical	F	53	Sterile site aspirate	>64
A83	2023/05/20	Adult multidisciplinary ICU	F	34	Urine	4
A84	2023/01/04	Adult multidisciplinary ICU	M	53	Urine	>64

Table B2: Demographic data of colistin-resistant *Klebsiella pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa

No	Date	Ward	Gender	Age (years)	Specimen type	Colistin MIC (µg/ml)
K88	2024/05/19	Paediatric surgery	M	1 month 6 days	Blood	>64
K29	2024/05/05	Adult multidisciplinary ICU	M	24	Blood	4
K69	2024/03/16	Adult oncology	F	27	Blood	>64
K89	2024/02/24	Other surgical	F	24	Sterile site aspirate	16
K87	2024/06/05	Other surgical	F	46	Blood	16
K83	2024/04/17	Adult multidisciplinary ICU	F	51	Respiratory tract sample	16
K90	2024/02/24	Adult multidisciplinary ICU	F	42	Urine	16
K82	2024/05/10	Adult multidisciplinary ICU	F	23	Blood	8
K71	2024/03/23	Adult multidisciplinary ICU	F	23	Respiratory tract sample	32
K6	2024/05/23	Adult multidisciplinary ICU	F	36	Blood	64
A18	2024/03/17	Trauma unit	M	27	Respiratory tract sample	8
K66	2024/03/10	General surgery	M	25	Urine	32
K91	2024/03/07	Trauma unit	F	50	Blood	16
K2	2024/02/08	Adult multidisciplinary ICU	F	28	Sterile site aspirate	>64
K92	2024/05/13	Adult multidisciplinary ICU	M	33	Blood	>64
K93	2024/02/03	Trauma unit	M	35	Sterile site aspirate	64
K94	2024/04/19	Trauma unit	M	51	Respiratory tract sample	16
K95	2024/03/09	Adult multidisciplinary ICU	M	48	Blood	4
K27	2024/01/20	Adult multidisciplinary ICU	M	22	Blood	4
K8	2024/01/08	Adult multidisciplinary ICU	F	37	Blood	16
K16	2023/12/19	Other surgical	F	59	Sterile site aspirate	32
K96	2024/01/20	Adult medical	M	70	Sterile site aspirate	>64
K97	2024/02/06	General surgery	F	52	Sterile site aspirate	16

Table B2: Demographic data of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	Date	Ward	Gender	Age (years)	Specimen type	Colistin MIC (µg/ml)
K18	2023/12/23	Trauma unit	M	37	Blood	64
K75	2024/03/31	Adult multidisciplinary ICU	M	78	Blood	>64
K98	2023/12/27	Neonatal unit	F	2 months 6 days	Blood	64
K26	2024/05/15	Adult medical	F	85	Urine	8
K86	2024/04/15	Trauma unit	M	21	Sterile site aspirate	64
K99	2023/08/03	General surgery	F	57	Blood	8
K68	2024/03/15	Adult multidisciplinary ICU	M	35	Sterile site aspirate	>64
K76	2024/03/26	Gynaecology	F	48	Sterile site aspirate	32
K100	2023/04/08	Paediatric medical	M	14 days	Blood	8
K101	2023/07/01	Paediatric surgery	M	4 months 6 days	Blood	>64
K102	2024/02/28	Adult multidisciplinary ICU	F	31	Respiratory tract sample	64
K9	2023/12/21	Adult medical	M	53	Respiratory tract sample	16
K103	2023/02/07	Adult multidisciplinary ICU	M	42	Respiratory tract sample	4
K1	2024/03/04	General surgery	F	72	Blood	8
K104	2022/12/29	Adult medical	F	15	Sterile site aspirate	>64
K105	2023/01/18	Other surgical	M	53	Sterile site aspirate	64
K106	2023/01/19	Adult multidisciplinary ICU	F	63	Blood	64
K107	2023/05/04	Other surgical	M	64	Sterile site aspirate	>64
K57	2024/05/28	Adult medical	M	38	Urine	>64
K108	2022/10/26	Adult multidisciplinary ICU	M	33	Blood	>64
K109	2024/05/13	Adult multidisciplinary ICU	F	56	Blood	32
K110	2023/02/06	Adult multidisciplinary ICU	M	58	Urine	>64

Table B2: Demographic data of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	Date	Ward	Gender	Age (years)	Specimen type	Colistin MIC (µg/ml)
K111	2024/03/25	Adult multidisciplinary ICU	M	66	Respiratory tract sample	4
K112	2024/01/22	Adult medical	M	57	Blood	4
K113	2022/11/10	Adult multidisciplinary ICU	F	60	Blood	>64
K114	2022/08/29	General surgery	F	22	Sterile site aspirate	32
K115	2022/09/08	Trauma unit	M	25	Swab	>64
K116	2022/11/09	Gynaecology	F	60	Sterile site aspirate	32
K117	2023/04/28	Adult multidisciplinary ICU	F	36	Blood	>64
K118	2022/07/31	Neonatal unit	M	14 days	Blood	16
K119	2022/08/01	General surgery	M	33	Swab	>64
K120	2022/12/15	Adult multidisciplinary ICU	F	46	Blood	4
K121	2022/08/24	Adult multidisciplinary ICU	M	33	Blood	>64
K122	2023/05/26	Adult multidisciplinary ICU	F	31	Urine	32
K123	2022/07/11	Adult multidisciplinary ICU	F	29	Blood	16
K124	2022/06/24	General surgery	M	29	Blood	32
K125	2022/08/28	Adult multidisciplinary ICU	F	35	Urine	8
K126	2023/04/01	Adult multidisciplinary ICU	F	73	Blood	32
K127	2022/05/28	Adult multidisciplinary ICU	M	34	Blood	>64
K128	2024/05/27	Adult multidisciplinary ICU	M	64	Blood	4
K129	2022/02/10	Adult multidisciplinary ICU	M	28	Blood	4
K130	2022/05/12	Neonatal unit	M	4 months 12 days	Sterile site aspirate	8
K131	2022/07/16	Adult multidisciplinary ICU	F	29	CSF	>64
K132	2022/02/09	Other surgical	M	53	Blood	>64
K133	2022/02/01	Paediatric surgery	F	1 month 18 days	Blood	4

