UNIVERSITY OF KWAZULU-NATAL
COLLEGE OF HEALTH SCIENCES
SCHOOL OF LABORATORY MEDICINE AND MEDICAL SCIENCES
DEPARTMENT OF MEDICAL MICROBIOLOGY

Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19

Principal investigator: Dr Nitesh Brijlal
Student number: 207500990

Supervisor: Prof Khine Swe Swe-Han
Co-supervisor: Dr Sandra Maphumulo

Submitted in partial fulfilment of the academic requirements for the degree of Master of Medicine (MMed) in Medical Microbiology

November 2025

Declaration of authorship

I, Dr Nitesh Brijlal, declare as follows:

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

2. That my contribution to the project is as follows:
   (i) Study concept formulation, methodological design, and research protocol writing.
   (ii) Submission of protocol for obtaining the various approvals required – BREC, Department of Health, site/facility, and departmental.
   (iii) Application for project funding, conducting of laboratory work, and analysis of results.
   (iv) Dissertation write-up, with feedback and rectification assistance from both supervisors.

3. That the contributions of others to the project are as follows:
   (i) Prof Khine Swe Swe-Han – evaluation of and approval of proposed study idea, aim and objectives, review and corrections of protocol and dissertation, and provision of departmental consent.
   (ii) Dr Sandra Maphumulo – project design guidance, appraisal and feedback of protocol and dissertation, and continuous progress monitoring.
   (iii) Dr Ravesh Singh and Mr Diyothan Pillay – assistance with undertaking molecular characterisation of isolates and subsequent capturing of results.

[redacted] 07/11/2025

Dr Nitesh Brijlal (student) **Date**

We certify that the contents of this dissertation represent the original work of Dr Nitesh Brijlal and, as the candidate's supervisor and co-supervisor, have approved it for submission.

[redacted] 07/Nov/2025

Prof K Swe Swe-Han (supervisor) **Date**

[redacted] 7/11/2025

Dr S Maphumulo (co-supervisor) **Date**

Dedication

"Behind every young child who believes in himself is a parent who believed first."
– Matthew L. Jacobson

To my parents, Anitha and Vinesh Brijlal, your limitless sacrifices, unconditional love, and disciplined upbringing has nurtured me into the individual I am today.

To my grandparents, whether passed on or still beside me, your guidance is forever grateful.

I dedicate this dissertation to you all – my heroes of the past and present – thank you for everything!

Acknowledgements

Thank you, God, for instilling within me endless strength and determination in completing my MMed journey. *Jai Shri Ram.*

- To my supervisor and co-supervisor, your unwavering support, leadership and professional input throughout this research project is greatly appreciated.

- The Academic Affairs and Research Department of the National Health Laboratory Service, for financial support by providing K-Project funding to purchase consumables.

- Mr Malcolm Ellappen, laboratory manager at the Department of Virology, Inkosi Albert Luthuli Central Hospital, for meticulous guidance in completing the funding application.

- Mr Brian Brummer, epidemiologist at the National Institute for Communicable Diseases, for expertise and assistance with statistical analyses and data visualisations.

- The Department of Medical Microbiology laboratory staff at Inkosi Albert Luthuli Central Hospital, for storing the CRE isolates utilised in this project.

- Mrs Marshagne Smith, laboratory manager at the Antimicrobial Resistance Laboratory, National Institute for Communicable Diseases, for the timeous provision of control strains required to undertake the molecular laboratory work.

- I am eternally grateful to my beloved wife, Sumeshni Birbal, for bestowing unremitting encouragement and motivation from the inception of this research project, despite tackling the challenges and demands of completing her own PhD degree.

- To my Brijlal and Birbal families and loved ones, including my dearest yorkies, Ava and Mia, your constant patience and understanding during these years will always be cherished.

List of presentations and awards

- Oral presentation: University of KwaZulu-Natal (UKZN), 2025 College of Health Sciences (CHS) Research Symposium, **Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19**, K-RITH Tower Building, Nelson R Mandela School of Medicine (NRMSM) – Durban, KwaZulu-Natal, 25 August 2025 – by **N Brijlal**

- Virtual presentation: National Health Laboratory Service (NHLS), KwaZulu-Natal Academic Complex, Department of Medical Microbiology, Research Update Presentation, **Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19** – Durban, KwaZulu-Natal, 30 October 2025 – by **N Brijlal**

- UKZN College of Health Sciences certificate of excellence: registrar award for best research/study – Durban, KwaZulu-Natal, 14 November 2025

- Abstract accepted (poster): European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Global 2026 Congress, **Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19** – Munich, Germany (17-21 April 2026) – to be presented by **N Brijlal**

Abbreviations

AMR: Antimicrobial resistance

AMS: Antimicrobial stewardship

AST: Antimicrobial susceptibility testing

β: Beta

BL/BLI: β-lactam/β-lactamase inhibitor

bla: β-lactamase

CLSI: Clinical and Laboratory Standards Institute

CPE: Carbapenemase-producing Enterobacterales

CRE: Carbapenem-resistant Enterobacterales

DHP: Dehydropeptidase

DTR-GNB: Difficult-to-treat resistant gram-negative bacteria

ECDC: European Centre for Disease Prevention and Control

ESBL: Extended spectrum β-lactamases

GIT: Gastrointestinal tract

GNB: Gram-negative bacteria

HAI: Hospital-acquired infection

IALCH: Inkosi Albert Luthuli Central Hospital

ICU: Intensive care unit

IDSA: Infectious Diseases Society of America

IMP: Imipenemase metallo-β-lactamase

IPC: Infection prevention and control

GES: Guiana extended-spectrum β-lactamase

KPC: *Klebsiella pneumoniae* carbapenemase

KZN: KwaZulu-Natal

MBL: Metallo-β-lactamase

MDRO: Multidrug-resistant organism

MGE: Mobile genetic element

MIC: Minimum inhibitory concentration

NDM: New Delhi metallo-β-lactamase

Non-CPE: Non-carbapenemase-producing Enterobacterales

Non-CRE: Non-carbapenem-resistant Enterobacterales

OXA: Oxa-type carbapenemase/Oxacillinase

PBP: Penicillin binding protein

PPE: Personal protective equipment

SA: South Africa

SBL: Serine-β-lactamase

SOP: Standard operating procedure

US: United States

VIM: Verona integron-encoded metallo-β-lactamase

WHO: World Health Organization

Table of contents

CHAPTER 1:

INTRODUCTION

1.1 Background

Carbapenems are often considered one of the most effective and reliable last-resort antibiotics in the treatment of diverse drug-resistant bacterial infections.[1-3] Their widespread use, however, has resulted in the rapidly increasing occurrence and distribution of difficult-to-treat resistant gram-negative bacteria (DTR-GNB), such as carbapenem-resistant Enterobacterales (CREs), worldwide.[1-2,4] Infections caused by CREs pose a serious public health threat and are associated with significant patient morbidity and mortality.[5] A matter of extreme concern for more than the past decade, they have been categorised as "critical" according to the first published global priority pathogen list in 2017 by the World Health Organization (WHO).[6-7]

Enterobacterales refer to a group of gram-negative bacteria (GNB), some of which colonise the gastrointestinal tract (GIT) of humans and many animals.[8-9] They are currently recognised as the leading cause of serious community-acquired and nosocomial infections, and are among the commonest bacteria isolated from clinical specimens.[8,10] The production of beta(β)-lactamase (*bla*) enzymes, called carbapenemases, is the most frequently-mediated mechanism by which Enterobacterales develop carbapenem resistance.[2,10-12] Carbapenemases belong to the Ambler structural classification, being serine β-lactamases (Class A and D) and metallo-β-lactamases (Class B), which differ in their response to antimicrobial agents, impacting selection choices in patient management.[5,11,13]

The landscape of antimicrobial resistance (AMR) was greatly challenged during the outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), owing to the global mass consumption of broad-spectrum antibiotics.[14-16] Whatever the rationale for use – be it empirically or directed therapy for suspected or confirmed bacterial co-infection – this practise had mostly elevated the prevalence of multidrug-resistant organisms (MDROs), including CRE colonisation and infections harbouring carbapenemase-encoding genes.[6,15,17-18]

However, some studies report a reduced MDRO prevalence during the COVID-19 pandemic due to the strict implementation of policies related to hand hygiene, personal protective equipment (PPE) use, isolation precautions, and heightened infection control awareness.[19-20]

1.2 Microbiology of Enterobacterales

1.2.1 Taxonomy, biochemical characteristics and habitat

The order Enterobacterales (formerly Enterobacteriales) is comprised of seven families, over 80 genera, and more than 250 species – the largest group of clinically-relevant GNB.[21-22] These rod-shaped, non-spore-forming, facultative anaerobes attain undemanding nutritional requirements and, biochemically, are oxidase negative (except *Plesiomonas shigelloides*), catalase positive, reduce nitrate to nitrite, and ferment glucose with acid production.[23-24] In addition to being enteric flora, many are ubiquitous in ecological and healthcare environments, with hand carriage and contamination of surfaces, food or water sources facilitating easy spread, necessitating a One Health approach to successfully combat AMR.[25-30]

1.2.2 Species and disease spectrum

In the United States (US), one-third of Enterobacterales-associated nosocomial infections in adults are due to *Klebsiella* spp., *Enterobacter* spp., and *Escherichia coli (E. coli)*, while pathogens such as *Citrobacter* spp., *Proteus* spp., and *Serratia* spp. are less commonly implicated.[21] These microorganisms are isolated from both sterile and non-sterile sites and are responsible for a broad range of medical conditions of infectious aetiology; such as bacteraemia, ventilator-associated pneumonia, meningitis, gastroenteritis, as well as intra-abdominal, skin and soft tissue, and urinary tract infections, among others.[8-9,31] They account for approximately 80% of gram-negatives encountered in clinical microbiology laboratories and have the potential to cause disease in both healthy and immunocompromised individuals, with isolation of CREs often resulting in poorer clinical outcomes.[7,10,23]

1.2.3 Virulence factors and contribution to pathogenicity

The interplay between host factors and virulence characteristics of the bacterial species involved is contributory to strategies employed by majority of Enterobacterales in disease progression, invasiveness, and immune system evasion.[32] Motility is enabled by peritrichous flagella (except *Klebsiella* spp. and *Shigella* spp.), while an armoury of other significant virulence factor categories – commonly encountered in *Klebsiella pneumoniae* (*K. pneumoniae*), for example – entail secretion systems, siderophores, adhesins, fimbriae, lipopolysaccharides, capsules, and toxin production.[23] The emergence of AMR, as a result of biofilm formation or various other mechanisms (e.g. enzymatic inactivation, acquisition of resistance genes, etc.), further worsens the global crisis of effectively combating these infections. [23,26,32]

1.3 Carbapenem antibiotics

1.3.1 Class, chemical structure and spectrum of activity

Carbapenems (e.g. ertapenem, meropenem, imipenem and doripenem) are members of the β-lactam class of antibiotics.[33] They possess a β-lactam ring and a five-membered ring which differs in structure from penicillin by virtue of being unsaturated and attaining a carbon atom in place of the sulfur atom.[13,34] This distinctly unique molecular configuration confers notable stability and resistance to hydrolysis (inactivation) by most β-lactamase enzymes, inclusive of extended spectrum β-lactamases (ESBLs), but not carbapenemases.[13,34] They exhibit a broad spectrum of activity against many gram-positive, gram-negative and anaerobic organisms.[13,34] These potent antibiotics are often reserved as a last resort in treatment modalities of MDROs, particularly for serious or complicated infections caused by Enterobacterales, with the exception of CREs.[2,21,33,35-36]

1.3.2 Pharmacokinetics and adverse effects

Poor oral bioavailability and absorption through the GIT necessitates parenteral administration, achieving good penetration into body fluids and tissues.[36] Imipenem, the first in the carbapenem class, undergoes significant hydrolysis by renal dehydropeptidase (DHP), forming a potentially nephrotoxic inactive metabolite – this drawback is overcome by co-administration with cilastatin, a renal DHP-1 inhibitor.[33] Similar to other β-lactams, elimination of carbapenems predominately occurs by glomerular filtration, thus requiring dose adjustments in the presence of kidney dysfunction.[36] Nausea, vomiting and diarrhoea are the commonest adverse effects, while some patients may experience skin rashes and infusion-site reactions.[34,36] High imipenem doses have been associated with seizures, occurring in less than 2% of patients.[34,36] Alteration of the intestinal microbiota may also occur, resulting in the selection of carbapenem-resistant isolates.[36]

1.3.3 Mechanism of action

Similar to other β-lactam antibiotics, carbapenems bind to penicillin binding proteins (PBPs) – enzymes that catalyse peptidoglycan formation – thereby interfering with transpeptidation or cross-linkage, resulting in inhibition of bacterial cell wall biosynthesis and subsequent cell lysis.[25,34,36-37] The acylation of the PBPs is irreversible and bactericidal action occurs in a time-dependent manner.[33,36] Since the hydrophilic nature of carbapenems limits outer membrane permeability of GNB, antibiotic entry into the periplasmic space is facilitated by outer membrane protein channels, called porins.[33,37]

1.4 Carbapenem resistance in Enterobacterales

The extensive use (and often misuse) of carbapenem antibiotics has given rise to the emergence and increased spread of CREs – a serious and significant concern for healthcare systems worldwide, with adverse impacts in medical expenditure, human and economic loss.[8-9,23,35] On the World Health Organization (WHO) global priority pathogen list, CREs rank among the top three, emphasizing their importance from a public health perspective.[38-39]

1.4.1 Definition of carbapenem-resistant Enterobacterales

A CRE is defined as a bacterium belonging to the Enterobacterales order that is resistant to at least one carbapenem.[35] According to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing (33rd edition, 2023), the minimum inhibitory concentration (MIC) clinical breakpoint defining resistance to Enterobacterales is ≥2μg/mL for ertapenem and ≥4μg/mL for doripenem, imipenem and meropenem.[40] For bacteria that are intrinsically less susceptible to imipenem (e.g. *Proteus* spp., *Providencia* spp. and *Morganella* spp.), resistance to a carbapenem other than imipenem is required to classify as a CRE.[41]

1.4.2 Risk factors for CRE acquisition and mortality

Predominant risk factors for CRE infections include recent exposure to healthcare facilities or broad-spectrum antibiotics, extensive invasive or surgical procedures, medical indwelling device usage, and the presence of underlying patient comorbidities.[10,42-43] CRE exposure, admission to an intensive care unit, or receiving mechanical ventilation, are other notable predispositions.[37,44-45]

Greater mortality rates in CRE-infected individuals may be attributed to a variety of factors; these can be patient-related (e.g. demographic profile), organism-related (e.g. bacterial strain isolated or virulence characteristics), infection-related (e.g. site/location), and treatment-related (e.g. inappropriate empirical therapy choice).[31,46]

1.4.3 Major mechanisms of resistance in CREs

Carbapenem resistance among Enterobacterales arises mainly via three mechanisms (Figure 1).[47] This constitutes (1) the production of β-lactamase enzymes called carbapenemases, as well as non-enzymatic methods comprising (2) porin loss or modification and (3) efflux pump overactivity.[12,33,47]

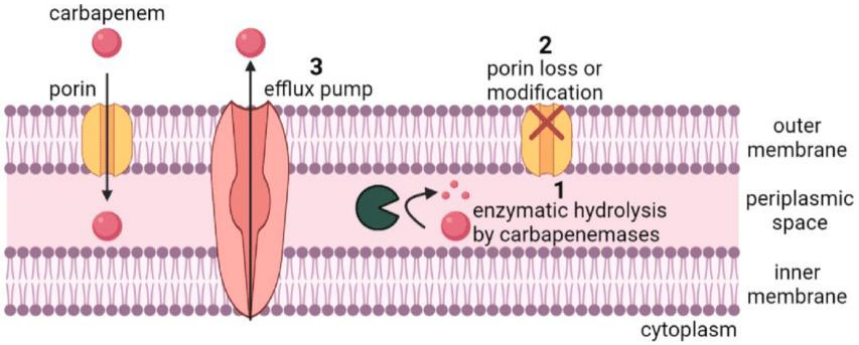


Figure 1: Carbapenem resistance mechanisms in Enterobacterales (adapted from Dixon *et al.*, 2022)

Depending on the resistance mechanism(s) involved, CREs can be divided into carbapenemase-producing Enterobacterales (CPEs) and non-carbapenemase-producing Enterobacterales (non-CPEs), with the former usually being the chief contributor to CRE isolates in clinical settings of high CRE prevalence.[12,41,46]

1.4.3.1 Upregulation of efflux pumps

The upregulation or overexpression of efflux pumps, belonging primarily to the Resistance-Nodulation-Division (RND) family, facilitates the active expulsion (pumping out) of the carbapenem from the periplasmic space of the bacterium, effectively reducing antibiotic cellular concentrations.[12,34,36,47] The AcrAB-TolC system is the chief mechanism implicating multidrug resistance in Enterobacterales which, together with the CusABC efflux complex, is found in *E. coli*.[12,25] The efflux pump genes OqxA and OqxB, as detected by Bedenić et al in *K. pneumoniae* isolates, can confer resistance to multiple antimicrobials.[6]

1.4.3.2 Porin-mediated resistance

Chromosomal mutations that lead to porin loss or modification result in diminished outer membrane permeability – this inhibits or reduces carbapenem uptake/influx into the periplasm, preventing the antibiotic from reaching their PBPs.[25,33,36,46] Examples include ompK35, ompK36, and ompK3 found in *K. pneumoniae*, while ompC, ompF and PhoE are attributed to *E. coli*.[29] This mechanism can also occur concurrently with amplification of ESBLs or hyperproduction of AmpC β-lactamases, contributing synergistically to carbapenem resistance.[8,29,41,46]