Table B2: Demographic data of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	Date	Ward	Gender	Age (years)	Specimen type	Colistin MIC (µg/ml)
K134	2024/01/08	Trauma unit	M	39	Sterile site aspirate	4
K135	2022/06/28	Adult oncology	F	42	Sterile site aspirate	>32
K136	2024/05/17	Other surgical	M	45	Sterile site aspirate	>64
K137	2023/05/16	Adult oncology	M	67	Blood	>64
K138	2023/05/12	Adult medical	F	47	Urine	64
K139	2023/04/03	Adult oncology	F	18	Swab	64
K140	2023/01/13	General surgery	F	47	Sterile site aspirate	32
K141	2023/03/08	General surgery	M	41	CSF	>64
K142	2023/02/02	Adult oncology	M	67	Blood	64
K143	2022/08/09	General surgery	M	40	Urine	64
K144	2022/11/10	Adult multidisciplinary ICU	F	49	Sterile site aspirate	>64
K145	2022/07/24	Adult multidisciplinary ICU	F	20	Blood	>64
K146	2022/11/21	Paediatric surgery	F	4	Sterile site aspirate	64
K147	2023/12/05	Adult multidisciplinary ICU	F	34	Sterile site aspirate	>64
K148	2022/01/24	Trauma unit	M	32	Respiratory tract sample	>64
K70	2024/04/24	Adult multidisciplinary ICU	M	36	Sterile site aspirate	32
K149	2024/03/20	Adult medical	F	42	Blood	32
K150	2022/08/16	Adult multidisciplinary ICU	M	41	Blood	32
K151	2023/04/05	Adult multidisciplinary ICU	M	30	Blood	8
K152	2022/06/06	Adult multidisciplinary ICU	M	31	Blood	>64
K153	2023/04/01	Trauma unit	M	39	Blood	64
K154	2022/05/07	Adult multidisciplinary ICU	M	73	Blood	4
K5	2024/01/04	Adult medical	M	57	Blood	>64
K15	2024/05/26	General surgery	M	74	Blood	16

Table B2: Demographic data of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	Date	Ward	Gender	Age (years)	Specimen type	Colistin MIC (µg/ml)
K155	2022/03/13	Adult multidisciplinary ICU	M	25	Sterile site aspirate	8
K156	2022/07/23	Adult multidisciplinary ICU	M	67	Blood	>64
K157	2022/08/23	Adult multidisciplinary ICU	F	38	Respiratory tract sample	16
K158	2022/11/11	Other surgical	F	67	Sterile site aspirate	64
K45	2024/05/24	Trauma unit	M	67	Blood	32
K159	2022/07/10	Adult multidisciplinary ICU	M	64	Blood	32
K160	2022/09/22	Adult multidisciplinary ICU	M	37	Sterile site aspirate	64
K161	2022/11/14	Adult oncology	M	47	Sterile site aspirate	>64
K162	2023/03/29	Adult multidisciplinary ICU	M	67	Blood	>64
K163	2023/10/30	General surgery	M	57	Respiratory tract sample	>64
K164	2022/02/26	Adult multidisciplinary ICU	M	68	Sterile site aspirate	>64
K165	2022/11/29	Adult medical	F	26	Sterile site aspirate	4
K166	2022/12/20	Adult multidisciplinary ICU	F	68	Swab	>64
K167	2023/01/09	Trauma unit	M	29	Sterile site aspirate	8
K168	2022/12/10	Adult multidisciplinary ICU	M	34	Sterile site aspirate	>64
K12	2024/04/26	Paediatric surgery	F	8	Urine	32
K65	2024/01/29	Other surgical	M	83	Blood	>64
K170	2023/04/25	Paediatric medical	M	2 months 15 days	Urine	4
K171	2022/01/17	Paediatric medical	F	13	Sterile site aspirate	>64
K172	2023/05/15	Adult multidisciplinary ICU	F	41	Blood	8
K173	2023/05/21	Other surgical	M	78	Urine	32
K174	2023/05/26	Adult multidisciplinary ICU	M	36	Blood	4
K175	2022/12/07	Adult medical	M	52	Blood	>64
K176	2023/08/07	Adult medical	F	79	Blood	>64
K177	2022/02/04	General surgery	M	54	Blood	32

Table B3: Susceptibility profile of colistin-resistant *A. baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa

NO	COLISTIN	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME ORAL	CEFUROXIME PARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance profile
A19	R					S						I						Non-MDR
A5	R		R	R		R			R	R		R			R	R	S	MDR
A20	R		R	R		S			R	R		I			R	S	I	MDR
A6	R		R	R		R			R	R		R			R	R	S	XDR
A21	R		R	R		S			R	R		R			R	S	I	MDR
A22	R		R	R	S	S	S		R			R			R	S		XDR
A23	R		R	R		I			R	R		R			R	R	S	XDR
A24	R		R	R		R			R	R		R			R	R	S	XDR
A25	R		R	R		R			R	R		R			R	R	S	XDR
A26	R		R	R	R	R			R			R			R	R	S	MDR
A27	R		R	R	R	R			R			R			R	R	R	XDR
A28	R		R	R	R	R	R		R			R				R		MDR
A29	R		R	R	R	R	R		R			R			R	R		MDR
A30	R		R	R		R			R			R			R	R	S	XDR
A31	R		R	R	R	R			R			R			R	R	S	XDR
A32	R		R	R		R			R	R		R			R	R	S	XDR
A33	R		R	R		R			R	R		R			R	R	I	XDR
A34	R		R	R		R			R	R		R			R	R	S	MDR
A35	R		R	R	R	R	R		R			R			R	R		XDR

R = Resistant; I = Intermediate; S = Susceptible

Table B3: Susceptibility profile of colistin-resistant *A. baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

NO	COLISTIN	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME ORAL	CEFUROXIME PARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance profile
A36	R		R	R	R	R	R		R			R			R	R		XDR
A37	R		R	R		R	R								R	R	S	XDR
A38	R		R	R	R	R			R	R		R			R	R	S	XDR
A39	R		R	R	R	R			R			R			R	R	S	XDR
A40	R		R	R	R	R	R		R	R		R			R	R	S	XDR
A41	R		R	R		R			R			R			R	R	I	XDR
A42	R		R	R	R	R	R		R	R		R			R		S	XDR
A43	R		R	R	R	R	R		R			R			R	R		XDR
A44	R		R	R	R	R			R			R			R	R	S	XDR
A45	R		R	R	R	R	R		R			R				R		XDR
A46	R		R	R		R			R			R			R	R	S	XDR
A47	R		R	R		R	R		R						R	R	S	XDR
A48	R		R	R		S			R	R		R			R	R	S	XDR
A49	R		R	R	S	S	S		R	R		R			R	S	S	XDR
A50	R		R	R	R	R			R			R			R	R	I	XDR
A51	R		R	R	R	R	R		R			R			R	R	I	MDR
A52	R		R	R	R	R			R			R			R	R		XDR
A53	R		R	R	R	R	R		R			R			R	R	S	MDR
A54	R		R	R	R	R	R		R			R				R		MDR

R = Resistant; I = Intermediate; S = Susceptible

Table B3: Susceptibility profile of colistin-resistant *A. baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

NO	COLISTIN	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME ORAL	CEFUROXIME PARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance profile
A55	R		R	R		R			R	R		R			R	R	S	XDR
A56	R											R			R			MDR
A57	R		R	R	R	R			R			R			R	R		XDR
A58	R		R	R		R			R			R			R	R		MDR
A59	R		R	R	R	R			R			R			R	R		XDR
A60	R		R	R	R	R	R		R			R				R		XDR
A61	R		R	R		R			R			R			R	R	S	XDR
A62	R		R	R		R			R	R		R			R	R	S	XDR
A63	R		S	S	S	S	R		R			R			S	R	S	MDR
A64	R		R	R		R			R	R		R			R	R	S	XDR
A65	R		R	R		R			R	R		R			R	R	S	XDR
A66	R		R	R	R	R			R			R			R	R	S	MDR
A67	R		R	R	R	R	R		R			R				R		XDR
A68	R		R	R	R	R			R			R			R	R	S	MDR
A69	R		R	R	R	R	R		R			R			R	R		XDR
A70	R		R	R		R			R			R				R	S	XDR
A71	R		R	R	R	R	R		R			R			R	R		MDR
A72	R			R	R	R	R		R			R				R		XDR
A73	R		R	R	R	R	R		R			R			R	R		XDR

R = Resistant; I = Intermediate; S = Susceptible

Table B3: Susceptibility profile of colistin-resistant *A. baumannii* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

NO	COLISTIN	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME ORAL	CEFUROXIME PARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance profile
A1	R		R	R		S			R	R		R			R	R	S	XDR
A74	R		R	R	R	R			R			R			R	R	S	XDR
A75	R		R	R	R	R			R			R			R	R	I	MDR
A76	R		R	R	R	R			R			R			R		S	MDR
A77	R		R	R	R	R	R		R			R			R	R	S	XDR
A78	R		R	R		R			R			R			R	R	S	MDR
A79	R		R	R	R	R	R		R			R				R		MDR
A80	R		R	R	R	R			R			R			R	R		XDR
A81	R		R	R		R			R			R			R	R	S	MDR
A82	R		R	R	R	R	R		R			R			R	R		MDR
A83	R		R	R	R	R			R			R			R	R	S	XDR
A84	R		R	R		R			R			R			R	R	R	XDR

R = Resistant; I = Intermediate; S = Susceptible;

Table B4: Susceptibility profile of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa

No	COLISTIN INTREPRETATION	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIMEORAL	CEFUROXIMEPARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance Profile
K88	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K29	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K69	R	S	S	S	S	R			R	R	S	R	R	R	R	R	S	XDR
K89	R		R	R	R	S			R	R	R	R	R	R	R	R	S	XDR
K87	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K83	R	R	R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K90	R	S	S	S	S	R			R	R	S	R	R	R	R	R	S	MDR
K82	R		R	R	R	I			R	R	R	R	R	R	R	R		MDR
K71	R	R	R	R	R			R	R	R	R	R	R	R	R	R		XDR
K6	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
A18	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K66	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K91	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K2	R	S	S	S	S	S			S	S	R	S	R	R	S	S	S	non-MDR
K92	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K93	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K94	R		R	R		R			R	R	R	R	R	R	R	R	S	XDR
K95	R		I	S	S	R			R	R	R	R	R	R	R	R		XDR

R = Resistant; I = Intermediate; S = Susceptible; blank spaces = not tested

Table B4: Susceptibility profile of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	COLISTIN INTREPRETATI	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME_ CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME_ ORAL	CEFUROXIME_ PARENTERAL	PIPERACILLIN_ TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance Profile
K27	R	R	I	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K8	R	R	I	R	R	I			R	R	R	R	R	R	R	R	S	XDR
K16	R	R	R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K96	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K97	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K18	R	R	R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K75	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K98	R	R	R	R	S	R			R	R	R	R	R	R	R	R	S	MDR
K26	R		I	I					S		S		R	R	R		S	MDR
K86	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K99	R	R	R	R	R	R			R	R	R	R	R	R	R	R	I	XDR
K68	R		R	S	S	R			R	R	R	R	R	R	R	R	S	XDR
K76	R		R	R	R	R			R	R	R	R	R	R	R	R	S	MDR
K100	R	R	R	R	R	S			R	R	R	R	R	R	R	R	R	XDR
K101	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K102	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K9	R	R	R	R	R	S			R	R	R	R	R	R	R	R	S	XDR
K103	R	R	S	S	S	S			R	R	R	R	R	R	R	R	S	XDR
K1	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR

R = Resistant; I = Intermediate; S = Susceptible; blank spaces = not tested

Table B4: Susceptibility profile of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	COLISTIN INTREPRETATI	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME_ CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME_ ORAL	CEFUROXIME_ PARENTERAL	PIPERACILLIN_ TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance Profile
K104	R	R	I	S	I	R			R	R	R	R	R	R	R	S	I	MDR
K105	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R		XDR
K106	R	R	I	I	S	S			R	R	R	R	R	R	R	R	S	MDR
K107	R		S	R	R	R	R		R	R		R		R	R	R		MDR
K57	R	R																non-MDR
K108	R	R	R	R	R	R			R	R	R	R	R	R	R	R		MDR
K109	R	R	R	R	R	R			R	R	R	R	R	R	R	R		XDR
K110	R	R	I	R	R	R			S	I	R	S	R	R	R	R	S	MDR
K111	R	R	R	R														non-MDR
K112	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K113	R	R	R	R	R	R			R	R	R	R	R	R	R	R		XDR
K114	R	R	R	R	R	R			R	R	R	R	R	R	R	R	R	XDR
K115	R	R	S	I	S	R			R	R	R	R	R	R	R	R	I	XDR
K116	R	R	R	R	I	I			R	R	R	R	R	R	R	R	S	XDR
K117	R	R	I	R	R	R			R	R	R	R		R	R	R	I	XDR
K118	R	R	I	R	S	R			R	R	R	R	R	R	R	R	S	XDR
K119	R	R	R	S	I	S			R	R	R	R		R	R	R		MDR
K120	R	R	I	R	S	R			R	R	R	R	R	R	R	R	S	XDR
K121	R	R	R	R	R	R			R	R	R	R	R	R	R	R	I	MDR
K122	R	R	R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K123	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R	MDR

R = Resistant; I = Intermediate; S = Susceptible; blank spaces = not tested

Table B4: Susceptibility profile of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	COLISTIN INTREPRETATI	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME_ CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME_ ORAL	CEFUROXIME_ PARENTERAL	PIPERACILLIN_ TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance Profile
K124	R	R	R	R	R	R			R	R	R	S	S	R	R	R	S	XDR
K125	R	R	R	R	R	S			R	R	R	R	R	R	R	R	R	XDR
K126	R	R	R	R	R	R			R	R	R	R	R	R	R	R	R	MDR
K127	R	R	R	R	R	R			R	R	R	R	R	R	R	R	R	XDR
K128	R		R	S	S	R			R	R	R	R	R	R	R	R	S	MDR
K129	R	R	R	R	R	I			R	R	R	R	R	R	R	R	R	MDR
K130	R	R	R	R	S	S	R		R	R	I	R	R	R	R	I	S	XDR
K131	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	XDR
K132	R	R	R	R	S	R			R	R	R	R	R	R	R	R	I	XDR
K133	R	R	R	R	S	R			R	R	R	R	R	R	R	R	R	MDR
K134	R	S	S	S	S	S			S	S	S	S	S	S	R	S		non-MDR
K135	R	R	R	R	R	S			R	R	R	R	R	R	R	R	R	XDR
K136	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K137	R	R	R	R	R	R			R	R	R	R	R	R	R	R	I	XDR
K138	R	R	I	R	R	R			R	R	R		R	R	R	R	S	MDR
K139	R	R	R	R														NEITHER
K140	R	R	S	S	S	R	R		R	R	R	R		R	R	R	S	MDR
K141	R	S	S	S	S	S			S	S	R	S	R	R		S		non-MDR
K142	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R	S	XDR
K143	R	R	R	R	R	R			R	R	R	R	R	R	R	R	S	MDR