1.4.3.3 Enzymatic inactivation by carbapenemase(s)

This highly potent group of β-lactamases hydrolyse a broad range of β-lactam antibiotics, including carbapenems, rendering them ineffective.[2,11,47] This is the commonest and most epidemiologically-relevant resistance method, posing a great threat to CRE management strategies.[21,34,46] Carbapenemase-encoding genes are located on mobile genetic elements (MGEs), including but not limited to transposons, integrons, and plasmids.[11,36] Acquisition of this can occur via horizontal gene transfer, thus facilitating their dissemination within and between different bacteria.[21,30,34]

1.5 Classification of β-lactamases

Nomenclature for classifying β-lactamases can be based on amino acid sequence (molecular structure) – defined as the Ambler classification (Figure 2)[48] – which comprises four major classes (A-D), or on functional characteristics (substrate profile and inhibitor response) – defined as the Bush-Jacoby-Medeiros classification.[5,11] In relation to these two schemes, carbapenemases belong to Ambler classes A and D (Bush-Jacoby-Medeiros group 2) and Ambler class B (Bush-Jacoby-Medeiros group 3).[5] Ambler class C β-lactamases are cephalosporinases that belong to Bush-Jacoby-Medeiros group 1.[5]

Class A and D enzymes are termed serine β-lactamases (SBLs) due to the possession of a serine residue at their active site to enable β-lactam ring opening.[11,34,49-50] Class B enzymes are termed metallo-β-lactamases (MBLs) since they utilise zinc ions at their active site to mediate broad hydrolytic properties against most β-lactam antibiotics (except monobactams).[11,34,49-50] MBLs are not inhibited by classical β-lactamase inhibitors and require divalent cation chelators [e.g. ethylenediaminetetraacetic acid (EDTA)] for inhibition.[12,25,49,51]

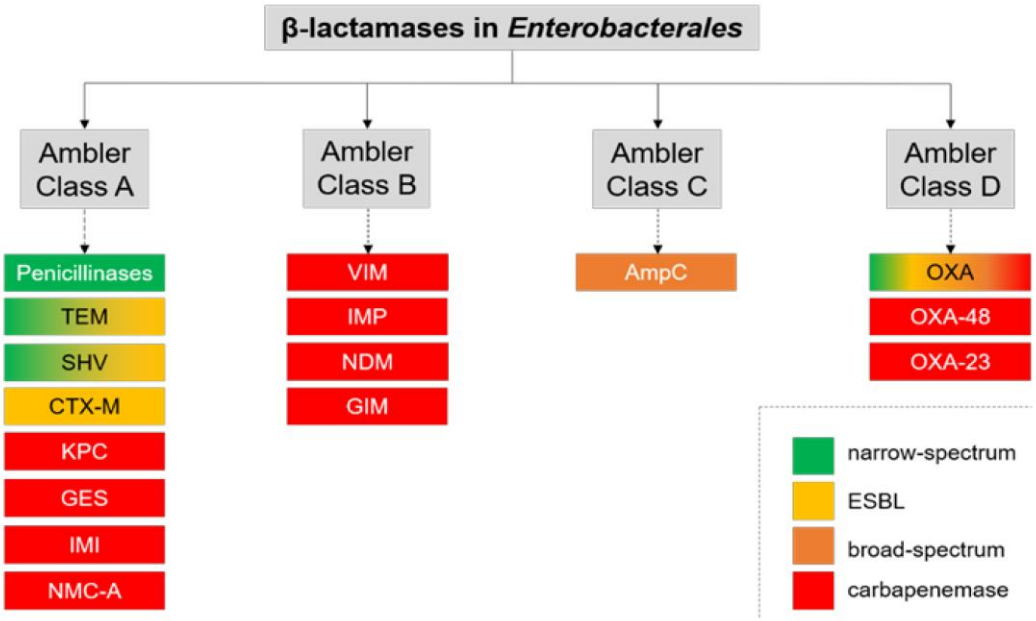


Figure 2: Ambler classification of the main β-lactamases relevant to Enterobacterales (adapted from Noster *et al.*, 2021)

KPC, *Klebsiella pneumoniae* carbapenemase; GES, Guiana extended-spectrum β-lactamase; IMI, imipenem-hydrolysing β-lactamase; NMC-A, not metalloenzyme carbapenemase A; VIM, Verona integron-encoded metallo-β-lactamase; IMP, imipenemase metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; GIM, German imipenemase; OXA, oxacillin carbapenemase/oxacillinase

1.6 Diagnostic methods for carbapenemase detection and identification

The various methods (Table 1)[28] for confirming suspected carbapenemase production in CRE-defined isolates encompass phenotypic, genotypic, immunological, proteomic, and other emerging assays (such as biosensor technology).[28,52-53]

1.6.1 Consideration factors in test selection

The increasing incidence and versatility of carbapenemases among the family of β-lactamase enzymes underpins the need for microbiology diagnostic laboratories to employ optimal CPE detection techniques.[25,54] However, appropriate test selection may be fraught with various challenges; such as local prevalence and epidemiology, diagnostic performance factors (e.g. sensitivity, specificity, complexity and turnaround time), workflow integration considerations (e.g. infrastructure and staffing), regulatory status and feasibility, among others.[52] Health institutions should therefore utilise methods tailored to their individualistic antimicrobial stewardship (AMS) and infection prevention and control (IPC) programmes.[52]

1.6.2 Phenotypic methods

These tests determine carbapenemase presence or absence by detecting enzyme activity (functional expression) and are suitable for initial screening, cost-efficient, relatively easy to perform, but mostly unable to identify the specific carbapenemase(s) present.[50,53]

1.6.2.1 Antimicrobial susceptibility testing (AST)-based methods

In the double-disk synergy test (DDST), a disk approximation method, two individual disks – one a carbapenem and one a carbapenemase class-specific inhibitor (e.g. boronic acids for KPC, or EDTA or dipicolinic acid for MBLs) – are placed near each other, whereby an enlargement of the zones of inhibition between the two disks (halo effect) is indicative of a positive result.[1,28,48,50,54] In the combined disk test (CDT), an inhibitor-based disk synergy test, two individual disks – one a carbapenem and one a carbapenem combined with a carbapenemase class-specific inhibitor (as mentioned above) – are placed near each other whereby the observation of synergy between the latter disk (zone diameter ≥5mm difference compared to the former) is indicative of a positive result.[1,28,48,50,54-55] These methods, which are performed on Muller–Hinton agar (MHA) that is streaked with a test strain, can differentiate between certain carbapenemase classes (e.g. SBLs versus MBLs).[28,48,50,52,54]

1.6.2.2 Selective and chromogenic media

Routinely-available laboratory media (e.g. MacConkey agar) can be supplemented with a carbapenem (e.g. 1μg/mL imipenem), allowing for selective growth of carbapenem strains that are non-susceptible, rendering a cost-effective screening method.[48,52,54] Commercially-available chromogenic media involve catalysis of the chromogenic substrate by the carbapenemase enzyme, forming a product with a colour change.[28,48,50,52,54] Though more expensive and unable to identify the specific carbapenemase enzyme(s), this selective and differential medium combines CRE detection with presumptive organism identification.[28,48,50,52,54]

1.6.2.3 Modified Hodge test (MHT)

In this well-known method, a carbapenem-susceptible *E. coli* indicator strain is inoculated onto a MHA plate, after which a carbapenem disk (ertapenem or meropenem) is placed in the centre.[28,48,50-52] The suspected clinical isolate and control strains are then streaked in a line away from the edge of the disk to the periphery of the plate.[28,48,50-52] Following overnight incubation, a cloverleaf-like indentation arises alongside the streak line of the test isolate if it

is a carbapenemase producer.[28,48,50-52] This is due to a decreased local antibiotic concentration following carbapenem hydrolysis, enabling uninhibited growth of the carbapenem-susceptible *E. coli*.[28,48,50-52] Due to limitations of its poor sensitivity for detecting MBLs, as well as high false-positive results in ESBL or hyperproducing AmpC β-lactamases with porin loss, this test is no longer recommended by the CLSI.[1,28,34,48,50,52-54,56]

1.6.2.4 Carbapenem inactivation method (CIM)

A carbapenem disk (usually 10µg meropenem) becomes hydrolysed following incubation for two hours in a suspension of water that contains colonies of a suspected carbapenemase-producing organism.[1,28,48,50-52] This disk is subsequently placed on a MHA plate lawned with a carbapenem-susceptible *E. coli* indicator strain and incubated overnight.[1,28,48,50-52] The diameter of the inhibition zone is then measured – the absence of an inhibition zone, or a narrow zone (6-15mm) is indicative of carbapenemase activity, whereas the presence of a zone diameter ≥19mm (clear zone) indicates preservation of meropenem disk activity (i.e. the test isolate is negative for carbapenemase production).[40,48,50-52]

To address limitations of reduced sensitivity in detecting CPEs attaining reduced hydrolytic activity (e.g. OXA-48-like carbapenemases) and MBLs, modifications to this assay were made by the CLSI to enhance overall performance – this included using a 1µL inoculating loop (versus 10µL), the use of 2mL tryptic soy broth (TSB) instead of water, and an increased incubation time from two to four hours.[40,48,50-51] This CLSI-endorsed method is termed the modified carbapenem inactivation method (mCIM), while the EDTA carbapenem inactivation method (eCIM), which uses 20µL of 0.5M EDTA in addition to the TSB, distinguishes MBLs from SBLs.[40,48,50-51] Although the mCIM may be done alone, if the eCIM is conducted, it must be performed simultaneously with the mCIM and is only interpreted/valid if the mCIM is positive (a ≥5mm increase in zone diameter for eCIM versus mCIM zone diameter is positive for MBL production).[40,48,50-51]

1.6.2.5 Carba NP test

This colorimetric microtube assay is based on the principle that, upon carbapenemase-induced imipenem hydrolysis, a drop in pH ensues, which is detected by the indicator (phenol red), resulting in a visible colour change from red to yellow.[1,34,40,50,54,56] The test is rapid and cost-efficient; however, operator interpretation subjectivity, false negatives with OXA-48-like

carbapenemases, and the need for reagent preparation with a short shelf-life, entail some drawbacks.[1,34,50,56]

The CLSI recommends performing the mCIM (± eCIM) or CarbaNP test when indicated for treatment (as per institutional policies/guidelines), IPC procedures, or for epidemiological purposes.[40]

1.6.3 Genotypic methods

Molecular methods are the gold standard for carbapenemase-encoding gene detection and identification.[1,25,34] However, the need for specialised infrastructure and skilled personnel, including high costs, represent some of the contributory factors making availability in many laboratories scarce.[1,25,34] A wide array of in-house and commercial manual and automated nucleic acid amplification tests (NAATs) exist.[1,34,50,54] Polymerase chain reaction (PCR)-based platforms are the commonest; such as conventional simplex and multiple, real-time, combined-nested, quantitative PCR (qPCR), and reverse-transcriptase PCR (RT-PCR).[1,50,52]

Advancements in genomics have led to techniques like loop-mediated isothermal amplification (LAMP), fluorescence in situ hybridization (FISH), microarrays, next generation sequencing (NGS), whole genome sequencing (WGS), among others.[28,48,50,54] These technologies are quick and display excellent sensitivity and specificity, but their labour-intensiveness, need for expertise, and cost factors, are notable limitations to routine implementation.[28,34,52-53,56]

1.6.4 Lateral flow immunoassays (LFIAs) and immunological methods

Ready-to-use immunochromatographic assays are based on membrane technology and utilise colloidal gold nanoparticles to enable rapid detection and identification of various epidemiologically-relevant carbapenemases via antibody methods.[51-52] Serological techniques [e.g. enzyme-linked immunosorbent assay (ELISA)] are also available. [28]

Table 1: Current and emerging methods for the detection of common carbapenemase enzymes (adapted from Caliskan-Aydogan and Alocilja, 2023)

Techniques	Advantages	Limitations
Culture-based methods	**Simple and cost-effective**	**Time-consuming (>24 h)**
1. Improved AST tests: E-test or disk diffusion test	Detect KPC and MBLs with good sensitivity (>82%) and specificity (>95%)	Insufficient for OXA-48; requires specific reagents and pure culture
2. Modified Hodge Test (MHT)	Detects KPC with good sensitivity (>69%) and specificity (>90%)	Insufficient for MBLs; requires pure culture
3. Carbapenem-inactivation methods (CIM)	Detect all carbapenemases with higher sensitivity (>90%) and specificity (>95%)	Requires pure culture
4. Selective media: SUPERCARBA, Colorex KPC, ID Carba, etc.	Detect carbapenemases from direct patient samples; SUPERCARBA has higher sensitivity (>96.5%)	Variable sensitivity (40–96.5%) and specificity (>50%)
Rapid phenotypic methods	**Rapid (<24 h)**	**Costly equipment**
1. Colorimetric assay: CarbaNP test and its automated kits	Detect carbapenemases with good sensitivity (>70%) and specificity (>80%) Simple, rapid (<2 h), and cost-effective; no equipment requirement	Insufficient for OXA-48 Requires pure culture
2. MALDI-TOF MS	Rapidly (1–4 h) detects KPC and MBLs with good sensitivity (>72.5%) and specificity (>95%) Low-measurement cost and simple	Requires data analysis; insufficient for OXA-48 Requires single isolated colonies
3. Emerging techniques: Flow cytometry, microfluidic techniques, and Raman spectroscopic techniques	Simple and rapid (<4 h) Good sensitivity (>80%) and specificity (>90%) from pure culture	Lower applicability on specimens Insufficient work on carbapenemases
Genotypic methods	**Rapid and highly specific (>90%) and sensitive (>90%)**	**Costly and complex equipment**
1. PCR-based methods: qPCR, RT-PCR, mPCR, automated PCR (Xpert system, Check-Direct, and Carba-R-assay)	Gold standard and rapid (<4 h) Detect and type all carbapenemases directly from specimens	High technical requirements and specific reagents High measurement cost
2. Loop-mediated isothermal amplification (LAMP)	Simple and moderate cost; applicable in low-resource settings	Specific reagents and complex primer design
3. Whole genome sequencing (WGS)	Discovers a new resistance mechanism	Longer turn-around time; complex data management
4. Emerging techniques: Fluorescence in situ hybridization (FISH), and microarray techniques	Rapid (<6 h) Detect carbapenemases	Require specific equipment and reagents Insufficient work on carbapenemases
Immunological methods		
1. Enzyme-linked immunosorbent assay (ELISA), an immunochromatographic assay	Rapid and moderate cost Poor sensitivity and specificity directly from specimens	Complex and difficult antibody design due to antigenic site modification
Biosensors: emerging technology	**Rapid, simple, and cost-effective**	**Specific equipment**
1. Electrochemical assays: Impedimetric, potentiometric, and voltametric	Detect carbapenemases Moderate cost	Require equipment for signal processing and data analysis Insufficient work on AMR and carbapenemase detection from pure culture and specimens
2. Optical assays: Raman scattering, surface plasmon resonance (SPR), and plasmonic biosensors	Rapid, simple, and cost-effective; no equipment requirement Detect carbapenemases with good sensitivity (78%) and specificity (97%)	Insufficient work on AMR and carbapenemase detection from pure culture and specimens

1.6.5 Proteomic-based and spectrophotometric methods

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a bioanalytical proteomic-based assay which can detect carbapenem antibiotic degradation products by carbapenemase-hydrolysing enzymes via peak-based spectra formation.[34,48,50-52,54] Apart from being expensive and requiring skilled personnel, difficulty exists in identifying OXA-48 carbapenemases, while mucoid isolates can produce false negative results.[34,52,56]

Ultraviolet-visible (UV) spectrophotometry is a cheap method capable of confirming carbapenemase activity by detecting β-lactam ring (carbapenem) hydrolysis.[34,54] In addition, it can differentiate CREs into CPEs or those having combined or other resistance mechanisms (e.g. ESBLs, overproduction of AmpC, or porin loss).[54] It is unable to identify the type of carbapenemase enzyme, while its time-consuming and laborious process warrants implementation only in reference laboratories.[54,56]

1.6.6 Other methods and emerging technologies

A broad spectrum of efficient, innovative and alternative techniques in development attain potential for future use – these include molecular typing to analyse clonal relatedness (particularly for CPE surveillance programmes), pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), microsphere-based flow cytometry, biosensor technology (e.g. electrochemical, optical and plasmonic), microfluidics, and bioinformatic tools.[1,28,48,54]

1.7 Therapeutic implications of carbapenemase identification

The inability of AST results alone to reliably distinguish CPEs from non-CPEs impacts clinical decision-making, as patient outcomes differ depending on the particular carbapenemase(s) implicated.[49,51] DTR-GNB may also co-express multiple carbapenemases, making treatment selection troublesome, further reinforcing the need to undertake enzyme identification in CRE isolates – a practise that clinical microbiology laboratories are being encouraged to implement.[33,41,51]

The resistance profile to β-lactam agents differs among the carbapenemase classes; therefore, identification and knowledge of the carbapenemase-encoding gene(s) present in clinical settings – either for surveillance, AMS, or management purposes – is fundamental to both

preventing CRE proliferation and guiding the selection of appropriate targeted antibiotic regimens that may only be effective against certain carbapenemases.[5,11,38,41,53]

1.8 Epidemiological burden of CRE infections

Carbapenemases are the primary drivers implicated in the increasing worldwide prevalence of CREs (Figure 3).[31,57-58] Although endemicity of certain enzymes exist in particular geographical regions, this landscape is currently expanding at an extraordinary rate.[5,29,31,59] The evolving epidemiology and dissemination of strains containing multiple enzymes attain the potential to complicate management, including selection choices of the novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations.[29]

CRE occurrence in companion animals and livestock is also concerning, with a documented prevalence of 2-26% in Africa, posing a considerable public health risk.[29] This, together with environmental AMR gene contamination via bacterial propagation in the food chain, establishes the critical need for monitoring systems from a One Health viewpoint.[30]

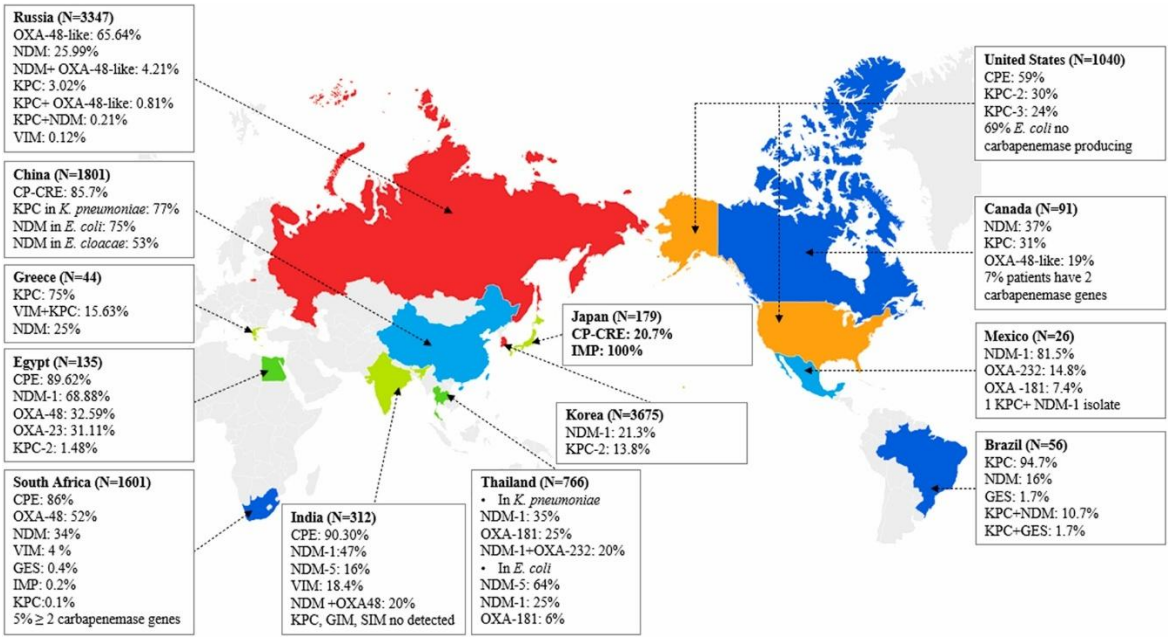


Figure 3: Global distribution of carbapenemases (adapted from Ma *et al.*, 2023)

1.8.1 Global

The prevalence of CRE infection, faecal carriage, and hospital outbreaks over time is well-described.[5,29,60-61] Globalisation and access to international travel have undoubtedly led to AMR spread that transcends boundaries.[13] Countries attaining high overall CRE rates include

Brazil, China, Greece, Italy, Colombia and the US.[31] Discovered in North Carolina in 1996, KPC is the most commonly encountered CRE globally, primarily due to clonal expansion of *K. pneumoniae* strains, and is responsible for a dramatic increase in disease burden and major hospital outbreaks.[5,29,44] In the US, CPEs account for 35-83% of CRE infections with an estimated 26% mortality, coupled with exceedingly high inpatient costs.[5,41,62] Other KPC-endemic regions include Greece, Israel and Latin America.[5,63]

IMP, the first carbapenemase discovered, was isolated in a *Serratia marcescens* isolate in 1990 that was responsible for an outbreak in seven Japanese hospitals.[12] IMP-type MBL-containing Enterobacterales are endemic only in Taiwan and Japan, with single reports or sporadic outbreaks described in other countries.[5,12] The commonest VIM-type MBL worldwide is currently VIM-2, with around 46 variants further catalogued.[5] Following the discovery of NDM in 2008 in a Swedish patient that received medical treatment in New Delhi, there has been a global propagation of NDM-type MBLs with rapid gene transfer between species, including environmental spread among communities belonging to poorer income countries.[5,12] Regarding Ambler class D β-lactamase enzymes, $bla_{\text{OXA-48}}$ was discovered in 2001 in Turkey, where it is now endemic, including in other Middle Eastern and North African countries.[5,64] In the Indian subcontinent, $bla_{\text{OXA-181}}$ is commonly found.[5,8]

In terms of demographics, CRE prevalence in Europe occurs mostly in the 19-64 year age group, affecting more males than females.[58] The European Centre for Disease Prevention and Control (ECDC) documented in 2019 that 43% of countries reported inter-regional spread of CREs, a large population of which were invasive CRE *E. coli* infections.[60] According to a study by Han *et al.* in 2020, CPE in Chinese paediatric patients was dominated by NDM, followed by KPC.[58,65] There was also a notable absence of multi-enzyme genes in this population – a comparison that significantly differed from adults.[58,65]

The WHO 2021 Global Antimicrobial Resistance and Use Surveillance System (GLASS) study showed that among African countries, Egypt, Uganda and Madagascar attain prominently high resistance rates to carbapenems.[58,66] A study in Thailand encompassing CRE strains from blood, sputum and urine revealed 80% of samples to be carbapenemase-producers, with 17% producing more than one enzyme.[58] Similarly, a phenotype-genotype correlation among CREs received from four Egyptian hospitals elicited the co-harbouring of carbapenemases in over half the isolates.[67] A surveillance study from the Antimicrobial Testing Leadership and

Surveillance (ATLAS) programme (2018-2020) describing CRE distribution in Africa and the Middle East identified NDM-1 as the commonest MBL.[68] During the same time period, over 80% of CREs cultured in a cross-sectional prospective study in a Moroccan hospital were carbapenemase-producers, majority of which had been isolated from the neonatal unit.[9]

1.8.2 South Africa (SA) and KwaZulu-Natal (KZN)

In the limited studies evaluating CRE infection, screening and carbapenemase gene characterisation in SA, Perovic *et al.* demonstrated 68% of clinical isolates to be CPEs in 2016 (majority *Klebsiella pneumoniae*), while the commonest enzymes identified were OXA-48 (52%) and NDM (32%) in hospital-acquired CRE bacteraemia patients during 2015-2020.[10,35] Of all the countries included in the ATLAS programme study, VIM-1 (five isolates in total) was only detected in SA.[68] An increase in isolation of NDM-producing *Klebsiella pneumoniae* and *Serratia marcescens* (26 CREs in total) was described among 22 children in a retrospective analysis at a Cape Town paediatric hospital in 2022.[69] Tootla *et al* identified $bla_{\text{OXA-48-like}}$ (80%) and bla_{NDM} (11%) in a multicentre Western Cape study comprising 117 participants from six hospitals.[45]

Screening and genomic investigation at a public sector hospital in the uMgungundlovu District of KZN found $bla_{\text{OXA-48}}$-producing *Klebsiella pneumoniae* to be endemic in the ICU setting, causing patient colonisation.[10-11,35] A retrospective evaluation of CREs in the neonatal unit of a tertiary hospital in Durban established 32 cases over a two-year period – a prevalence of five per 1000 admissions, with 34% of patients having prior carbapenem exposure.[70]

1.9 CRE and COVID-19

It has been postulated that CPE spread may be accelerated by COVID-19 due to the virus promoting bacterial attachment and respiratory tract colonisation, leading to increased rates of CRE-related infection.[58] Additionally, high-risk patients with COVID-19 and carbapenem-resistant *Klebsiella pneumoniae* dual infections might attain a poorer prognosis and increased mortality.[58] Data from six countries revealed KPC, OXA-48 and NDM to be the dominant genes during such infections, comprising predominantly of respiratory and bloodstream specimen types.[58,71] According to the ECDC, the COVID-19 pandemic has impacted negatively on the implementation of AMS programmes, escalating the prevalence of MDROs secondary to greater antimicrobial use (and misuse).[60,72] In a multicentre study conducted in Croatia during COVID-19, 80% of CRE isolates (*Klebsiella pneumoniae and Enterobacter*

cloacae) were found to harbour multiple carbapenemases, demonstrating the ability of microorganisms to acquire various resistance determinants under the selection pressure of broad-spectrum antibiotics widely used in the pandemic.[6,73]

However, although COVID-19 has been associated with an increased incidence in hospital-acquired resistant bacterial co-infections,[17-18,74] some global data depict that prolonged PPE use and strict, well-enforced IPC policies during this period, have been beneficial in minimising the spread of MDR pathogens in certain healthcare environments.[14,19-20]

1.10 CRE treatment options and clinical outcome

Systematic reviews show various studies correlating the timely diagnosis and management of CPEs to having profound patient and healthcare implications – each hour delayed attracts an increased mortality rate of 8%, while prolonged hospital stays, escalated treatment costs, and a reduced probability of being discharged, add to the burden.[28-29,31]

1.10.1 Current management difficulties

The alarming surge in resistance to the traditionally-used antibiotics for CRE treatment in SA is a cause for grave concern.[4,75] Polymyxins (e.g. colistin) and tigecycline are typically used as salvage therapy, while agents such as aminoglycosides, double carbapenems, fluoroquinolones, among others, may also be options.[76] In all instances, selection choice depends on organism identification and accurate AST results, while host factors, pathogen inherent resistance, infection site efficiency, and adverse effect potential, pose notable limitations to their use.[76] The Infectious Diseases Society of America (IDSA) 2024 guidance document advises against the previous standard practice of administering combination therapy consisting of an extended-infusion carbapenem and a second agent.[41] This is due to trial data revealing increased mortality and excess nephrotoxicity in aminoglycoside- or polymyxin-based regimens.[41] Major challenges therefore prevail in the context of waning antimicrobial options and the ability to improve CRE infection-related patient clinical outcomes.[4,26]

1.10.2 Novel therapeutic agents

The recent development and approval of therapeutic agents (Figure 4)[77] such as the new BL/BLI combinations and cefiderocol have afforded much relief in providing combative strategies to treat DTR-GNB, including CREs.[1,4,60,75] Ceftazidime-avibactam is a third generation cephalosporin combined with a BLI and is active against Ambler class A (bla_{KPC})

and Ambler class D (*bla*$_{\text{OXA-48-like}}$) carbapenemases, while meropenem-vaborbactam is effective only against *bla*$_{\text{KPC}}$.[49] Both drugs are ineffective against MBLs, for which aztreonam is utilised (in combination with avibactam to overcome hydrolysis by the organism often coproducing SBLs; such as ESBLs, AmpCs, *bla*$_{\text{KPC}}$, and/or *bla*$_{\text{OXA-48-like}}$).[49] Cefiderocol, a siderophore cephalosporin that is active against SBLs and MBLs, binds to PBPs and inhibits cell wall biosynthesis following bacterial entry using active iron-transport systems.[77]

In conjunction to their enhanced potency/efficacy and reduced side effect/toxicity profile, the rationale for preferring these agents entail an ability to optimise AMS practices, ensure longevity of existing broad-spectrum antimicrobials, and, rather importantly, augment favourable patient outcomes.[41,79] Since drug selection is influenced by knowledge of the carbapenemase-encoding gene(s) present, enzyme detection and identification is imperative, as is accessibility and regulatory approval to these newer antibiotics.[1,4,60]

Agent	KPC-producer	NDM-producer	OXA-48-like-producer	Carbapenem-resistant *Pseudomonas aeruginosa*	Carbapenem-resistant *Acinetobacter baumannii*	*Stenotrophomonas maltophilia*
Aztreonam-avibactam						
Cefiderocol						
Ceftazidime-avibactam[1]						
Ceftolozane-tazobactam[1]						
Eravacycline[1,2]						
Fosfomycin (intravenous)						
Imipenem-relebactam[3]						
Meropenem-vaborbactam[1]						
Plazomicin[1,4]						
Polymyxin B[1,5] or Colistin[1,5]						
Tigecycline[1,2]						

Figure 4: Select antibiotics with activity against carbapenem-resistant organisms, including CREs (adapted from Tamma and Hsu, 2019)

Green, susceptibility anticipated to be >80%; yellow, susceptibility anticipated to be 30-80%; red, intrinsic resistance or susceptibility anticipated to be <30%. [1]US Food and Drug Administration (FDA)-approved agent; [2]synthetic tetracycline derivative; [3]imipenem-cilastatin–relebactam; [4]synthetic aminoglycoside; [5]polymyxin class

1.11 Summation

Carbapenemase-producing bacterial strains are often resistant to multiple antibiotics and attain high ability for dissemination in healthcare settings, creating significant management

challenges.[2,38] The current landscape of limited treatment options, particularly in resource-deprived settings, compounded by the upsurge in CRE-associated infections post COVID-19, supports the urgent need for access to these recently-developed agents.[4,8,10,80]

2. Problem statement

Antimicrobial resistance to carbapenem antibiotics represents a substantial healthcare burden, contributing greatly to patient morbidity and mortality.[5,61] The global epidemiology of CRE-related infections, gut colonisation, genotypic characterisation of carbapenemase genes, including patient clinical outcomes, is well-documented.[5,29,60-61] Surveillance of high-risk patients for CRE carriage is imperative to facilitate the timely implementation of appropriate protective measures, thereby reducing the spread of bacterial resistance.[1,11,61] Although many recent studies have reported a substantial increase in MDROs, including CREs, following mass usage of antibiotics during the COVID-19 pandemic,[6,15,17-18] some research demonstrates the opposite effect owing to the practise of rigid IPC protocols.[19-20]

Previous CRE data in SA studies have isolated the carbapenemases *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{OXA-181}}$ or *bla*$_{\text{NDM}}$ in either retrospective or screening studies.[10,35,42,69] Maphumulo et al. demonstrated a prevalence of *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$ carbapenemase genes in a retrospective study comprising 194 clinical CRE isolates from IALCH during 2013 to 2017, which was prior to COVID-19.[80] However, whether the paradigm has now shifted to encompass other or multiple carbapenemase-encoding genes in this adult and paediatric population, especially following amplified antibiotic prescribing practises during the COVID-19 pandemic, remains unknown. Additionally, a holistic evaluation of demographic, microbiological and molecular information relative to patient clinical outcomes within this institution, is yet to be established.

3. Research question

How have antibiotic prescribing practices during the COVID-19 pandemic influenced the prevalence, demography, molecular characterisation, and patient clinical outcomes of CRE infection at a quaternary public hospital in KZN, South Africa?

4. Study rationale

An analysis of CRE infections and patient clinical outcome correlation comparing multiple pre- and post-COVID-19 data from a South African public health sector perspective in the

KZN academic complex has, to date, been lacking. The deficiency of this baseline information also hinders optimal management of patients with CRE infection or colonisation due to the absence of standard operating procedures (SOPs) to (1) routinely identify carbapenemase genes from clinical CRE specimens and (2) ascertain CRE colonisation status in high-risk patients via surveillance.

The paucity of such significant data at IALCH, a specialised referral health facility in Durban, impedes the implementation of swift IPC measures, as well as timely adjustment of empirical treatment via the selection of appropriate directed therapy, where available. Therefore, a need existed to quantify the local prevalence of CREs, in conjunction to correlating patient risk factors to different clinical outcomes. Molecular characterisation has provided valuable information into the resistance mechanisms (being a CPE or not), including identification of the carbapenemase-encoding gene(s) present within this clinical setting. Availability of these results will further pivot future directions of strengthening AMS interventions focused at curtailing the spread of CREs, whilst simultaneously leveraging for the currently unavailable new therapeutic agents from a governance and policy influence perspective.

5. Study impact and clinical relevance

This novel study in our setting affords insight into the pre- and post-COVID-19 demographic profile of patients at IALCH who are infected with CREs, including their clinical outcome. The description of organism profiles and identification of carbapenemase-encoding genes helps delineate the molecular characteristics and distribution of locally circulating carbapenemase enzymes in this health institution. The study results also serve as a baseline in providing recommendations on the need for establishing SOPs for introducing a routine rapid test for carbapenemase identification and initiating the practice of performing CRE surveillance in selected patients.

A comparative trend analysis of CRE-related infections relative to the COVID-19 pre- and post-pandemic eras has also been established – a first for this specialised referral hospital. This enables critique of the efficiency of current IPC procedures, whilst simultaneously evaluating the need for guideline reforms. In addition to building a repository of CRE isolates for use during further research purposes, the study has, rather importantly, ascertained local antimicrobial prescribing practises and developed CRE antibiograms. In essence, fundamental

scientific data has been provided in advocating for the new appropriate drugs that are active against CREs, which are currently not routinely available in the KZN public health sector, but urgently required.

6. Aim

To determine the prevalence, carbapenemase-encoding genes, and clinical outcome of CRE-associated infection in a quaternary public sector hospital in KZN, South Africa, both prior to and following the COVID-19 pandemic.

7. Objectives

7.1 To determine the prevalence of CRE infections at IALCH, both during the pre- and post-COVID-19 time periods.

7.2 To describe the demographic characteristics and clinical outcome of patients in whom CREs are isolated, inclusive of any specimen site.

7.3 To analyse the antimicrobial susceptibility profiles and molecularly identify the carbapenemases present (if any) from both stored and prospectively-collected CRE isolates.

7.4 To utilise the study findings to advocate for the availability of the novel BL/BLI combinations and other drugs effective against CREs in this clinical setting.

8. References

1. Cui X, Zhang H, Du H. Carbapenemases in Enterobacteriaceae: detection and antimicrobial therapy. Frontiers in microbiology. 2019 Aug 20;10:1823.