R = Resistant; I = Intermediate; S = Susceptible; blank spaces = not tested

Table B4: Susceptibility profile of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	COLISTIN INTREPRETATIO	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME ORAL	CEFUROXIME PARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance Profile
K144	R	R	R	R	R	S			R	R	R	R	R		R	R	S	MDR
K145	R	R	R	R	R	I			R	R	R	R	R	R	R	R	R	MDR
K146	R	R	R	R	S	I			R	R	R	R	R	R	R	R	S	XDR
K147	R	R	R	R	R	R			R	R	R	R	R	R	R	R	R	XDR
K148	R	R	R	R	I	R			R	R	R	R	R	R	R	R	S	XDR
K70	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K149	R		R	R	R	S			R	R	R	R	R	R	R	R	S	XDR
K150	R	R	R	R	R	I			R	R	R	R	R	R	R	R	R	XDR
K151	R	R	R	R	R	S			R	R	R	R	R	R	R	R	R	XDR
K152	R	S	S	S	S	S			S	S	S	S	S	S	S	S	S	non-MDR
K153	R	R	I	S	R	R			R	R	R	R	R	R	R	S	R	XDR
K154	R	R	R	R	R	R			R	R	R	R	R	R	R	S	S	MDR
K5	R	R	I	R	R	S			R	R	R	R	R	R	R	R	S	XDR
K15	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K155	R	R	R	R	R	R	R		R	R	R		R	R	R	R	R	XDR
K156	R	R	R	R	R	R			R	R	R	R	R	R	R	R	R	XDR
K157	R	R	R	R	I	R			R	R	R	R	R	R	R	R	S	MDR
K158	R	R	R	R	R	R			R	R	R	R	R	R	R	R		XDR
K45	R		R	R	R	R			R	R	R	R	R	R	R	R	S	XDR
K159	R	R	R	R	R	I	R		R	R	R	R	R	R	R	R	I	MDR
K160	R	R	R	R	R	I			R	R	R	R	R	R	R	R	R	MDR

R = Resistant; I = Intermediate; S = Susceptible; blank spaces = not tested

Table B4: Susceptibility profile of colistin-resistant *K. pneumoniae* isolates from a tertiary-level hospital in Johannesburg, South Africa continued

No	COLISTIN INTREPRETATION	ERTAPENEM	IMIPENEM	MEROPENEM	AMIKACIN	GENTAMICIN	TOBRAMYCIN	CEFAZOLIN	CEFEPIME	CEFOTAXIME CEFTRIAXONE	CEFOXITIN	CEFTAZIDIME	CEFUROXIME ORAL	CEFUROXIME PARENTERAL	PIPERACILLIN TAZOBACTAM	CIPROFLOXACIN	TIGECYCLINE	Resistance Profile
K161	R	R	R	I	R	R	R		R	R	R	R	R		R	R		XDR
K162	R	R	R	R	R	S			R	R	R	R	R	R	R	R	I	MDR
K163	R	R	I	S	S	R			R	R	S	R	R	R	R	R	S	XDR
K164	R	R	R	R	R	R			R	R	R	R	R	R	R	I	R	XDR
K165	R	R	R	R	R	S			R	R	R	R	R	R	R	R	R	MDR
K166	R	S	S	S	S	R			S	R	S	R		R	R	S		MDR
K167	R	R	S	S	S	S	R		R	R	R	R		I	R	R		XDR
K168	R	R	I	R	S	R			R	R	R	R		R	R	R		MDR
K12	R		R	R	R	R			R	R	R	R	R	R	R	R		XDR
K65	R	R	R	R	R	I			R	R	R	R	R	R	R	R	I	MDR
K170	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		XDR
K171	R	R	R	S	S	R			R	R	R	R	R	R	R	R		XDR
K172	R	R	R	R	R	S			R	R	R	R	R	R	R	R	R	MDR
K173	R	R	I	R	S	S			R	R	R	R	R	R	R	R		XDR
K174	R	R	R	R	R	R			R	R	R	R	R	R	R	R	I	MDR
K175	R	S	S	S	S	R			R	R	S	R	R	R	R	R		XDR
K176	R	R	R	R	R	S			R	R	R	R	R	R	R	R	S	XDR
K177	R	R	R	R	R	R			R	R	R	R	R	R	R	R	S	non-MDR