2. Gatya Al-Mayahie SM, Al-Guranie DR, Hussein AA, Bachai ZA. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic *Escherichia coli* phylogroup B2 isolates from outpatients in Wasit Province/Iraq. PLOS One. 2022 Jan 25;17(1):e0262984.

3. Chatterjee N, Nirwan PK, Srivastava S, Rati R, Sharma L, Sharma P, Dwivedi P, Jaggi N. Trends in carbapenem resistance in Pre-COVID and COVID times in a tertiary care hospital in North India. Annals of clinical microbiology and antimicrobials. 2023 Jan 3;22(1):1.

4. Brink AJ, Coetzee J, Richards GA, Feldman C, Lowman W, Tootla HD, Miller MG, Niehaus AJ, Wasserman S, Perovic O, Govind CN. Best practices: Appropriate use of the new β-lactam/β-lactamase inhibitor combinations, ceftazidime-avibactam and ceftolozane-tazobactam in South Africa. Southern African Journal of Infectious Diseases. 2022 Oct 20;37(1):453.

5. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases. 2017 Feb 15;215(suppl_1):S28-36.

6. Bedenić B, Luxner J, Car H, Sardelić S, Bogdan M, Varda-Brkić D, Šuto S, Grisold A, Beader N, Zarfel G. Emergence and Spread of Enterobacterales with Multiple Carbapenemases after COVID-19 Pandemic. Pathogens. 2023 May 3;12(5):677.

7. Asokan GV, Ramadhan T, Ahmed E, Sanad H. WHO global priority pathogens list: a bibliometric analysis of Medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahrain. Oman medical journal. 2019 May;34(3):184.

8. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of clinical microbiology and antimicrobials. 2016 Dec;15(1):1-1.

9. Dilagui I, Loqman S, Hanchi AL, Soraa N. Antibiotic resistance patterns of carbapenemase-producing enterobacterales in Mohammed VI University Hospital of Marrakech, Morocco. Infectious Diseases Now. 2022 Sep 1;52(6):334-40.

10. Perovic O, Ismail H, Quan V, Bamford C, Nana T, Chibabhai V, Bhola P, Ramjathan P, Swe Swe-Han K, Wadula J, Whitelaw A. Carbapenem-resistant Enterobacteriaceae in patients with bacteraemia at tertiary hospitals in South Africa, 2015 to 2018. European Journal of Clinical Microbiology & Infectious Diseases. 2020 Jul;39:1287-94.

11. Madni O, Amoako DG, Abia AL, Rout J, Essack SY. Genomic investigation of carbapenem-resistant *Klebsiella pneumonia* colonization in an intensive care unit in South Africa. Genes. 2021 Jun 22;12(7):951.

12. Suay-García B, Pérez-Gracia MT. Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. Antibiotics. 2019 Aug 19;8(3):122.

13. Hansen GT. Continuous evolution: perspective on the epidemiology of carbapenemase resistance among Enterobacterales and other Gram-negative bacteria. Infectious Diseases and Therapy. 2021 Mar;10:75-92.

14. Wong SC, Chau PH, So SY, Chiu KH, Yuen LL, AuYeung CH, Lam GK, Chan VW, Chen JH, Chen H, Li X. Epidemiology of multidrug-resistant organisms before and during COVID-19 in Hong Kong. Infection Prevention in Practice. 2023 Jun 1;5(2):100286.

15. Rehman S. ReviewA parallel and silent emerging pandemic: Antimicrobial resistance (AMR) amid COVID-19 pandemic. Journal of Infection and Public Health. 2023 Feb 21.

16. Farfour E, Lecuru M, Dortet L, Le Guen M, Cerf C, Karnycheff F, Bonnin RA, Vasse M, Lesprit P. Carbapenemase-producing Enterobacterales outbreak: another dark side of COVID-19. American Journal of Infection Control. 2020 Dec 1;48(12):1533-6.

17. Shbaklo N, Corcione S, Vicentini C, Giordano S, Fiorentino D, Bianco G, Cattel F, Cavallo R, Zotti CM, De Rosa FG. An observational study of MDR hospital-acquired infections

and antibiotic use during COVID-19 pandemic: a call for antimicrobial stewardship programs. Antibiotics. 2022 May 20;11(5):695.

18. Miftode IL, Leca D, Miftode RS, Roşu F, Plesca C, Loghin I, Timpau AS, Mitu I, Mititiuc I, Dorneanu O, Miftode E. The clash of the titans: COVID-19, carbapenem-resistant enterobacterales, and first mcr-1-mediated colistin resistance in humans in Romania. Antibiotics. 2023 Feb 3;12(2):324.

19. Howard-Jones AR, Sandaradura I, Robinson R, Orde SR, Iredell J, Ginn A, van Hal S, Branley J. Multidrug-resistant OXA-48/CTX-M-15 *Klebsiella pneumoniae* cluster in a COVID-19 intensive care unit: salient lessons for infection prevention and control during the COVID-19 pandemic. Journal of Hospital Infection. 2022 Aug 1;126:64-9.

20. Elliott TM, Hurst C, Doidge M, Hurst T, Harris PN, Gordon LG. Unexpected benefit of COVID-19 hospital restrictions: Reduction in patients isolating with multidrug resistant organisms after restrictions were lifted. Infection, Disease & Health. 2022 Feb 1;27(1):14.

21. Shugart A, Mahon G, Huang JY, Karlsson M, Valley A, Lasure M, Gross A, Pattee B, Vaeth E, Brooks R, Maruca T. Carbapenemase production among less-common Enterobacterales genera: 10 US sites, 2018. JAC-Antimicrobial Resistance. 2021 Sep 1;3(3):dlab137.

22. Adeolu M, Alnajar S, Naushad S, S. Gupta R. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International journal of systematic and evolutionary microbiology. 2016 Dec;66(12):5575-99.

23. Azam MW, Zarrilli R, Khan AU. Updates on the virulence factors produced by multidrug-resistant Enterobacterales and strategies to control their infections. Microorganisms. 2023 Jul 27;11(8):1901.

24. Morales-López S, Yepes JA, Prada-Herrera JC, Torres-Jiménez A. Enterobacteria in the 21st century: a review focused on taxonomic changes. The Journal of Infection in Developing Countries. 2019 Apr 30;13(04):265-73.

25. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm!. Trends in molecular medicine. 2012 May 1;18(5):263-72.

26. Tilahun M, Kassa Y, Gedefie A, Ashagire M. Emerging carbapenem-resistant Enterobacteriaceae infection, its epidemiology and novel treatment options: a review. Infection and drug resistance. 2021 Oct 21:4363-74.

27. Velazquez-Meza ME, Galarde-López M, Carrillo-Quiróz B, Alpuche-Aranda CM. Antimicrobial resistance: one health approach. Veterinary world. 2022 Mar 28;15(3):743.

28. Caliskan-Aydogan O, Alocilja EC. A review of carbapenem resistance in Enterobacterales and its detection techniques. Microorganisms. 2023 Jun 3;11(6):1491.

29. Brink AJ. Epidemiology of carbapenem-resistant Gram-negative infections globally. Current opinion in infectious diseases. 2019 Dec 1;32(6):609-16.

30. Ramsamy Y, Mlisana KP, Amoako DG, Abia AL, Ismail A, Allam M, Mbanga J, Singh R, Essack SY. Mobile genetic elements-mediated Enterobacterales-associated carbapenemase antibiotic resistance genes propagation between the environment and humans: A One Health South African study. Science of the Total Environment. 2022 Feb 1;806:150641.

31. Zhou R, Fang X, Zhang J, Zheng X, Shangguan S, Chen S, Shen Y, Liu Z, Li J, Zhang R, Shen J. Impact of carbapenem resistance on mortality in patients infected with Enterobacteriaceae: a systematic review and meta-analysis. BMJ open. 2021 Dec 1;11(12):e054971.

32. Bujňáková D, Puvača N, Ćirković I. Virulence factors and antibiotic resistance of enterobacterales. Microorganisms. 2022 Aug 7;10(8):1588.

33. Armstrong T, Fenn SJ, Hardie KR. JMM Profile: Carbapenems: a broad-spectrum antibiotic. Journal of medical microbiology. 2021 Dec 10;70(12):001462.

34. Elshamy AA, Aboshanab KM. A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options. Future science OA. 2020 Mar 1;6(3):FSO438.

35. Lowe M, Shuping L, Perovic O. Carbapenem-resistant Enterobacterales in patients with bacteraemia at tertiary academic hospitals in South Africa, 2019-2020: An update. South African Medical Journal. 2022 Aug 1;112(8):55-62.

36. Nepal, H. P., & Paudel, R. (2020). An overview of carbapenem, its resistance and therapeutic options for infections caused by carbapenem resistant bacteria. International Journal of Basic & Clinical Pharmacology, 9(9), 1454–1461.

37. Codjoe FS, Donkor ES. Carbapenem resistance: a review. Medical Sciences. 2017 Dec 21;6(1):1.

38. Bianco G, Boattini M, Comini S, Casale R, Iannaccone M, Cavallo R, Costa C. Occurrence of multi-carbapenemases producers among carbapenemase-producing Enterobacterales and in vitro activity of combinations including cefiderocol, ceftazidime-avibactam, meropenem-vaborbactam, and aztreonam in the COVID-19 era. European Journal of Clinical Microbiology & Infectious Diseases. 2022 Apr;41(4):573-80.

39. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet infectious diseases. 2018 Mar 1;18(3):318-27.

40. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.

41. Tamma PD, Heil EL, Justo JA, Mathers AJ, Satlin MJ, Bonomo RA. Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections. Clinical infectious diseases. 2024 Aug 7:ciae403.

42. Nel P, Roberts LA, Hoffmann R. Carbapenemase-producing Enterobacteriaceae colonisation in adult inpatients: A point prevalence study. Southern African Journal of Infectious Diseases. 2019 Mar 1;34(1):1-5.

43. Kedišaletše M, Phumuzile D, Angela D, Andrew W, Mae NF. Epidemiology, risk factors, and clinical outcomes of carbapenem-resistant enterobacterales in Africa: a systematic review. Journal of Global Antimicrobial Resistance. 2023 Dec 1;35:297-306.

44. van Loon K, Voor in 't holt AF, Vos MC. A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant Enterobacteriaceae. Antimicrobial agents and chemotherapy. 2018 Jan;62(1):10-128.

45. Tootla HD, Prentice E, Moodley C, Marais G, Nyakutira N, Reddy K, Bamford C, Niehaus A, Whitelaw A, Brink A. Carbapenem-resistant Enterobacterales among hospitalized patients in Cape Town, South Africa: clinical and microbiological epidemiology. JAC-Antimicrobial Resistance. 2024 Apr;6(2):dlae051.

46. Ambretti S, Bassetti M, Clerici P, Petrosillo N, Tumietto F, Viale P, Rossolini GM. Screening for carriage of carbapenem-resistant Enterobacteriaceae in settings of high endemicity: a position paper from an Italian working group on CRE infections. Antimicrobial Resistance & Infection Control. 2019 Aug 13;8(1):136.

47. Dixon B, Ahmed WM, Felton T, Fowler SJ. Molecular phenotyping approaches for the detection and monitoring of carbapenem-resistant Enterobacteriaceae by mass spectrometry. Journal of mass spectrometry and advances in the clinical lab. 2022 Nov 1;26:9-19.

48. Noster J, Thelen P, Hamprecht A. Detection of multidrug-resistant Enterobacterales—from ESBLs to carbapenemases. Antibiotics. 2021 Sep 21;10(9):1140.

49. MacDonald JW, Chibabhai V. Evaluation of the RESIST-4 OKNV immunochromatographic lateral flow assay for the rapid detection of OXA-48, KPC, NDM and VIM carbapenemases from cultured isolates. Access Microbiology. 2019 Jul;1(5):e000031.

50. Rabaan AA, Eljaaly K, Alhumaid S, Albayat H, Al-Adsani W, Sabour AA, Alshiekheid MA, Al-Jishi JM, Khamis F, Alwarthan S, Alhajri M. An overview on phenotypic and genotypic characterisation of carbapenem-resistant enterobacterales. Medicina. 2022 Nov 19;58(11):1675.

51. Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. Journal of clinical microbiology. 2018 Nov;56(11):10-128.

52. Simner PJ, Pitout JD, Dingle TC. Laboratory detection of carbapenemases among Gram-negative organisms. Clinical Microbiology Reviews. 2024 Dec 10;37(4):e00054-22.

53. Villegas MV, Jiménez A, Esparza G, Appel TM. Carbapenemase-producing Enterobacteriaceae: A diagnostic, epidemiological and therapeutic challenge. Infectio: Revista de la Asociación Colombiana de Infectología. 2019 Dec;23(4):388-97.

54. Hammoudi D, Moubareck CA, Sarkis DK. How to detect carbapenemase producers? A literature review of phenotypic and molecular methods. Journal of microbiological methods. 2014 Dec 1;107:106-18.

55. Solgi H, Badamchi A, Shahcheraghi F, Badmasti F, Akbari M, Behzadfar M. A comparative evaluation of five phenotypic methods for identification of carbapenemase-producing Enterobacteriaceae: a modified carbapenemase detection test. Microbiology Spectrum. 2024 Jul 2;12(7):e00386-24.

56. Thomas TS, Duse AG. Epidemiology of carbapenem-resistant Enterobacteriaceae (CRE) and comparison of the phenotypic versus genotypic screening tests for the detection of carbapenemases at a tertiary level, academic hospital in Johannesburg, South Africa. Southern African Journal of Infectious Diseases. 2018 Sep 11.

57. Miao M, Wen H, Xu P, Niu S, Lv J, Xie X, Mediavilla JR, Tang YW, Kreiswirth BN, Zhang X, Zhang H. Genetic diversity of carbapenem-resistant Enterobacteriaceae (CRE) clinical isolates from a tertiary hospital in Eastern China. Frontiers in Microbiology. 2019 Jan 15;9:3341.

58. Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, Zhang W, Zhang Y, Shen A, Sun H, Li L. Global spread of carbapenem-resistant Enterobacteriaceae: epidemiological features, resistance mechanisms, detection and therapy. Microbiological Research. 2023 Jan 1;266:127249.

59. Oka K, Matsumoto A, Tetsuka N, Morioka H, Iguchi M, Ishiguro N, Nagamori T, Takahashi S, Saito N, Tokuda K, Igari H. Clinical characteristics and treatment outcomes of carbapenem-resistant Enterobacterales infections in Japan. Journal of Global Antimicrobial Resistance. 2022 Jun 1;29:247-52.

60. Coppola N, Maraolo AE, Onorato L, Scotto R, Calò F, Atripaldi L, Borrelli A, Corcione A, De Cristofaro MG, Durante-Mangoni E, Filippelli A. Epidemiology, Mechanisms of Resistance and Treatment Algorithm for Infections Due to Carbapenem-Resistant Gram-Negative Bacteria: An Expert Panel Opinion. Antibiotics. 2022 Sep 17;11(9):1263.

61. Willems RPJ, van Dijk K, Vehreschild MJ, Biehl LM, Ket JC, Remmelzwaal S, Vandenbroucke-Grauls CM. Incidence of infection with multidrug-resistant Gram-negative bacteria and vancomycin-resistant enterococci in carriers: a systematic review and meta-regression analysis. The Lancet Infectious Diseases. 2023 Jan 30.

62. Jean SS, Harnod D, Hsueh PR. Global threat of carbapenem-resistant gram-negative bacteria. Frontiers in Cellular and Infection Microbiology. 2022:236.

63. Tadese BK, Darkoh C, DeSantis SM, Mgbere O, Fujimoto K. Clinical epidemiology of carbapenem-resistant Enterobacterales in the Greater Houston region of Texas: a 6-year trend and surveillance analysis. Journal of Global Antimicrobial Resistance. 2022 Sep 1;30:222-7.

64. Pitout JD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. Clinical microbiology reviews. 2019 Dec 18;33(1):10-128.

65. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, Zheng Y, Guo Y, Zhang R, Hu F, China Antimicrobial Surveillance Network (CHINET) Study Group. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant Enterobacteriaceae isolated from adult and children patients in China. Frontiers in cellular and infection microbiology. 2020 Jul 3;10:314.

66. Global antimicrobial resistance and use surveillance system (GLASS) report 2021. Geneva: World Health Organization (WHO); 2021. Licence: CC BY-NC-SA 3.0 IGO.

67. Abdelaziz NA. Phenotype-genotype correlations among carbapenem-resistant Enterobacterales recovered from four Egyptian hospitals with the report of SPM carbapenemase. Antimicrobial Resistance & Infection Control. 2022 Dec;11(1):1-0.

68. Karlowsky JA, Bouchillon SK, El Mahdy Kotb R, Mohamed N, Stone GG, Sahm DF. Carbapenem-resistant Enterobacterales and *Pseudomonas aeruginosa* causing infection in Africa and the Middle East: A surveillance study from the ATLAS programme (2018–20). JAC-Antimicrobial Resistance. 2022 Jun;4(3):dlac060.

69. Greybe L, Nuttall JJ, Brink AJ, Tootla HD. Descriptive analysis of infections due to New Delhi metallo-β-lactamase-producing Enterobacterales in children at Red Cross War Memorial Children's Hospital. Southern African Journal of Infectious Diseases. 2022 Jul 27;37(1):440.

70. Mzimela BW, Nkwanyana NM, Singh R. Clinical outcome of neonates with Carbapenem-resistant Enterobacteriaceae infections at the King Edward VIII Hospital's neonatal unit, Durban, South Africa. Southern African Journal of Infectious Diseases. 2021 Jan 21;36(1):223.

71. Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, Dever LL, Kreiswirth BN. Colistin-and carbapenem-resistant *Escherichia coli* harboring mcr-1 and

bla NDM-5, causing a complicated urinary tract infection in a patient from the United States. mBio. 2016 Sep 7;7(4):10-128.

72. Ansari S, Hays JP, Kemp A, Okechukwu R, Murugaiyan J, Ekwanzala MD, Ruiz Alvarez MJ, Paul-Satyaseela M, Iwu CD, Balleste-Delpierre C, Septimus E. The potential impact of the COVID-19 pandemic on global antimicrobial and biocide resistance: an AMR Insights global perspective. JAC-antimicrobial resistance. 2021 Jun 1;3(2):dlab038.

73. Yu H, González Molina MK, Carmona Cartaya Y, Hart Casares M, Aung MS, Kobayashi N, Quiñones Pérez D. Multicenter Study of Carbapenemase-Producing Enterobacterales in Havana, Cuba, 2016–2021. Antibiotics. 2022 Apr 12;11(4):514.

74. Montrucchio G, Corcione S, Sales G, Curtoni A, De Rosa FG, Brazzi L. Carbapenem-resistant *Klebsiella pneumoniae* in ICU-admitted COVID-19 patients: Keep an eye on the ball. Journal of Global Antimicrobial Resistance. 2020 Dec 1;23:398-400.

75. Vrancianu CO, Dobre EG, Gheorghe I, Barbu I, Cristian RE, Chifiriuc MC. Present and future perspectives on therapeutic options for carbapenemase-producing Enterobacterales infections. Microorganisms. 2021 Mar 31;9(4):730.

76. Mbele S, Vasaikar SD. Risk factors for Carbapenem-resistant Enterobacterales infections: A case-control study. South African Family Practice. 2025 Feb 7;67(1):6029.

77. Tamma PD, Hsu AJ. Defining the role of novel β-lactam agents that target carbapenem-resistant gram-negative organisms. Journal of the Pediatric Infectious Diseases Society. 2019 Jul;8(3):251-60.

78. Amod F, Cassimjee H, Driver C, Govender S, Govind C, Ishwar C, Khan S, Lander I, Peer AKC, Ramdass R, Skinner D, Subrayen D, Ubbink A, Wadee R. Carbapenem Resistant Enterobacterales (CRE) blood stream infections in KZN (private sector): Guideline for targeted antimicrobial therapy. KZN Specialist Network Antimicrobial Stewardship Initiative. Nov 2022.

79. Jing N, Yan W, Zhang Q, Yuan Y, Wei X, Zhao W, Guo S, Guo L, Gao Y, Zhao L, Shi C. Epidemiology and genotypic characteristics of carbapenem resistant Enterobacterales in Henan, China: a multicentre study. Journal of Global Antimicrobial Resistance. 2022 Jun 1;29:68-73.

80. Maphumulo SL, Mlisana K, Singh, R, Swe Swe-Han K. Laboratory Screening and prevalence of Carbapenemase Producing Enterobacteriaceae isolates from Inkosi Albert Luthuli Central Hospital (IALCH). Unpublished data. MMed dissertation. University of KwaZulu-Natal, 2019.

CHAPTER 2:

SUBMISSION-READY MANUSCRIPT

Prepared according to the Instructions for Authors of PLOS One

Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19

Nitesh Brijlal[1,2*], Sandra Lihle Maphumulo[1,2], Khine Swe Swe-Han[1,2]

[1]Department of Medical Microbiology, National Health Laboratory Services, KwaZulu-Natal, Durban, South Africa

[2]School of Laboratory Medicine and Medical Sciences, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa

[*]Corresponding author:
E-mail: niteshbrijlal@yahoo.com (NB)

Abstract

Background

Extensive carbapenem use has resulted in an increasing occurrence of difficult-to-treat resistant gram-negative bacteria (DTR-GNB), such as carbapenem-resistant Enterobacterales (CREs). The international antimicrobial resistance (AMR) landscape was profoundly impacted due to varying antibiotic prescribing practices following coronavirus disease 2019 (COVID-19). The resultant evolution in multidrug-resistant organism (MDRO) trends underscored this novel study's aim in establishing the COVID-19 pandemic's influence on the prevalence, molecular characterisation, and clinical outcome of CRE infections at a South African quaternary public hospital.

Methods

Demographics, microorganism identification, antibiograms, and patient outcomes with associated factors were extracted retrospectively from electronic records at Inkosi Albert Luthuli Central Hospital (IALCH). Carbapenemase-encoding genes from CRE isolates were prospectively identified by conventional multiplex polymerase chain reaction. Comparisons between the pre-COVID-19 (01 January 2018 to 05 March 2020) and post-COVID-19 (06 March 2020 to 31 May 2023) periods were conducted.

Results

A total of 1481 (n) CRE samples were analysed from 985 patients, with an age range of 2 days to 91-years-old. The post-COVID-19 CRE prevalence (13.58%) was more than double the pre-pandemic prevalence (6.72%). *Klebsiella pneumoniae* (85.7%; 1269/1481) was the predominant microbial species, followed by *Enterobacter cloacae* (6.4%; 95/1481), both of which exhibited *in vitro* resistance to majority of antibiotic classes. Of the 357 molecularly-tested isolates, 322 (90.2%) were carbapenemase-producers, with an upsurge in $bla_{\text{OXA-48}}$ (20.8%; 15/72 versus 61.2%; 137/224, $p < 0.001$) and decrease in bla_{NDM} (69.4%; 50/72 versus 17.9%; 40/245, $p < 0.001$) in *Klebsiella pneumoniae* relative to the COVID-19 periods. Overall, majority of deaths (42.2%; 57/135) occurred within 7 days of CRE isolation, while linear regression analysis showed that intensive/high-care unit admission and sterile site infection were significantly associated with increased all-cause mortality.

Conclusion

There is a rising CRE burden and shift in carbapenemase-encoding gene prevalence within this referral healthcare institution following COVID-19. This study highlights the need for ongoing epidemiological surveillance and suggests conducting routine carbapenemase testing for CRE infections amongst selected patients. Multisectoral collaborative approaches streamlining accessibility to the newer β-lactam/β-lactamase inhibitor (BL/BLI) drug combinations, such as ceftazidime-avibactam, including agents like aztreonam and cefiderocol, are urgently required to provide directed therapy, thereby potentially improving patient clinical outcomes.

Keywords

Carbapenem-resistant Enterobacterales; carbapenemase; COVID-19; β-lactam/β-lactamase inhibitor

Introduction

The widespread use of carbapenems has resulted in the rapidly increasing occurrence and distribution of difficult-to-treat resistant gram-negative bacteria (DTR-GNB), such as carbapenem-resistant Enterobacterales (CREs), worldwide.[1-3] Infections caused by CREs pose a serious public health threat and are associated with significant patient morbidity and mortality.[4] Additionally, they have been categorised as "critical" according to the first published global priority pathogen list in 2017 by the World Health Organization (WHO).[5]

Enterobacterales are currently recognised as the leading cause of serious community-acquired and nosocomial infections and are among the commonest bacteria isolated from clinical specimens.[6-7] The production of beta(β)-lactamase (*bla*) enzymes, called carbapenemases, is the most frequently-mediated mechanism by which Enterobacterales develop carbapenem resistance.[2,7-9] Carbapenemases belong to the Ambler structural classification, being serine β-lactamases (Class A and D) and metallo-β-lactamases (Class B), which differ in their response to antimicrobial agents, impacting selection choices in patient management.[4,8,10]

The landscape of antimicrobial resistance (AMR) was greatly challenged during the outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[11-13] The COVID-19 pandemic had impacted negatively on the implementation of antimicrobial stewardship (AMS) programmes, escalating the prevalence of multidrug-resistant organisms (MDROs) secondary to the mass consumption (and misuse) of broad-spectrum antibiotics.[14-15] Global data revealed bla_{KPC}, $bla_{\text{OXA-48}}$ and bla_{NDM} to be the dominant carbapenemase-encoding genes in these CRE infections, comprising predominantly of respiratory and bloodstream specimen types, with some isolates harbouring multiple carbapenemases.[16-19]

However, although COVID-19 had been associated with an increased incidence in hospital-acquired resistant bacterial co-infections,[20-22] some studies revealed that prolonged personal protective equipment (PPE) use and strict, well-enforced infection prevention and control (IPC) strategies during this time period, were beneficial in minimising the spread of MDROs in certain healthcare environments.[11,23-24]

Maphumulo et al. demonstrated a prevalence of *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$ carbapenemase genes in a retrospective study comprising 194 clinical CRE isolates from Inkosi Albert Luthuli Central Hospital (IALCH), a quaternary public sector hospital in Durban, South Africa (SA), during 2013 to 2017, which was prior to COVID-19.[25] Whether the paradigm had now shifted to encompass other or multiple carbapenemase-encoding genes in this adult and paediatric population, especially following amplified antibiotic prescribing practises during the COVID-19 pandemic, remains unknown.

The paucity of such significant data within this specialised referral health facility impedes the implementation of swift IPC measures, the strengthening of AMS interventions, and the provision of baseline information on currently circulating carbapenemases to advocate for newer therapeutic agents active against certain carbapenemase-producing Enterobacterales (CPEs). This underscored the aim of this novel study in determining the influence of COVID-19 on the comparative prevalence, demography, molecular characterisation, and patient clinical outcomes of CRE infections at IALCH.

Methodology

Study design and setting

The study comprised a retrospective, descriptive, single-centre chart review using electronically-stored microbiological and patient clinical records, as well as a prospective, laboratory-based molecular characterisation of carbapenemase-encoding genes from stored CRE clinical isolates at IALCH.

Study period

The total study period (01 January 2018 to 31 May 2023) was divided into the pre-COVID-19 period, defined from 01 January 2018 to 05 March 2020 (first COVID-19 case in SA), while the period from 06 March 2020 to 31 May 2023 resembled the post-COVID-19 period.

Study definitions

A CRE was defined as a bacterium belonging to the Enterobacterales order that was resistant to at least one carbapenem antibiotic (ertapenem, imipenem or meropenem) in accordance to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing (33rd edition, 2023) guidelines.[26] For bacteria

intrinsically less susceptible to imipenem (e.g. *Proteus* spp., *Providencia* spp. and *Morganella* spp.), resistance to a carbapenem other than imipenem was required to classify as a CRE.

Study population and CRE isolate selection

Inclusion criteria

IALCH patients of all ages in whom CREs were identified from any anatomical site and laboratory-stored isolates conforming to the CLSI minimum inhibitory concentration (MIC) clinical breakpoint interpretative criteria for Enterobacterales against carbapenems, were included. A CRE cultured after 21 days of the last CRE infection for the same patient, obtained from the same specimen type/site, and attaining the same antibiogram, was deemed a new case.

Exclusion criteria

Patient information not attributable to CRE infections, microbiological specimen workup results not demonstrating *in vitro* resistance to any carbapenem (including intermediate susceptibility), and data not applicable to the study period, were excluded.

Data collection and management

Retrospective microbiological and patient clinical data were extracted from the hospital's integrated software platform, MEDITECH, and the National Health Laboratory Service (NHLS) Laboratory Interface System (LIS) database, LabTrak/TrakCare. This included patient demographics, specimen characteristics, microorganism identification, antibiogram profiles, and clinical outcome.

Following eligibility criteria application and filtering, continuous, de-duplicated data encompassing the total study period was derived and stratified per the relative COVID-19 time periods (Figure 1). A password-protected Microsoft Excel® Workbook document was used for data compilation, thereby ensuring restricted access. Data was securely stored electronically and will be destroyed after five years following research completion and publication.

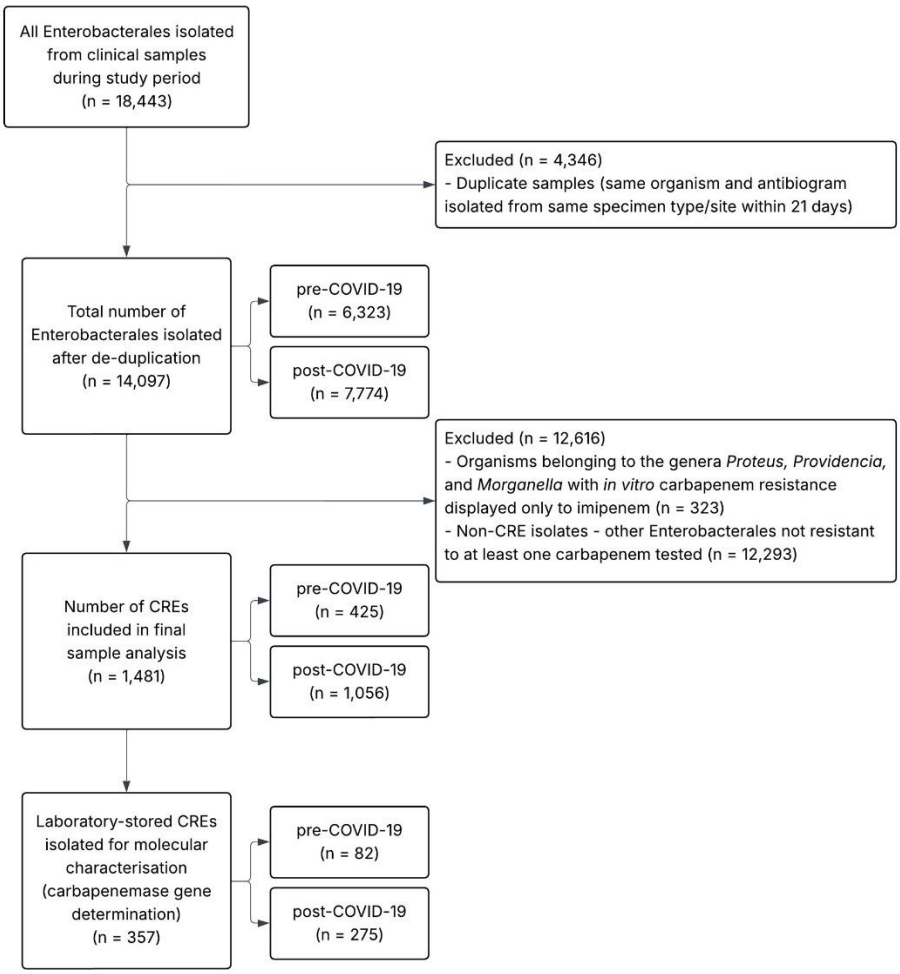


Figure 1: Flowchart depicting study sample derivation

Data analysis and statistical planning

Descriptive statistics were performed on patient demography, CRE isolate characteristics and AST results. Frequencies and percentages were calculated to summarise categorical variables. Measures of central tendency and dispersion of numerical data were calculated using means and standard deviations for normally-distributed variables, or medians and interquartile ranges for skewed variables. Susceptibility and resistance were calculated as the percentage relative to the proportion of species resulted against each antibiotic tested. Pearson's chi-squared test, Fisher's exact test, or the Wilcoxon rank sum test (Mann-Whitney U test) were used to test for association between characteristics of interest and patient clinical outcome in the pre- and post-COVID-19 periods. Logistic regression models were performed to identify factors associated with in-hospital mortality and odds ratios (ORs) were reported with a 95% confidence interval (95% CI). Data was analysed in R Statistical Software (version 4.5.0) and a *p*-value less than or equal to 0.05 ($p \leq 0.05$) was considered statistically significant.

Phenotypic characterisation

Microbiological identification of clinical specimens was done using either the VITEK® 2 (bioMérieux, Marcy-l'Étoile, France) automated system, the VITEK® MS PRIME MALDI-TOF [Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (bioMérieux, Marcy-l'Étoile, France)] automated system, or a combination of the above. Antimicrobial susceptibility testing (AST) was done either via Kirby-Bauer disk diffusion, the VITEK® 2 automated system, or an Epsilometer test (E-test).

Genotypic (molecular) characterisation

The identification of carbapenemase-encoding genes was done via conventional multiplex polymerase chain reaction (PCR), as outlined in Figure 2.

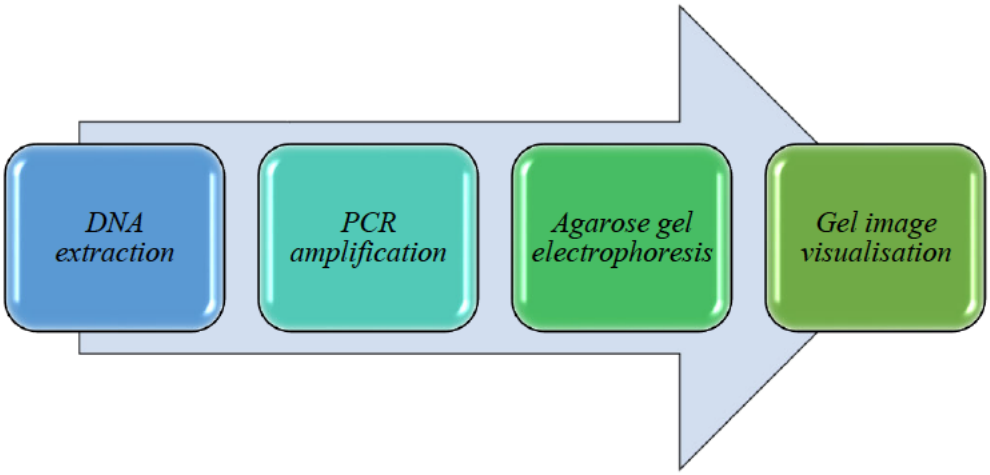


Figure 2: PCR workflow process (source: Author's own illustration)

DNA extraction

Following thawing and subculturing of stored CRE isolates, DNA was extracted using the Lucigen MA150E-QuickExtract™ DNA Extraction Solution protocol (Diagnostech, South Africa). Multiple colonies of each isolate were suspended in 100 µL of nuclease-free water (Qiagen, Hilden, Germany) to make a bacterial suspension in a 1.5 mL microcentrifuge tube. Thereafter, 150 µL of this solution was transferred into a separate PCR tube containing 150 µL of DNA Extraction Solution. This was subsequently mixed by vortexing for 15 seconds, heated at 98°C for 15 minutes, and centrifuged for 15 minutes at 4°C at 12,000 rpm. The DNA template for PCR was extracted from the resultant supernatant and stored in a -20°C freezer (Defy, Midrand, South Africa) until further downstream use.

Oligonucleotide primers

Previously-published oligonucleotide primer sequences for the amplification of carbapenemase-encoding genes (*bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{GES}$) were used for the assay (Table 1).

Table 1: Oligonucleotide primers used in the study (adapted from Watahiki et al., 2020) [27]

Targeted gene groups	Primer name	Sequence (5' to 3' direction)	Length (bases)	Amplicon size (bp)
***bla*$_{KPC}$**	mc-kpc-f	CGGAACCATTCGCTAAACTCG	21	322
	mc-kpc-r	AACAAATTGGCGGCGGCGT	19	
***bla*$_{IMP}$**	mc-imp-f	TCTCRATCTATCCCCACGTATGC	23	269
	mc-imp-r	GCGGACTTTGGCCAAGCTTCTA	22	
***bla*$_{NDM}$**	mc-ndm-f	CGGTTTGGCGATCTGGTTTT	20	207
	mc-ndm-r	GACCGGCAGGTTGATCTCC	19	
***bla*$_{VIM}$**	mc-vim-f	GTTTGGTCGCATATCGCAAC	20	155
	mc-vim-r	CCAATTTGCTTYTCAATCTCCG	22	
***bla*$_{OXA-48}$**	mc-oxa48-f	GCTCTGGAATGAGAATAAGCAGCA	24	125
	mc-oxa48-r	TAACCACGCCCAAATCGAG	19	
***bla*$_{GES}$**	mc-ges-f	CTGTGGCTAAAGTCCTCTATGGCG	24	94
	mc-ges-r	GTCGCGTCTCCCGTTTGGTT	20	

PCR amplification

The multiplex reactions were prepared using 12.5 µL of Thermo Scientific™ DreamTaq™ PCR Master Mix (2X) (Thermo Fisher Scientific, Waltham, Massachusetts, United States), 0.5 µL (1 µM) of each forward and reverse primer (Inqaba Biotech, Pretoria, South Africa) specific to *bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{GES}$, 1.5 µL of nuclease-free water (Qiagen, Hilden, Germany), and 5 µL of template DNA.

PCR amplification was carried out using a GeneAmp® PCR System 9700 Thermal Cycler (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and comprised the following conditions: an initial activation step at 95°C for 5 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 1 minute 30 seconds, extension at 72°C for 1 minute, and a final extension step at 68°C for 10 minutes.[27]

Positive control strains confirmed to carry the target carbapenemase-encoding genes were obtained from the National Institute for Communicable Diseases (NICD) of South Africa and, together with negative control (non-CRE) strains, were included in the PCR assays, thereby ensuring accuracy and reliability of the results.

Agarose gel electrophoresis

To confirm successful amplification and visualise the PCR amplicons, agarose gel electrophoresis was performed. The PCR products were loaded onto a 1.5% agarose gel (TopVision Agarose Tablets 0.5g and Invitrogen™ 10X TBE Buffer, Thermo Fisher Scientific, Waltham, Massachusetts, United States). Each well was loaded with 2 µL of 6X DNA Loading Dye mixed with GelRed dye (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and 6 µL of the PCR product. The gel electrophoresis was run at 80 V for 2 hours (Bio-Rad PowerPac™ Universal Electrophoresis Power Supply, California, United States) and the resultant gel image visualised under an ultraviolet (UV) transilluminator (Bio-Rad GelDoc™ Go Imaging System, California, United States) and photographed.

A 100 bp size ladder (Invitrogen™, Thermo Fisher Scientific, Waltham, Massachusetts, United States) was used as a marker for amplicon detection. The results obtained were compared against the control band sizes to ascertain if the sample DNA contained any carbapenemase-encoding gene(s) of interest, which was documented.

Ethical considerations

Ethical approval for this study was obtained in full from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN) (protocol reference number BREC/00005907/2023) and the KwaZulu-Natal Department of Health (KZN DoH) Ethics Committee (NHRD Ref: KZ_202307_012). The project was also supported by the Academic Affairs and Research Management System (AARMS) of the NHLS (K-Project PR2448733).

Gatekeeper permission was obtained from the medical manager at IALCH and departmental permission from the Head of Department of Medical Microbiology in the KZN Academic Complex. Patient consent was not required as this was a retrospective, descriptive analysis with molecular characterisation where patient personal or identifier details from the electronic databases were not shown, thereby ensuring and maintaining confidentiality.

Results

Prevalence of CRE infections at IALCH

The overall prevalence of CRE infections (Figure 2) was 10.5% (1,481/14,097). Notably, the post-COVID-19 prevalence was more than double the pre-COVID-19 prevalence, at 13.58% and 6.72%, respectively. During the initial five years of the study, a CRE surge was followed by a stable plateau, occurring at annual intervals. However, a concerning upward continuous trend from January 2022 was evident, necessitating the need for awareness and ongoing surveillance.

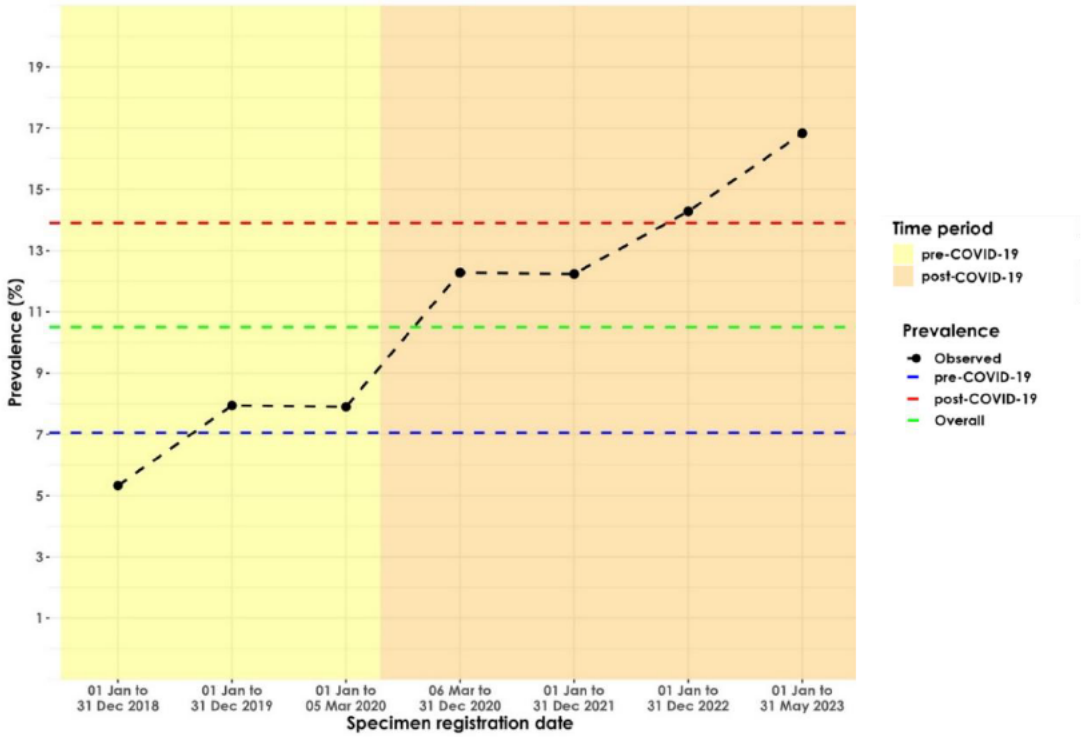


Figure 3: Prevalence of CREs at IALCH (2018 to 2023)

Following the onset of COVID-19 in SA (declared on 06 March 2020), the CRE prevalence of 12.29% (Table 1) had risen by 4.39% compared to the pre-pandemic period of the same year (7.90%). Thereafter, the number of CREs at IALCH had increased on a yearly basis, averaging 29 CRE isolates per month (n = 841, 29 months).

Table 2: CREs isolated as a proportion of de-duplicated Enterobacterales (2018 to 2023)

Time period	01 Jan to 31 Dec 2018	01 Jan to 31 Dec 2019	01 Jan to 05 Mar 2020	06 Mar to 31 Dec 2020	01 Jan to 31 Dec 2021	01 Jan to 31 Dec 2022	01 Jan to 31 May 2023
CREs isolated (n)	157	227	41	215	273	395	173
De-duplicated Enterobacterales (n)	2,946	2,858	519	1,750	2,230	2,766	1,028
Percentage (%)	**5.33**	**7.94**	**7.90**	**12.29**	**12.24**	**14.28**	**16.83**

Demographic profile

A total of 1,481 de-duplicated CRE isolates belonging to two study periods were analysed: a pre-COVID-19 period (n = 425 isolates) and a post-COVID-19 period (n = 1,056 isolates). The comparative general characteristics of the study population are summarised in Table 3.

Table 3: Patient characteristics relative to number of CRE isolates

Variable	Overall N = 1,481	pre-COVID-19 N = 425	post-COVID-19 N = 1,056
	n (%)	n (%)	n (%)
Age categorisation			
Neonates and infants	**508 (34.3)**	**172 (40.5)**	**336 (31.8)**
0-6 days (early neonates)	17 (1.1)	8 (1.9)	9 (0.9)
7-27 days (late neonates)	110 (7.4)	51 (12.0)	59 (5.6)
28-364 days (post-neonatal infants)	381 (25.7)	113 (26.6)	268 (25.4)
Children	**155 (10.5)**	**32 (7.5)**	**123 (11.6)**
1-4 years (young children)	99 (6.7)	19 (4.5)	80 (7.6)
5-9 years (older children)	56 (3.8)	13 (3.1)	43 (4.1)
Adolescents	**106 (7.2)**	**26 (6.1)**	**80 (7.6)**
10-14 years (young adolescents)	66 (4.5)	21 (4.9)	45 (4.3)
15-19 years (older adolescents)	40 (2.7)	5 (1.2)	35 (3.3)
Adults	**615 (41.5)**	**169 (39.8)**	**446 (42.2)**
20-24 years (young adults)	96 (6.5)	27 (6.4)	69 (6.5)
25-39 years (early adulthood)	252 (17.0)	64 (15.1)	188 (17.8)
40-59 years (middle-aged adults)	267 (18.0)	78 (18.4)	189 (17.9)
Elderly [≥60 years (older adults)]	**97 (6.5)**	**26 (6.1)**	**71 (6.7)**
Gender			
Female	707 (47.7)	194 (45.6)	513 (48.6)
Male	774 (52.3)	231 (54.4)	543 (51.4)
Patient location			
Intensive and high care units	**880 (59.4)**	**262 (61.6)**	**618 (58.5)**
Adult medical/surgical	163 (11.0)	50 (11.7)	113 (10.7)
Cardiothoracic	56 (3.8)	10 (2.4)	46 (4.3)
Neonatal	210 (14.2)	78 (18.3)	132 (12.5)
Neurosurgical	32 (2.2)	10 (2.4)	22 (2.1)
Paediatric	281 (19.0)	88 (20.7)	193 (18.3)
Transplant	20 (1.3)	2 (0.5)	18 (1.7)
Trauma	118 (7.9)	24 (5.6)	94 (8.9)
General wards	**508 (34.3)**	**135 (31.8)**	**373 (35.3)**
Adult haematology/oncology	80 (5.4)	20 (4.7)	60 (5.7)
Adult medical	152 (10.3)	44 (10.4)	108 (10.2)
Adult surgical	111 (7.5)	26 (6.1)	85 (8.1)
Obstetrics/gynaecology/labour ward	21 (1.4)	4 (0.9)	17 (1.6)
Paediatric haematology/oncology	52 (3.5)	12 (2.8)	40 (3.8)
Paediatric medical	68 (4.6)	19 (4.5)	49 (4.6)
Paediatric surgical	24 (1.6)	10 (2.4)	14 (1.3)
Outpatients/specialist clinics (all ages and disciplines)	**93 (6.3)**	**28 (6.6)**	**65 (6.2)**

Age groupings are represented via standardised age-disaggregated health data by life stage, as recommended by the 2030 Agenda for Sustainable Development Goals, allowing for global harmonisation.[28] Patient age distribution ranged from 2 days to 91 years old, with a median age of 12 years, 18 years and 16 years corresponding to the pre-pandemic, post-pandemic and overall period, respectively (IQR 0-39). Neonates and infants exhibited the solitary age category to obtain a reduction in CRE burden during the post-COVID-19 period (40.5%; 172/425 versus 31.8%; 336/1,056). In addition to males (52.3%, 774/1,481), most CREs were attributed to patients admitted to intensive and high-care units (59.4%, 880/1,481).

Table 4: Gender distribution relative to number of individual patients with CREs

Gender	Overall N = 985	pre-COVID-19 N = 310	post-COVID-19 N = 675
	n (%)	n (%)	n (%)
Female			
Total (any number of CREs)	454 (46.1)	136 (43.9)	318 (47.1)
Only 1 CRE	323 (32.8)	102 (32.9)	221 (32.7)
Multiple (≥2) CREs	131 (13.3)	33 (10.6)	100 (14.8)
Average number of CREs, if multiple (384 isolates)	2.93	2.76	2.92
Male			
Total (any number of CREs)	531 (53.9)	174 (56.1)	357 (52.9)
Only 1 CRE	393 (39.9)	137 (44.2)	256 (37.9)
Multiple (≥2) CREs	138 (14.0)	35 (11.3)	104 (15.4)
Average number of CREs, if multiple (381 isolates)	2.76	2.60	2.75

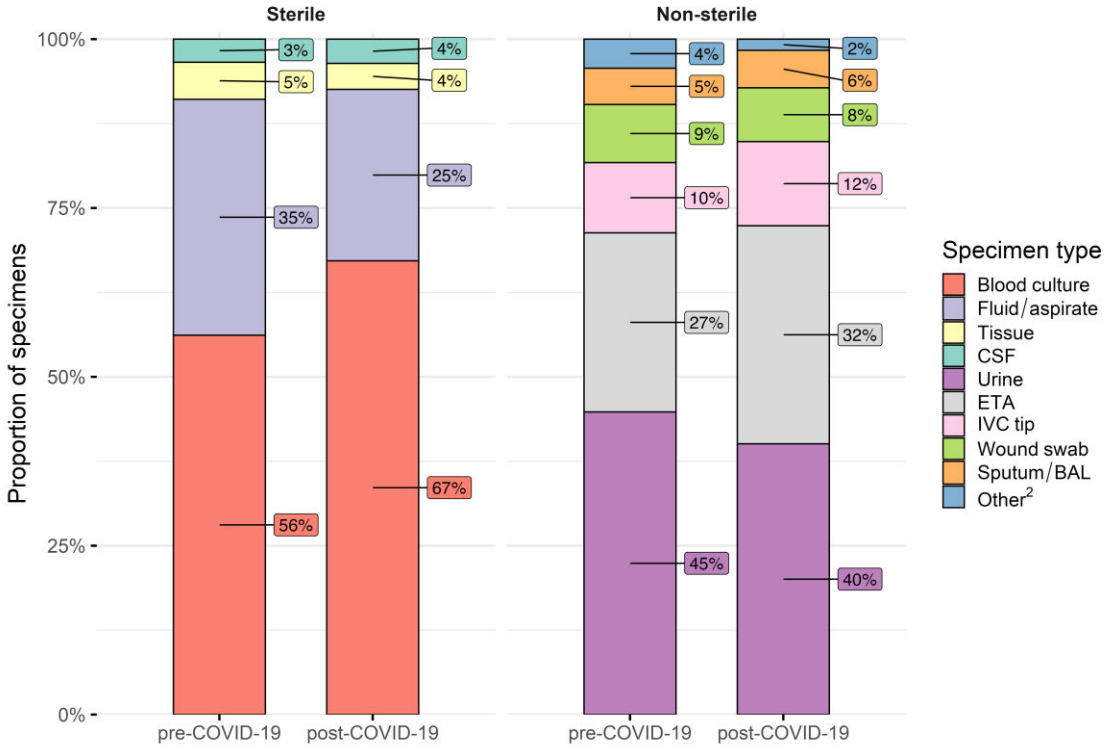
As displayed in Table 4, CREs isolated from laboratory specimens belonged to 985 patients, of which 46.1% (454/985) were females (regardless of isolate count per individual patient). Both genders elicited an increase (4.1-4.2%) in isolating multiple (≥2) CREs per patient in the post-COVID-19 period relative to the pre-COVID-19 period, while a decline (0.2-6.3%) was noted amongst patients attaining only one CRE in the same timeframes. For individual patients with multiple CREs, females averaged 2.93 isolates during the combined period, compared to males at 2.76 isolates.

Microbiology of CRE isolates

Specimen type

Depending on the anatomical site and nature of sample obtained, these were divided into sterile and non-sterile types (Figure 4), with the latter comprising the overall majority (63.8%; 945/1,481). In the respective pre- and post-COVID-19 periods, urine specimens predominated within this category (44.8%; 125/279 versus 40.1%; 267/666), followed by endotracheal

aspirates (26.5%; 74/279 versus 32.3%; 215/666). Blood cultures were the most prevalent sterile sample type (Supplementary Table 1), attaining a significant post-COVID-19 rise (56.2%; 82/146 versus 67.2%; 262/390, p = 0.018). Although fluid/aspirates ranked as the second commonest sterile sample type, a noticeable decrease was recorded in the post-pandemic period (34.9%; 51/146 versus 25.4%; 99/390, p = 0.028).