R = Resistant; I = Intermediate; S = Susceptible; blank spaces = not tested

ANNEXURE C: RAW OXFORD NANOPORE SEQUENCING DATA

Table C1: Genomic results of *Acinetobacter baumannii* isolates

Lab No.	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	Resistance profile	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	Wildtype colistin genes	AMR genes
A1*	Adult multidisciplinary ICUs	M	57	Urine	>64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
A2*	Other surgical	M	36	Tissue	8		Not detected	Not detected	Not detected	Not detected	Not detected
A3	Trauma unit	M	29	Blood Culture	>64		Not detected	Not detected	Not detected	Not detected	*sul*2; *cml*A1; *aad*A1; *sat2*; *dfr*A1; *aph*(6)-Id; bla_{NDM-1}; *ble*; *sul*1; *qac*E; bla_{PER-7}
A4	Adult multidisciplinary ICUs	M	70	Blood Culture	16		Not detected	Not detected	Not detected	Not detected	Not detected
A5	Trauma Unit	M	29	Blood Culture	>64	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
A6*	Other Surgical	M	36	Fluid aspirate	>64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
A7	Adult multidisciplinary ICUs	F	52	Respiratory tract sample	4		2	Not detected	pmrC_A304H; pmrC_A370S; pmrC_F166L; pmrC_K531T; pmrC_T246M; pmrC_V58I; pmrB_M1V; pmrC_HS543SQ; pmrC_I131V; pmrC_I535V; pmrC_PQ537NK; pmrC_R230Q; pmrC_V151A	pmrC_R125R; pmrA_E8E; pmrA_M12M; pmrB_A227A; pmrB_G315G; pmrB_I164I; pmrB_P170P; pmrB_P233P; pmrB_Q270Q; pmrB_R231R; pmrB_R263R; pmrB_S17S; pmrB_T232T; pmrB_T235T; pmrC_R125R; lpxA_D233D; lpxA_G68G; lpxA_H159H; lpxA_Q72Q; lpxC_P30P	*ant*(3")-Iia; *par*C_S84L; bla_{OXA-23}; armA; msr(E); mph(E); sul1; *qac*E; *cml*A5; *arr*-2; *sul2*; *tet*(B); *aph*(6)-Id; *aph*(3")-Ib; bla_{OXA}; *aac*(3)-Ia; *aad*A1; *gyr*A_S81L; bla_{ADC}
A8	Paediatric medical	M	40 days	Blood culture	4		2	Not detected	pmrC_H499R; pmrC_I131V; pmrB_M1V; pmrB_P360Q; pmrC_A54G; pmrC_AI28LF; pmrC_AI49SV; pmrC_F45N; pmrC_FM16LN; pmrC_HS543SQ; pmrC_I131V; pmrC_I535V; pmrC_IIA21LLS; pmrC_IL70LI; pmrC_PQ537NK; pmrC_QIS12DVT; pmrC_R230Q; pmrC_T40Q; pmrC_T63A; pmrC_V118F; pmrC_V151A; pmrC_V47I; pmrC_V61I; pmrC_YAVL65NLIF; pmrC_YHQV35FKKI; lpxA_D233D; lpxA_G68G; lpxA_H159H; lpxA_Q72Q	lpxC_P30P; pmrC_R125R; pmrA_E8E; pmrA_M12M; pmrB_A226A; pmrB_A227A; pmrB_G315G; pmrB_I164I; pmrB_P233P; pmrB_P170P; pmrB_Q270Q; pmrB_R231R; pmrB_R263R; pmrB_S17S; pmrB_T232T; pmrB_T235N; pmrC_R125R	*sul*1; *qac*E; *aad*A1; *aac*(3)-Ia; *aph*(3')-Ia; *cat*A1; *tet*(A); *par*C_S84L; *ant*(3")-Iia; *sul*2; *tet*(B); *mph*E; *msr*E; *arm*A; *qac*E; *cml*A5; *arr*-2; *sul*2; *aph*(6)-Id; *dfr*A1; *sat*2; *aad*A1; *gyr*A_S81L; *aph*(3')-Via; bla_{OXA-69}; bla_{ADC}; bla_{OXA-23}
A9*	Adult multidisciplinary ICUs	F	29	Respiratory tract sample	64		Not detected	Not detected	Not detected	Not detected	Not detected
A10*	Other Surgical	M	65	Tissue	4		Not detected	Not detected	Not detected	Not detected	Not detected
A11*	Trauma unit	M	39	Respiratory tract sample	32		Not detected	Not detected	Not detected	Not detected	Not detected
A12*	Trauma Unit	M	29	Blood Culture	65	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
A13*	Other Surgical	M	36	Fluid aspirate	65	XDR	Not detected	Not detected	Not detected	Not detected	Not detected

excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

Table C1: Genomic results of *Acinetobacter baumannii* isolates continued

Lab No.	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	Resistance profile	ST	Chromosomal colistin-resistant genes	Novel colistin-resistant genes	Wildtype colistin genes	AMR genes
A14*	Trauma unit	M	39	Respiratory tract sample		-	Not detected	Not detected	Not detected	Not detected	Not detected
A15*	Adult multidisciplinary ICUs	M	57	Urine	>64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
A16*	Gynaecology	F	38	Respiratory tract sample		-	Not detected	Not detected	Not detected	Not detected	Not detected
A17*	Cardiology	M	78	Blood		-	Not detected	Not detected	Not detected	Not detected	Not detected
A19	Neonatal unit	M	43 days	Respiratory tract sample	>64	-	231	*pmr*A M12I	pmrC_H390Y; pmrC_H499R; pmrC_I131V; pmrB_M1V; pmrB_P360Q; lpxA_G68G	pmrC_R125R; pmrA_E8E; pmrB_A226A; pmrB_A227A; pmrB_G315G; pmrB_I164I; pmrB_P170P; pmrB_P233P; pmrB_Q270Q; pmrB_R231R; pmrB_R263R; pmrB_S17S; pmrB_T232T; pmrB_T235T; lpxC_P30P; lpxA_D233D; lpxA_H159H; lpxA_Q72Q;	*aad*A1; *sat*2; *dfr*A1; aph(6)-Id; bla_{OXA-69}; ant(3")-Iia; *sul*2; *tet*(B); bla_{NDM-1}; bla_{ADC}; *sul*1; *qac*E; *aad*A1; aac(3)-Ia; aph(3')-Ia; bla_{OXA-23}; *mph*(E); *msr*(E); *arm*A; *cml*A5; *arr*-2; *gyr*A_S81L; aph(3")-Ib; aph(6)-Id; *bla*NDM-1; *ble*
A20	Other surgical	M	38	Urine	>64	-	231	Not detected	pmrC_H390Y: pmrC_H499R; pmrC_I131V; pmrB_HKTYHEED66S*NLPQ KN; pmrB_L56F; pmrB_P360Q; pmrB_TVSSKFD58NCKQ*VR	pmrC_R125R; pmrA_E8E; pmrA_M12M; pmrB_A226A; pmrB_A227A; pmrB_G315G; pmrB_I164I; pmrB_P170P; pmrB_P233P; pmrB_Q270Q; pmrB_R231R; pmrB_R263R; pmrB_T232T; pmrB_T235T;	bla_{ADC}; bla_{OXA-23}; *sul*1; *qac*E; aph(3')-Ia; *par*C_S84L; ant(3")-Iia; *sul*2; *ble*; bla_{NDM-1}; aph(6)-Id; aph(3")-Ib
A21	Adult Oncology	F	47	Fluid aspirate	>64	-	dcd2	Not detected	Not detected	Not detected	*gyr*A_S81L; *sul*2; ant(3")-Iia; aph(6)-Id; *arr*-2; *cml*A5; *qac*E; *sul*1; bla_{OXA-23}; bla_{OXA-23}; *par*C_S84L; aph(3')-Ia; bla_{OXA-69}; bla_{NDM-1}; *ble*; aph(6)-Id; aph(3")-Ib

*= excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

Table C2: Genomic results of *Klebsiella pneumoniae* isolates

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	ST	Resistance profile	Chromosomal colistin-resistant genes	Wildtype colistin genes	Novel colistin-resistant genes	AMR genes
K1	General surgery	F	72	Blood Culture	8	Not detected	XDR	pmrB_R256G: contig_2:4921920-4923014_point mutation	pmrB_ALNQLV129ALNQLV; pmrB_D313D; pmrB_L17L; pmrB_L82L; pmrB_R14R; pmrB_Y209Y; pmrB_T157T; pmrB_T140T; pmrB_R256G; pmrB_S208S; pmrB_S85S	pmrB_T246A	*bla*$_{OXA-232}$
K2	Adult multidisciplinary ICUs	F	28	Fluid aspirate	>64	Not detected	non-MDR	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$; *sul*2; aph(3")-Ib; aph(6)-Id; *bla*$_{TEM}$; *dfr*A
K3	Other surgical	M	45	Urine	>64	Not detected	XDR	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K4	General surgery	M	45	Fluid aspirate	>64	Not detected	XDR	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K5	Adult Medical	M	57	Blood Culture	>64	Not detected	XDR	Not detected	Not detected	Not detected	*bla*$_{OXA-181}$; *fos*A3; *qnr*S
K6	Adult multidisciplinary ICUs	F	36	Fluid aspirate	64	Not detected	XDR	Not detected	Not detected	Not detected	Not detected
K7	Adult multidisciplinary ICUs	F	29	Swab	64	Not detected	-	Not detected	Not detected	Not detected	Not detected
K8	Adult multidisciplinary ICUs	F	37	Fluid aspirate	16	a162	XDR	Not detected	Not detected	Not detected	aac(6')-Ib3; *arr*-3; *sul*1; *qac*E; *aad*A16; *dfr*A27; aph(6)-Id; aph(3")-Ib; *sul*2; aac(6')-Ib-cr5; *bla*$_{TEM-1}$; *bla*$_{CTX-M-15}$; *oqx*B25; *oqx*A; *fos*A; *qnr*S; *bla*$_{OXA-181}$
K9	Adult Medical	M	53	Respiratory tract sample	16	Not detected	XDR	Not detected	Not detected	Not detected	Not detected
K10	Other surgical	F	18	Blood Culture	16	Not detected	-	Not detected	Not detected	Not detected	Not detected
K11	Adult multidisciplinary ICUs	F	29	Sputum	64	Not detected	-	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K12	Paediatric Surgery	F	8	Urine	32	Not detected	XDR	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K13	Trauma unit	M	67	Blood Culture	64	Not detected	-	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K14	Trauma unit	M	48	Fluid aspirate	4	Not detected	-	Not detected	Not detected	Not detected	Not detected
K15	General Surgery	M	74	Blood Culture	16	Not detected	XDR	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K16	Other surgical	F	60	Blood Culture	32	Not detected	XDR	Not detected	Not detected	Not detected	Not detected
K17	Other surgical	F	73	Blood Culture	4	Not detected	-	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$

*= excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

Table C2: Genomic results of *Klebsiella pneumoniae* isolates continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	Resistance profile	ST	Chromosomal colistin-resistant genes	Wildtype colistin genes	Novel colistin-resistant genes	AMR genes
K18	Trauma unit	M	37	Fluid aspirate	64	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{CTX-M-15}$; aph(3")-Ib; *sul*1; aph(6)-Id; *sul*2; aac(6')-Ib-cr; aac(6')-Ib3; *aad*A16; *arr*-3; *dfr*A27; *qac*E
K19	Adult multidisciplinary ICUs	F	36	Blood Culture	64	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K20	Adult multidisciplinary ICUs	M	57	Tissue	64	-	Not detected	Not detected	Not detected	Not detected	Not detected
K21	Other Surgical	F	58	Blood Culture	32	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-181}$
K22	Other Surgical	M	37	Swab	64	-	Not detected	Not detected	Not detected	Not detected	aac(3)-Iid; aph(3")-Ib; aph(6)-Id; aac(6')-Ib3; *arr*-3; *dfr*A27; *aad*A16; *qac*E; *sul*1; *bla*$_{CTX-M-15}$; *bla*$_{OXA}$
K23	General Surgery	F	29	Fluid aspirate	8	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K24	Other Surgical	M	81	Blood Culture	4	-	Not detected	Not detected	Not detected	Not detected	Not detected
K25	Adult multidisciplinary ICUs	M	33	Respiratory tract sample	8	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K26	Other surgical	F	85	Blood Culture	8	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K27	Adult multidisciplinary ICUs	M	22	Blood Culture	4	XDR	Not detected	Not detected	Not detected	Not detected	*qnr*S1; *bla*$_{OXA-181}$
K28	Trauma unit	M	35	Respiratory tract sample	4	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K29	Adult multidisciplinary ICUs	M	24	Blood Culture	4	non-MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K30	Adult multidisciplinary ICUs	M	49	Blood Culture	64	-	Not detected	Not detected	Not detected	Not detected	aac(6')-Ib; *bla*$_{OXA-232}$
K31	Adult multidisciplinary ICUs	F	37	Blood Culture	32	-	Not detected	Not detected	Not detected	Not detected	Not detected
K32	Paediatric surgery	F	4	Tissue	16	-	Not detected	Not detected	Not detected	Not detected	Not detected
K33	Other Surgical	M	47	Tissue	64	-	Not detected	Not detected	Not detected	Not detected	Not detected

*= excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

Table C2: Genomic results of *Klebsiella pneumoniae* isolates continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	Resistance profile	ST	Chromosomal colistin-resistant genes	Wildtype colistin genes	Novel colistin-resistant genes	AMR genes
K34	Neonatal unit	M	218 days	Respiratory tract sample	8	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA}$
K35	Other surgical	F	19	Fluid aspirate	16	-	Not detected	Not detected	Not detected	Not detected	Not detected
K36	Other surgical	M	46	Blood Culture	16	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K37	General Surgery	M	62	Urine	64	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K38	Neonatal unit	F	58 days	Blood Culture	32	-	Not detected	Not detected	Not detected	Not detected	Not detected
K39	Other Surgical	M	47	Fluid aspirate	8	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K40	Paediatric Surgery	M	25 days	Blood Culture	4	-	Not detected	Not detected	Not detected	Not detected	
K41	Adult Oncology	M	60	Urine	64	-	Not detected	Not detected	Not detected	Not detected	Not detected
K42	Other Surgical	F	39	Blood Culture	16	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{CTX-M-15}$; *bla*$_{OXA-232}$
K43	General surgery	M	38	Urine	32	-	Not detected	Not detected	Not detected	Not detected	Not detected
K44	Adult multidisciplinary ICUs	M	57	Tissue	16	-	Not detected	Not detected	Not detected	Not detected	Not detected
K45	Trauma unit	M	67	Respiratory tract sample	32	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K46	Other surgery	F	44	Urine	32	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K47	Adult multidisciplinary ICUs	M	24	Blood	16	-	Not detected	Not detected	Not detected	Not detected	Not detected
K48	Adult multidisciplinary ICUs	M	59	Blood	8	-	69bd	Not detected	Not detected	Not detected	*qnr*B; *qac*E; *sul*1; *bla*$_{SHV-11}$; *bla*$_{OXA-232}$
K51	Gynaecology	F	36	Fluid aspirate	64	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA}$
K52	Adult multidisciplinary ICUs	M	64	Blood Culture	4	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K53	Other Surgical	F	59	Urine	416	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K55	General Surgery	M	74	Blood Culture	16	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K56	Adult multidisciplinary ICUs	M	45	Blood Culture	64	-	2497	Not detected	Not detected	Not detected	*fos*A; *bla*$_{SHV}$; *par*C_S80I; *gyr*A_S83I; *bla*$_{OXA-232}$; *oqx*A; *oqx*B; *bla*$_{TEM}$; aac(6')-Ib; *qnr*B; *dfr*A14
K65	Other surgical	M	83	Urine	>64	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K66	General surgery	M	25	Urine	32	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K68	Adult multidisciplinary ICUs	M	35	Fluid aspirate	>64	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K69	Other surgical	F	27	Blood Culture	>64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K70	General surgery	M	36	Fluid aspirate	32	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$

*= excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

Table C2: Genomic results of *Klebsiella pneumoniae* isolates continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	Resistance profile	ST	Chromosomal colistin-resistant genes	Wildtype colistin genes	Novel colistin-resistant genes	AMR genes
K71	Other surgical	F	23	Respiratory tract sample	32	XDR	2497	*pmr*B R256G	*pmr*B_ALNQLV129ALNQLV; *pmr*B_D313D; *pmr*B_L17L; *pmr*B_L82L; *pmr*B_R14R; *pmr*B_S208S; *pmr*B_S85S; *pmr*B_T140T; *pmr*B_T157T; *pmr*B_Y209Y; *pmr*A_G53G; *pmr*A_S42S; *pho*Q_A20A; *pho*Q_D266D; *pho*Q_D438D; *pho*Q_G385G; *pho*Q_L26L; *pho*Q_L348L; *pho*Q_L396L; *pho*Q_L96L; *pho*Q_R16R; *pho*Q_S174S; *pho*Q_S56S	*pmr*B G207V; *pmr*B T246A	*fos*A; *bla*$_{CTX-M-15}$; *cat*B3; *bla*$_{OXA-1}$; aac(6')-Ib-cr5; *cat*A1; *bla*$_{SHV-11}$; *gyr*A_S83I; *oqx*A; *oqx*B; *par*C_S80I; *qnr*B1; *dfr*A14; *tet*(D); *bla*$_{TEM-1}$; aac(6')-Ib; arr-2; *ere*(A); *aad*A1; *cml*A5; *ble*; *bla*$_{NDM-1}$; *mph*(E); *msr*(E); *arm*A; *sul*1; *qac*Edelta1; *dfr*A5; aph(6)-Id; aph(3")-Ib; *sul*2; *bla*$_{OXA-232}$
K72	Adult multidisciplinary ICUs	F	23	Respiratory tract sample	32	XDR	2499	*pmr*B R256R	*pmr*B_ALNQLV129ALNQLV; *pmr*B_D313D; *pmr*B_L17L; *pmr*B_L82L; *pmr*B_R14R; *pmr*B_S208S; *pmr*B_S85S; *pmr*B_T140T; *pmr*B_T157T; *pmr*B_Y209Y; *pmr*A_G53G; *pmr*A_S42S; *pho*Q_A20A; *pho*Q_D266D; *pho*Q_D438D; *pho*Q_G385G; *pho*Q_L26L; *pho*Q_L348L; *pho*Q_L396L; *pho*Q_L96L; *pho*Q_R16R; *pho*Q_S174S; *pho*Q_S56S; *pho*Q_V24V; *pho*P_D191D; *pho*P_I201I; *pho*P_V3V; *lap*B_V43V; *crr*B_F84F; *crr*B_G183G; *crr*B_L94L;*crr*B_N141N; *crr*B_P151P; *crr*B_Q10Q; *crr*B_S195S; *crr*B_W140W; *crr*B_Y31Y; *lpx*M_V30V; *arn*C_C161C	*pmr*B L213M; *pmr*B T246A; *pmr*A A41T; *lap*B N212S; *arn*C S30T	*fos*A; *bla*$_{SHV-28}$; *omp*K36_D135DGD; *gyr*A_S83I; *oqx*A; *oqx*B19; *par*C_S80I; *tet*(A); *qnr*B1; *sul*2; aph(3")-Ib; aph(6)-Id; *sul*1; *qac*E; *aad*A2; *dfr*A12; *cat*B3; aac(6')-Ib-cr5; *bla*$_{OXA-1}$; aac(3)-Iie; *bla*$_{CTX-M-15}$; *bla*$_{TEM-1}$; *mph*(A); *qnr*S1; *bla*$_{OXA-181}$
K73	General Surgery	M	74	Urine	16	XDR	2497	*pmr*B R256G			*fos*A; *cat*B3; *bla*$_{OXA-1}$; aac(6')-Ib-cr5; *cat*A1; *bla*$_{SHV-11}$; *gyr*A_S83I; *oqx*A; *oqx*B; *cml*A5; *aad*A1; ere(A); arr-2; *bla*$_{CTX-M-15}$; aac(6')-Ib; *bla*$_{TEM-1}$; tet(D); *dfr*A14; *qnr*B; *qac*E; *sul*1; *arm*A; *msr*E; *mph*E; *bla*$_{NDM-1}$; *ble*; *bla*$_{OXA-232}$; *dfr*A5; aph(6)-Id; aph(3")-Ib; *sul*2
K74	Adult multidisciplinary ICUs	M	78	Blood culture	>64	-	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$

*= excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

Table C2: Genomic results of *Klebsiella pneumoniae* isolates continued

Lab No	Wards	Sex	Age	Specimen Type	Colistin MIC (µg/ml)	Resistance profile	ST	Chromosomal colistin-resistant genes	Wildtype colistin genes	Novel colistin-resistant genes	AMR genes
K75	Adult multidisciplinary ICUs	M	78	Blood Culture	>64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K76	Gynaecology	F	48	Fluid aspirate	32	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K77	Gynaecology	F	48	Fluid aspirate	32	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K78	General Surgery	M	36	Respiratory tract sample	32	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K79	Adult multidisciplinary ICUs	M	36	Fluid aspirate	32	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K80	General Surgery	M	36	Blood Culture	32	XDR	Not detected	Not detected	Not detected	Not detected	*bla*$_{OXA-232}$
K81	General Surgery	M	74	Urine	16	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K82	Adult multidisciplinary ICUs	F	23	Fluid aspirate	8	MDR	Not detected	Not detected	Not detected	Not detected	Not detected
K83	Adult multidisciplinary ICUs	F	51	Respiratory tract sample	16	XDR	2497	*pho*Q G385S; *pmr*B R256G	Not detected	*mgr*B M1V; *lap*B N212T	gyrA_S83I; *bla*$_{CTX-M-15}$; *bla*$_{OXA-1}$; aac(6')-Ib-cr5; *cat*A1; *bla*$_{SHV-11}$; *fos*A; *par*C_S80I; *oqx*B; *oqx*A; *qnr*B1; *dfr*A14; *tet*(D); *bla*$_{TEM-1}$; aac(6')-Ib; *arr*-2; *ere*(A); *aad*A1; *cml*A5; aac(6')-Ib-cr5; *bla*OXA-1; *cat*B3; *ble*; *bla*NDM-1; *mph*E; *msr*E; *arm*A; *sul*1; *qac*E; *bla*$_{OXA-232}$
K84	Other surgical	M	45	Fluid aspirate	>64	XDR	2497	*pmr*B R256G	Not detected	Not detected	*bla*$_{SHV-11}$; *cat*A1; aac(6')-Ib-cr5; *bla*$_{OXA-1}$; *cat*B3; *bla*$_{CTX-M-15}$; *gyr*A_S83I; *oqx*A; *oqx*B; *par*C_S80I; *fos*A; *cat*B3; *bla*$_{OXA-1}$; aac(6')-Ib-cr5; *cml*A5; *aad*A1; *ere*(A); *arr*-2; *bla*$_{TEM-1}$; *tet*(D); *dfr*A14; *qnr*B; *qac*E; *sul*1; *arm*A; *msr*E; *mph*E; *bla*$_{NDM-1}$; *ble*; *bla*$_{OXA}$; *bla*$_{OXA-232}$
K85	Other surgical	M	45	Fluid aspirate	>64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K86	Trauma unit	M	21	Fluid aspirate	64	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K87	Other surgical	F	46	Respiratory tract sample	16	XDR	Not detected	Not detected	Not detected	Not detected	Not detected
K88	Trauma unit	M	27	Swab	8	XDR	Not detected	Not detected	Not detected	Not detected	Not detected

*= excluded from study; AMR = Antibiotic resistance; MDR = multidrug resistance; MIC = Minium inhibitory concentration; ST=sequence type; XDR = extensively drug-resistance

ANNEXURE D: APPROVAL LETTERS FROM THE FACULTY OF HEALTH SCIENCES, UNIVERSITY OF WITWATERSRAND, SOUTH AFRICA

1. Masters Committee: Approval Letters

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

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

30 January 2024
Person No: 1604479
PAG

Miss AS Mathew
5 Simbali Eco Estate
Wilkoppies
Klerksdorp
2571
South Africa

Dear Miss Anjali Mathew

Master of Science in Medicine: Approval of Title

We have pleasure in advising that your proposal entitled *Characterization of colistin-resistant Acinetobacter baumannii and Klebsiella pneumoniae isolates from tertiary-level hospitals in Johannesburg, South Africa* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Ms Sandra Munesar
E-mail: Sandra.munesar@wits.ac.za

05 May 2025
Person No: 1604479
TAA

Miss AS Mathew
5 Simbali Eco Estate
Wilkoppies
Klerksdorp
2571
South Africa

Dear Miss Anjali Mathew

Master of Science in Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Dissertation for the degree of **Master of Science in Medicine** has been approved:

From:	**Characterization of colistin-resistant Acinetobacter baumannii and Klebsiella pneumoniae isolates from tertiary-level hospitals in Johannesburg, South Africa**
To:	**Characterization of colistin- resistant Acinetobacter baumannii and Klebsiella pneumoniae isolates from a tertiary-level hospital in Johannesburg, South Africa**

Please take note that any study involving human or animal participants must receive ethics clearance from the relevant Research Ethics Committee before the study commences. It is the student's responsibility to apply for ethics clearance before starting any data collection/research activity involving human or animal participants.

Yours sincerely

Ms Sandra Munesar
Faculty Registrar
Faculty of Health Sciences

2. Ethics Committee: Approval Letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Anjali Susan Mathew

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M231162 M240415-B-0001

NAME: (Principal Investigator)	Miss Anjali Susan Mathew
DEPARTMENT:	Clinical Microbiology and Infectious Diseases, School of Pathology National Health Laboratory Service
DEGREE:	MSc in Clinical Microbiology and Infectious Diseases
PROJECT TITLE:	Characterization of colistin-resistant A. baumannii and K. pneumoniae isolates from tertiary-level hospitals in Johannesburg, South Africa
DATE CONSIDERED:	24-Nov-2023
DECISION:	Approved unconditionally
CONDITIONS:	N/A
NOTE:	Application Number: MED23-10-357
SUPERVISOR:	Dr Teena Thomas, Dr Michelle Lowe and Dr Trusha Nana

APPROVED BY: Prof P. Ruff, Chairperson, HREC (Medical)

DATE OF APPROVAL: 15-Apr-2024 **EXPIRY DATE:** 14-Apr-2029

This clearance certificate is valid for 5 years from data of approval. Extension may be applied for.

DECLARATION OF INVESTIGATOR(S)

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report** in the month date of convened meeting each year until study is closed. Failure to submit annual report will result in ethics clearance certificate suspension. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. Unreported changes to the application may invalidate the clearance given by the HREC (Medical). Email a signed copy of ethics clearance certificate to HREC-Medical.ResearchOffice@wits.ac.za

Signature of Principal Investigator

18 April 2024
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

3. Ethics Committee: Updated Approval Letter

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

22 July 2024

Ms AS Mathew
School of Pathology
Dept of Clinical Microbiology & Infectious Diseases
Medical School
University

Sent by e-mail to: anjali.mathew2201@gmail.com

Dear Ms Mathew

Protocol Ref No: M231162
Protocol Title: *Characterization of colistin-resistant A.baumannii and K.pneumoniae isolates from tertiary level hospitals in Johannesburg, South Africa*
Principal Investigator: Ms AS Mathew

Thank you for your letter of 2 July 2024.

I confirm that we have noted and approve of your proposal to extend your data collection window to include material collected up to and including 30 June 2024.

Thank you for keeping us informed.

Yours Sincerely

I Burns

Mr I Burns, for the Human Research Ethics Committee (Medical)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Ms AS Mathew

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M231162

NAME: (Principal Investigator)	Ms AS Mathew **DEGREE:** MSc (Med)
SCHOOL:	School of Pathology Dept of Clinical Microbiology & Infectious Diseases Medical School University
PROJECT TITLE:	*Characterization of colistin-resistant A.baumannii and K.pneumoniae isolates from a tertiary-level hospital in Johannesburg, South Africa* Change of study title noted on 2025/05/19
DATE CONSIDERED:	2023/11/24
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	For permission to use or contact student study participants, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISORS:	Drs T Thomas, M Lowe and T Nana
APPROVED BY:	Professor P Ruff, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2024/04/15 **EXPIRY DATE:** 2029/04/14

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report** in the format available at https://wits.ac.za/research/researcher-support/research-ethics/ethics-committees/. This report is due annually, for the duration of the Clearance Certificate, beginning on the first anniversary of Date of Approval above. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

20 May 2025
Date

ANNEXURE E: TURNIT IN REPORT

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Anjali Susan Silby
Student number: 1604479

A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of

Master of Science in Medicine in
Clinical Microbiology and Infectious Diseases

Johannesburg, August 2025

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