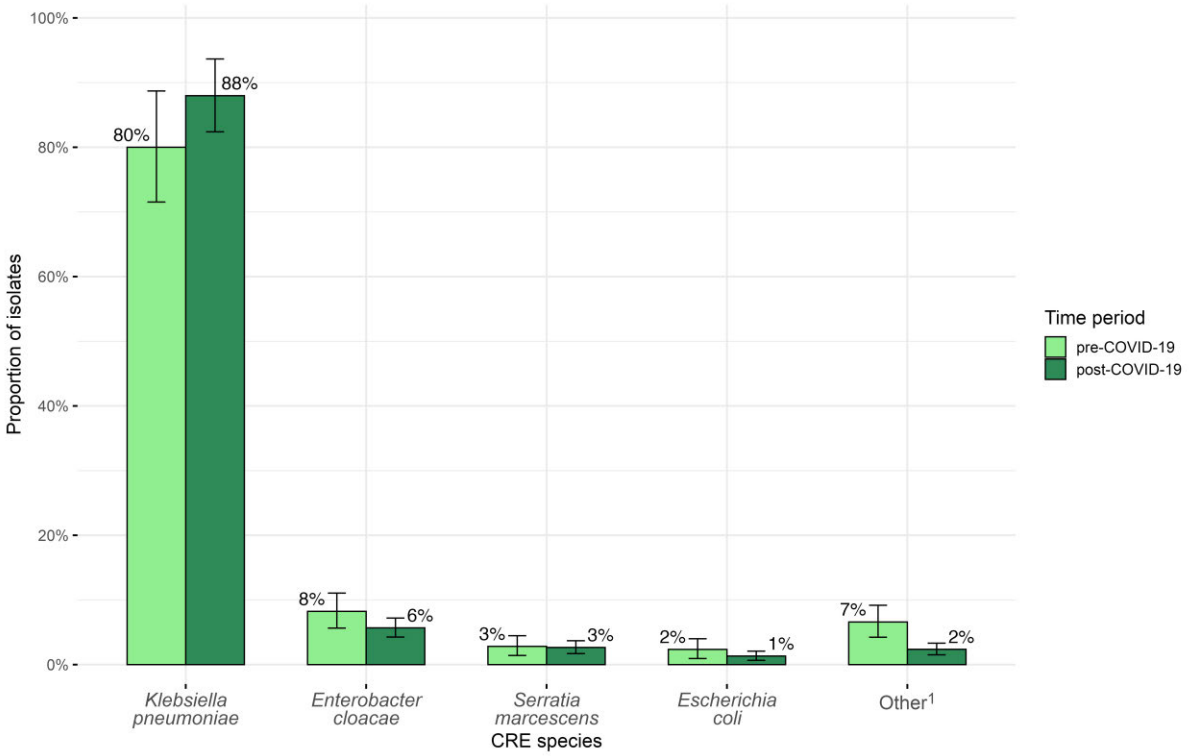
CSF, cerebrospinal fluid; ETA, endotracheal aspirate; IVC, intravascular catheter; BAL, bronchoalveolar lavage

[2]stool (n = 12), ventricular catheter tip (n = 11); overall period

Figure 4: Distribution of CREs according to type of specimen (n = 1,481)

CRE species

As depicted in Figure 5 and Supplementary Table 2, *Klebsiella pneumoniae*, which constituted the largest proportion of all CRE species overall (85.7%; 1,269/1,481), was identified in almost triple the number of specimens in the post-COVID-19 period (80%; 340/425 versus 88%; 929/1,056, p < 0.001). A declining insignificant prevalence, however, occurred amongst the other CRE species, namely *Enterobacter cloacae* (8.2%; 35/425 versus 5.7%; 60/1,056), *Serratia marcescens* (2.8%; 12/425 versus 2.6%; 28/1,056) and *Escherichia coli* (2.4%; 10/425 versus 1.3%; 14/1,056).



[1]*Enterobacter* species (n = 16), *Klebsiella* species (n = 9), *Citrobacter* species (n = 8), *Proteus mirabilis* (n = 6), *Morganella morganii* (n = 5), *Serratia* species (n = 4), *Providencia* species (n = 3), *Raoultella* species (n = 1), *Salmonella* species (n = 1)

Figure 5: Comparison of CRE species isolated (n = 1,481)

Antimicrobial susceptibility/resistance profile

Subsequent to the COVID-19 onset, more than half of the routinely-tested antibiotics demonstrated significantly increased resistance trends (Figure 6 and Supplementary Table 3). Ciprofloxacin was the dominant non-β-lactam agent eliciting this heightened resistance change (80.9%; 344/425 versus 88.3%; 926/1,049, $p < 0.001$). Although low overall, a notable 6.2% rise in tigecycline resistance relative to the pre-pandemic period (4%; 15/376 versus 10.2%; 100/979, $p < 0.001$), was manifest (excluding intrinsically-resistant *Proteus* spp., *Providencia* spp., and *Morganella* spp.).

Regarding carbapenems, the comparative resistance to imipenem and meropenem decreased slightly (3.7% and 4%, respectively), while ertapenem was associated with the highest escalation in resistance (16.6%). The aminoglycoside class represented the most considerable resistance decline following COVID-19; gentamicin resistance dropped by 15.6% (83.9%; 349/416 versus 68.3%; 709/1,038, $p < 0.001$) and amikacin by a substantial 26.1% (58.9%; 249/423 versus 32.8%; 342/1,042, $p < 0.001$).

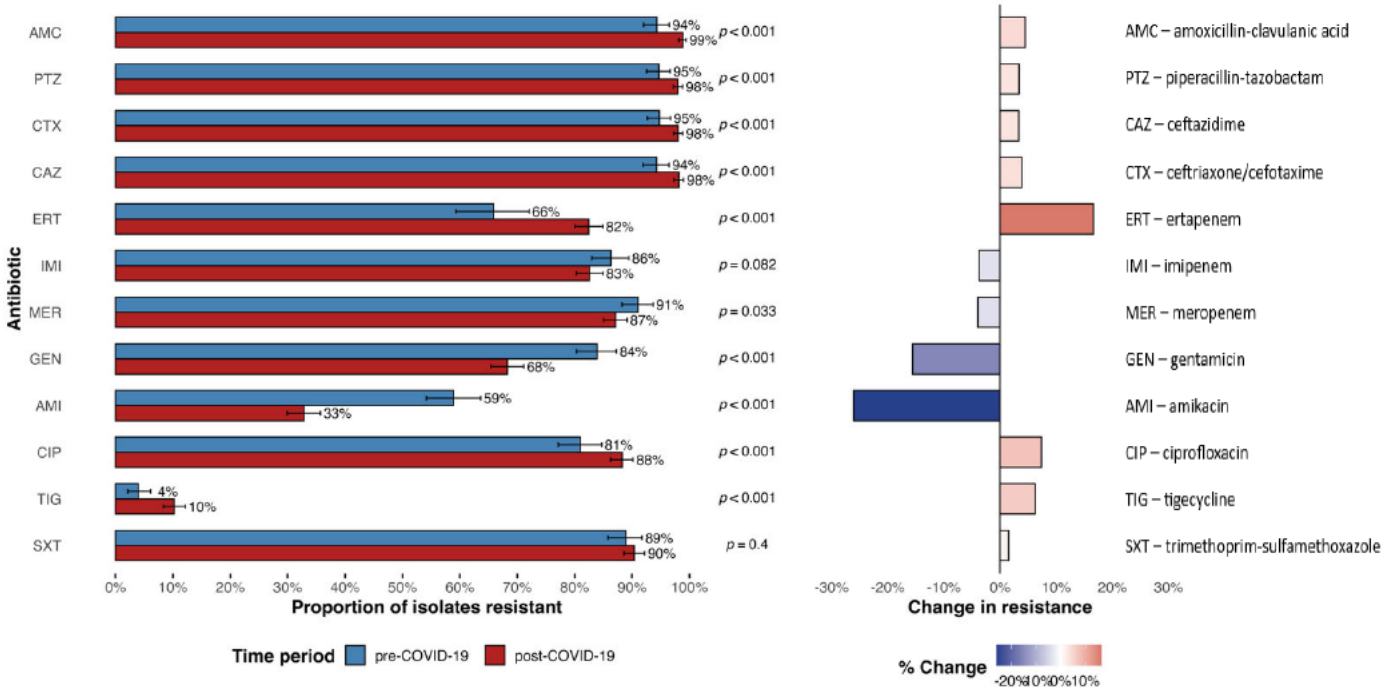


Figure 6: Comparative resistance profile trends of CREs against various antibiotics tested *in vitro*

Genotypic characterisation of CRE isolates

Conventional PCR was conducted on a total of 357 laboratory-stored CRE isolates, of which approximately three-quarter (77%; 275/357) belonged to the post-COVID-19 period.

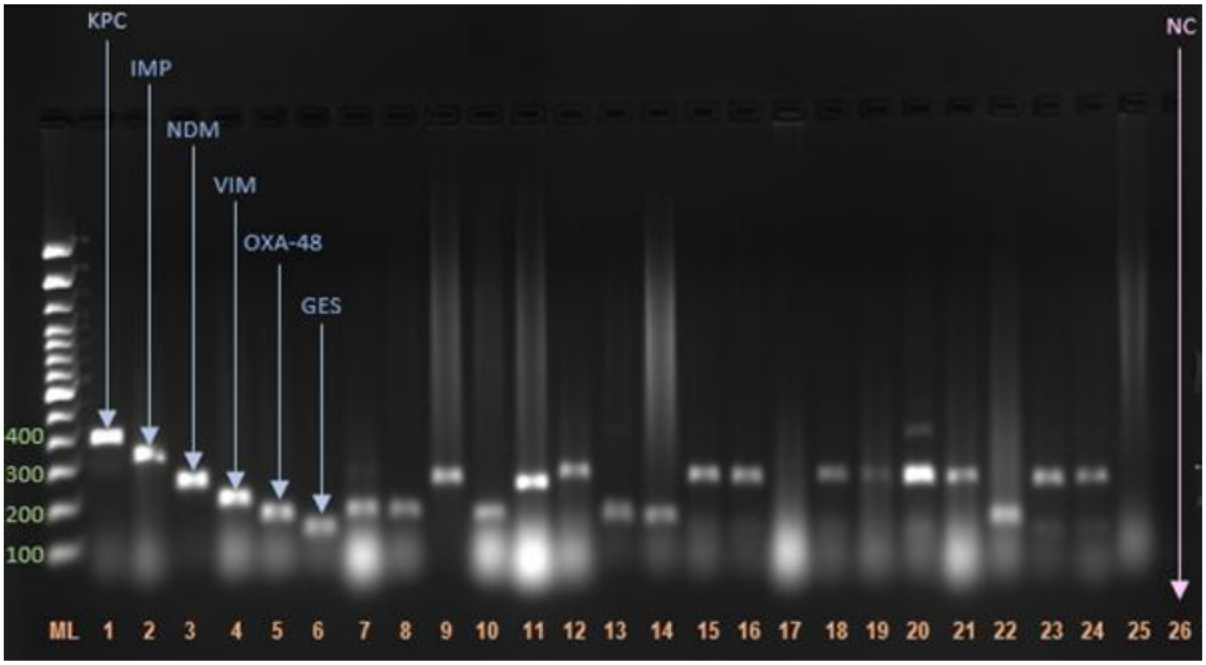
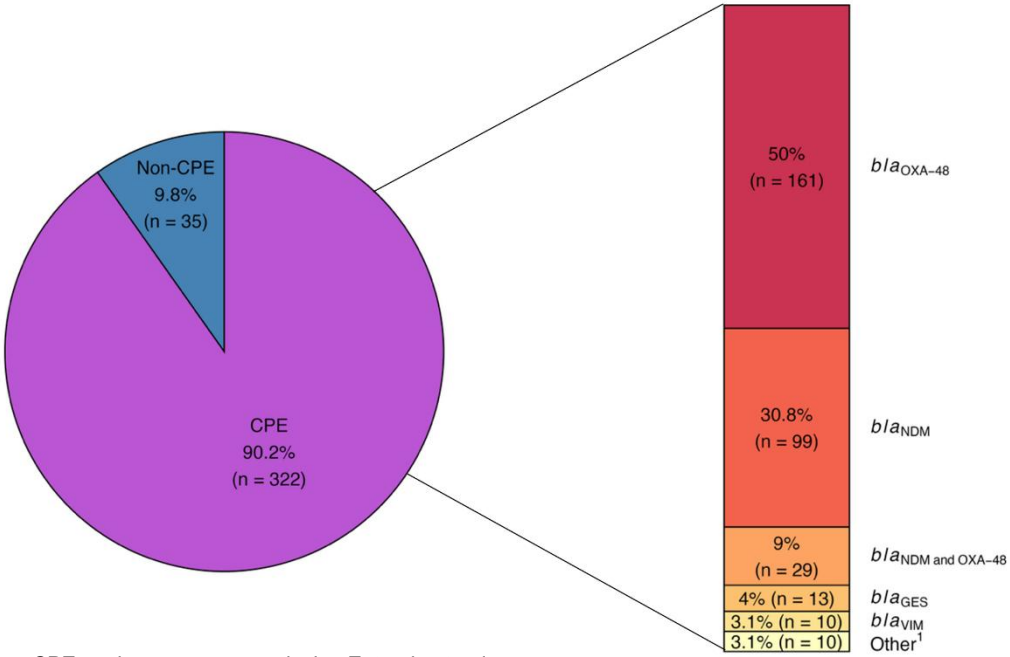


Figure 7: Representative gel electrophoresis results showing molecular detection of target carbapenemase-encoding genes (*bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{GES}$)

The detection of target PCR products is portrayed in Figure 7. Lane ML represents the 100 bp DNA molecular ladder, lanes 1-6 correspond to the positive controls (*bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, and *bla*$_{GES}$, as annotated), and lane 26 (blank) represents the negative control (NC). Lanes 7-25 resemble the CRE isolates tested – lanes 7-8, 10, 13-14 and 22 are positive for *bla*$_{OXA-48}$ only; lanes 9, 11, 15-16, 18-19, 21 and 23-24 are positive for *bla*$_{NDM}$ only; lane 12 is positive for *bla*$_{IMP}$ only; lanes 17 and 25 are negative for any of the six carbapenemase-encoding gene targets; and lane 20 is positive for *bla*$_{NDM}$ and *bla*$_{KPC}$ (dual carbapenemases).



CPE, carbapenemase-producing Enterobacterales

[1]*bla*$_{IMP}$ (n = 3), *bla*$_{NDM\ and\ KPC}$ (n = 2), *bla*$_{OXA-48\ and\ KPC}$ (n = 1), *bla*$_{OXA-48\ and\ VIM}$ (n = 1), *bla*$_{NDM\ and\ GES}$ (n = 1), *bla*$_{OXA-48\ and\ KPC\ and\ IMP}$ (n = 1), *bla*$_{OXA-48\ and\ KPC\ and\ VIM}$ (n = 1)

Figure 8: Frequency and distribution of carbapenemase-encoding genes in stored CRE isolates undergone molecular detection, total period (n = 357)

Molecular analysis revealed a vast majority of the isolates to be positive for carbapenemase production (90.2%; 322/357), with half being due to *bla*$_{OXA-48}$ (Figure 8). This was followed by *bla*$_{NDM}$ (30.7%; 99/322) and dual *bla*$_{NDM\ and\ OXA-48}$ (9%; 29/322). Amongst all the CRE isolates tested, two CPEs were found to contain three carbapenemases each.

The post-COVID-19 period demonstrated a 9.6% decline in CPE rate relative to the pre-pandemic period, denoting a significant molecular paradigm shift, particularly regarding the rise and decline for *bla*$_{OXA-48}$ and *bla*$_{NDM}$, respectively (Table 5). Notably, *Klebsiella pneumoniae* accounted for 91.9% (296/322) total CPEs, with a proportionate change reflecting

a greater than 2.9-fold increase in *bla*$_{\text{OXA-48}}$ (20.8%; 15/72 versus 61.2%; 137/224, $p < 0.001$) and 3.8-fold decrease in *bla*$_{\text{NDM}}$ (69.4%; 50/72 versus 17.9%; 40/245, $p < 0.001$) following COVID-19. In addition, CREs containing dual carbapenemases *bla*$_{\text{NDM and OXA-48}}$ attained a 5.3% rise during the combined period.

Table 5: Carbapenemase-encoding gene detection relative to predominant CRE species

	Total specimens for PCR N = 357			***Klebsiella pneumoniae*** N = 317			**Other CRE species[a]** N = 40		
Carbapenemase-encoding gene(s)	**pre-COVID-19** N = 82	**post-COVID-19** N = 275	***p*-value**	**pre-COVID-19** N = 72	**post-COVID-19** N = 245	***p*-value**	**pre-COVID-19** N = 10	**post-COVID-19** N = 30	***p*-value**
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
Total detected	**80 (97.6)**	**242 (88.0)**	**0.011[1]**	**72 (100)**	**224 (91.4)**	**0.006[1]**	**8 (80.0)**	**18 (60.0)**	**0.60[1]**
bla$_{\text{OXA-48}}$	16 (20.0)	145 (59.9)	<0.001[1]	15 (20.8)	137 (61.2)	<0.001[1]	1 (12.5)	8 (44.4)	0.19[2]
bla$_{\text{NDM}}$	55 (68.8)	44 (18.2)	<0.001[1]	50 (69.4)	40 (17.9)	<0.001[1]	5 (62.5)	4 (22.2)	0.078[1]
bla$_{\text{NDM and OXA-48}}$	4 (5.0)	25 (10.3)	0.15[1]	4 (5.6)	23 (10.3)	0.23[1]	0 (0)	2 (11.1)	>0.99[2]
Other	5 (6.2)	28 (11.6)	0.17[1]	3 (4.2)[b]	24 (10.7)[c]	0.093[2]	2 (25.0)[d]	4 (22.2)[e]	>0.99[2]
Not detected	**2 (2.4)**	**33 (12.0)**	**0.011[1]**	**0 (0)**	**21 (8.6)**	**0.006[2]**	**2 (20.0)**	**12 (40.0)**	**0.60[2]**

[1]Pearson's Chi-squared test

[2]Fisher's exact test

[a]*Enterobacter cloacae* (n = 23), *Serratia marcescens* (n = 10), *Escherichia coli* (n = 3), *Citrobacter freundii* (n = 2), *Klebsiella oxytoca* (n = 1), *Proteus mirabilis* (n = 1); overall period

[b]*bla*$_{\text{IMP}}$ (n = 1), *bla*$_{\text{NDM and KPC}}$ (n = 1), *bla*$_{\text{NDM and GES}}$ (n = 1)

[c]*bla*$_{\text{GES}}$ (n = 11), *bla*$_{\text{VIM}}$ (n = 8), *bla*$_{\text{OXA-48 and VIM}}$ (n = 1), *bla*$_{\text{OXA-48 and KPC}}$ (n = 1), *bla*$_{\text{NDM and KPC}}$ (n = 1), *bla*$_{\text{OXA-48 and VIM and KPC}}$ (n = 1), *bla*$_{\text{OXA-48 and IMP and KPC}}$ (n = 1)

[d]*bla*$_{\text{GES}}$ (n = 1), *bla*$_{\text{VIM}}$ (n = 1)

[e]*bla*$_{\text{IMP}}$ (n = 2), *bla*$_{\text{GES}}$ (n = 1), *bla*$_{\text{VIM}}$ (n = 1)

Patient clinical outcome

As denoted in Table 6, although overall mortality (all-cause) mostly occurred in the neonate and infant age group (36.3%; 49/135), the likelihood of dying amongst adults aged 20-59 years old was more than double during the post-COVID-19 period (AOR = 2.40; 95% CI: 1.09 to 5.42, $p = 0.032$). There was no gender predilection amid pre-COVID-19-related deaths; however, males attained a 5.2% lower death rate post-COVID-19, with the converse relationship occurring in females by the same measure. *Klebsiella pneumoniae* accounted for 100% (30/30) and 92.4% (97/105) of pre- and post-pandemic deaths, respectively.

Following COVID-19, univariable and multivariable logistic regression analyses revealed that *bla*$_{\text{OXA-48}}$ was associated with an increased odds of death (COR = 4.44; 95% CI: 2.02 to 10.9, $p < 0.001$ and AOR = 3.79; 95% CI: 1.67 to 9.48, $p = 0.002$) compared to *bla*$_{\text{NDM}}$ only. This association was also true for CREs bearing other uncommon or multiple carbapenemases.

Table 6: Clinical outcome of patients with carbapenemase-encoding gene-positive CRE isolates and factors associated with in-patient mortality

Variable	pre-COVID-19						post-COVID-19					
	Discharged N = 50	Died N = 30	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value	Discharged N = 137	Died N = 105	COR (95% CI)	*p*-value	AOR (95% CI)	*p*-value
	n (%)	n (%)					n (%)	n (%)				
Age categorisation, years												
<1 (neonates and infants)	33 (66.0)	19 (63.3)	Ref		Ref		50 (36.5)	30 (28.6)	Ref		Ref	
1-19 (children and adolescents)	6 (12.0)	3 (10.0)	0.87 (0.17 to 3.70)	0.85	1.99 (0.21 to 20.8)	0.54	40 (29.2)	27 (25.7)	1.13 (0.58 to 2.19)	0.73	1.53 (0.69 to 3.43)	0.30
20-59 (adults)	9 (18.0)	7 (23.3)	1.35 (0.42 to 4.22)	0.60	1.32 (0.21 to 8.65)	0.76	43 (31.4)	41 (39.0)	1.59 (0.85 to 2.98)	0.14	2.40 (1.09 to 5.42)	0.032
≥60 (elderly)	2 (4.0)	1 (3.3)	0.87 (0.04 to 9.66)	0.91	2.38 (0.07 to 56.8)	0.58	4 (2.9)	7 (6.7)	2.92 (0.81 to 11.9)	0.11	4.73 (1.16 to 22.5)	0.036
Gender												
Female	23 (46.0)	15 (50.0)	Ref		Ref		58 (42.3)	58 (55.2)	Ref		Ref	
Male	27 (54.0)	15 (50.0)	0.85 (0.34 to 2.12)	0.73	0.69 (0.20 to 2.32)	0.55	79 (57.7)	47 (44.8)	0.59 (0.36 to 0.99)	0.047	0.71 (0.41 to 1.24)	0.23
Patient location at time of CRE isolation												
General ward	19 (38.0)	6 (20.0)	Ref		Ref		65 (47.4)	39 (37.1)	Ref		Ref	
Intensive and high care unit	31 (62.0)	24 (80.0)	2.45 (0.88 to 7.59)	0.10	6.48 (1.17 to 48.2)	0.045	72 (52.6)	66 (62.9)	1.53 (0.91 to 2.58)	0.11	2.09 (1.07 to 4.17)	0.033
CRE specimen type												
Non-sterile	33 (66.0)	8 (26.7)	Ref		Ref		80 (58.4)	45 (42.9)	Ref		Ref	
Sterile	17 (34.0)	22 (73.3)	5.34 (2.03 to 15.2)	0.001	7.68 (2.26 to 31.0)	0.002	57 (41.6)	60 (57.1)	1.87 (1.12 to 3.14)	0.017	2.10 (1.21 to 3.70)	0.009
CRE species isolated												
Klebsiella pneumoniae	42 (84.0)	30 (100)	Ref		–		127 (92.7)	97 (92.4)	Ref		–	
Non-*Klebsiella pneumoniae*	8 (16.0)	0 (0)	0.00 (NA to NA)	>0.99			10 (7.3)	8 (7.6)	1.05 (0.39 to 2.75)	0.93		
Surgery during hospitalisation												
No	20 (40.0)	16 (53.3)	Ref		Ref		80 (58.4)	61 (58.1)	Ref		Ref	
Yes (any type/discipline)	30 (60.0)	14 (46.7)	0.58 (0.23 to 1.45)	0.25	0.24 (0.06 to 0.84)	0.031	57 (41.6)	44 (41.9)	1.01 (0.60 to 1.69)	0.96	1.01 (0.55 to 1.85)	0.99
Carbapenemase(s) detected												
bla_{NDM}	38 (76.0)	17 (56.7)	Ref		Ref		36 (26.3)	8 (7.6)	Ref		Ref	
bla_{OXA-48}	7 (14.0)	9 (30.0)	2.87 (0.92 to 9.32)	0.070	2.25 (0.56 to 9.30)	0.25	73 (53.3)	72 (68.6)	4.44 (2.02 to 10.9)	<0.001	3.79 (1.67 to 9.48)	0.002
bla_{NDM} and bla_{OXA-48}	2 (4.0)	2 (6.7)	2.24 (0.25 to 19.9)	0.44	8.78 (0.60 to 159)	0.11	16 (11.7)	9 (8.6)	2.53 (0.83 to 7.95)	0.10	1.89 (0.58 to 6.25)	0.29
Other	3 (6.0)[a]	2 (6.7)[b]	1.49 (0.18 to 9.80)	0.68	4.03 (0.36 to 46.2)	0.24	12 (8.8)[c]	16 (15.2)[d]	6.00 (2.12 to 18.3)	0.001	4.81 (1.60 to 15.5)	0.006

COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence Interval

[a] bla_{GES} (n = 1), bla_{VIM} (n = 1), $bla_{NDM\ and\ KPC}$ (n = 1)

[b] bla_{IMP} (n = 1), $bla_{NDM\ and\ GES}$ (n = 1)

[c] bla_{GES} (n = 6), bla_{VIM} (n = 3), bla_{IMP} (n = 1), $bla_{NDM\ and\ KPC}$ (n = 1), $bla_{OXA-48\ and\ IMP\ and\ KPC}$ (n = 1)

[d] bla_{GES} (n = 6), bla_{VIM} (n = 6), bla_{IMP} (n = 1), $bla_{OXA-48\ and\ VIM}$ (n = 1), $bla_{OXA-48\ and\ KPC}$ (n = 1), $bla_{OXA-48\ and\ VIM\ and\ KPC}$ (n = 1)

In addition to sterile specimen types, the isolation of CREs from patients located in intensive and high care units were, after controlling for age and gender, shown by linear regression analysis to significantly increase the odds of mortality.

Table 7: Time to death in patients with carbapenemase-producing CREs (n = 135)

Variable	Overall N = 135	pre-COVID-19 N = 30	post-COVID-19 N = 105
	n (%)	n (%)	n (%)
Time to death from specimen collection			
≤7 days	57 (42.2)	15 (50.0)	42 (40.0)
8-30 days	47 (34.8)	11 (36.7)	36 (34.3)
>30 days	31 (23.0)	4 (13.3)	27 (25.7)
≤30 days (combined)	104 (77.0)	26 (86.7)	78 (74.3)
≤7 days	57 (54.8)	15 (57.7)	42 (53.8)
8–30 days	47 (45.2)	11 (42.3)	36 (46.2)
Proportion of deaths	135/322 (41.9)	30/80 (37.5)	105/242 (43.4)

The all-cause in-hospital mortality rate for patients with CPEs was 41.9% (135/322) overall, with a 5.9% increase subsequent to COVID-19 (Table 7). Relative to the majority of total deaths that took place ≤30 days of specimen collection (77%; 104/135), over half had occurred ≤7 days during each COVID-19-related time period (57.7%; 15/30 versus 53.8%; 42/105).

CRE species	Carbapenemase-encoding gene	Antibiotic, n/N[1] (% susceptible)								
		ERT	IMI	MER	GEN	AMI	CIP	TIG[2]	COL[3]	SXT
Klebsiella pneumoniae (n = 127)	bla_{OXA-48} (n = 76)	0/65 (0)	0/76 (0)	0/76 (0)	24/75 (32)	33/76 (43)	1/76 (1)	56/69 (81)	53/64 (83)	3/73 (4)
	bla_{NDM} (n = 24)	0/12 (0)	0/24 (0)	0/24 (0)	0/24 (0)	1/24 (4)	2/24 (8)	23/24 (96)	22/24 (92)	1/24 (4)
	$bla_{OXA-48\ and\ NDM}$ (n = 10)	0/9 (0)	0/10 (0)	0/10 (0)	2/10 (20)	1/10 (10)	0/10 (0)	8/10 (80)	7/9 (78)	1/10 (10)
	Other (n = 17)	1/16 (6)	0/17 (0)	1/17 (6)	3/17 (18)	4/17 (24)	2/17 (12)	14/17 (82)	11/13 (85)	0/17 (0)
Enterobacter cloacae (n = 6)	bla_{OXA-48} (n = 3)	1/3 (33)	0/3 (0)	0/3 (0)	2/3 (67)	1/3 (33)	1/3 (33)	2/3 (67)	1/1 (100)	1/3 (33)
	bla_{NDM} (n = 1)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	NR	0/1 (0)
	$bla_{OXA-48\ and\ NDM}$ (n = 1)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	NR	0/1 (0)
	Other (n = 1)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)
Serratia marcescens (n = 2)	bla_{OXA-48} (n = 2)	1/2 (50)	NR	0/2 (0)	1/2 (50)	1/2 (50)	1/2 (50)	2/2 (100)	NR	1/2 (50)

> 80% susceptible
50-80% susceptible
30-49% susceptible
<30% susceptible

ERT, ertapenem; IMI, imipenem; MER, meropenem; GEN, gentamicin; AMI, amikacin; CIP, ciprofloxacin; TIG, tigecycline; SXT, trimethoprim-sulfamethoxazole; NR, no result/not reported

[1]n = isolates resulted as susceptible; N = total isolates where susceptibility testing result obtained

[2]US FDA (United States Food and Drug Administration) MIC breakpoints (susceptible ≤2 µg/mL; intermediate 4 µg/mL; resistant ≥8 µg/mL)

[3]Broth microdilution result interpreted as per CLSI (33rd ed.) MIC breakpoints (intermediate ≤2 µg/mL; resistant ≥4 µg/mL)

Figure 9: Heatmap depicting selected *in vitro* antibiotic susceptibility patterns of CRE species and associated carbapenemase-encoding genes in patients that died (n = 135)

As highlighted in the heatmap (Figure 9), the predominant antibiotic classes relative to the CRE species and carbapenemase-encoding gene(s) identified had attained poor *in vitro* susceptibility in patients that died. *Klebsiella pneumoniae* (94.1%; 127/135) harbouring lone $bla_{\text{OXA-48}}$ carbapenemase (56.3%; 76/135) was the rampant microbiological phenotype-genotype implicated in CRE-related mortality. Tigecycline and colistin (tested via broth microdilution) exhibited the most favourable antibiograms; this was followed by amikacin, although to a much lesser extent.

Discussion

The global spread of CRE-associated infections poses a substantial healthcare burden, with carbapenemase-encoding genes being the predominant driver of resistance. This study investigated the comparative pre- and post-COVID-19 prevalence of CREs in a quaternary public hospital in Durban, South Africa, providing insight into demographics, microbiological characteristics, antibiotic susceptibility profiles, molecular characterisation, and patient clinical outcomes. The phenotypic and genotypic paradigm shift ascertained relative to the multitude of aspects particularly analysed amid the COVID-19 time periods, represents, to date, a novel study not only for South Africa, but for the continent of Africa.

The overall CRE prevalence of 10.5% in this healthcare institution constituted 6.72% and 13.58% during the pre- and post-pandemic periods, respectively. This is strikingly alarming in relation to data from the Antimicrobial Testing Leadership and Surveillance (ATLAS) global surveillance programme that reported CRE detection rates in Latin America and Middle-East Africa to be between 4.7-5.7% in 2018 (before COVID-19), increasing only to 10.4-12.9% in 2022 (following COVID-19).[29] Additionally, a study by Sader et al comprising 64 medical centres worldwide, including Europe and Asia-Pacific regions, elicited a 4.1% CRE prevalence during 2020-2022,[30] which is less than one-third the prevalence in our findings (12.72%) for the same period.

Further to the SA government National State of Disaster declaration in respect of the COVID-19 pandemic onset, the CRE prevalence had declined slightly from 12.29% to 12.24% in the subsequent year, likely due to implementation of the various lockdown restrictions imposed. As supported by other studies, heightened IPC practices and more stringent isolation protocols entail other potential contributory factors for the reduced MDROs seen in some clinical settings

after COVID-19.[11,24,31] However, as evidenced by the continuous annual rise in prevalence by more than 2% thereafter, this initial trend in our study was unsustained – an outcome contrary to the verdicts by Linn et al in South-East Asian hospitals,[32] but in agreement with a Cape Town study where an almost 3-fold escalation in nosocomial infections occurred during the third COVID-19 wave.[33]

The median age of 16 years (IQR 0-39) for the total period differs greatly from other reports that included both paediatric and adult patients, where older populations were mainly affected by CRE infections.[34-37] The preponderance of males, in addition to intensive and high-care unit patient locations, are findings consistent with previous studies within the South African context.[7,37] The noteworthy reduction in CRE prevalence in neonates and infants following the pandemic may be parallel to a general observation in Hong Kong where diligent hand hygiene measures, in conjunction to other efforts, possibly mitigated drug-resistant bacterial burden.[11] Wielders et al indicated a median number of two isolates for patients having multiple CREs[38] – this similarity aligned with our study where the average number of isolates for the same cohort of individuals ranged between 2.76-2.93, overall.

Blood culture and urine encompassed the majority of sterile and non-sterile specimen types, respectively, regardless of the COVID-19 period, representing a finding synonymous with a vast expanse of national and global studies.[6,37,39-42] The increase in endotracheal aspirate and sputum samples – the former of which signifies the second commonest non-sterile specimen in this setting – is inferential of the post-pandemic surge in respiratory tract infections, conforming to the top two sample types amongst other research outputs.[43-47] *Klebsiella pneumoniae* accounted for the largest overall proportion (85.7%) of CRE species at IALCH, corresponding to the well-established dominance highlighted by various publications.[34-35,37,43,45-46,48-49] Moreover, its significant post-pandemic escalation synchronises with the results obtained by Jeon et al in four South Korean university hospitals during 2018-2021, denoting an important aspect that necessitates further monitoring.[50]

Amid the armamentarium of antibiotic classes tested for CREs, extensive *in vitro* resistance was exhibited. Barring carbapenems, this was most apparent for β-lactams (≥94%), followed by trimethoprim-sulfamethoxazole (≥89%) and ciprofloxacin (≥81%). The higher rate of resistance that prevailed after COVID-19 in the abovementioned instances may be a

consequence secondary to increased empiric antibiotic consumption[20] or enhanced surveillance and reporting systems during this period.[34] Similar to gentamicin, a converse association existed for amikacin and tigecycline, both of which, in accordance with findings from other studies,[21,34,37,45,47,51-52] recorded significantly lower degrees of resistance, thus reiterating the vital role attained by these agents for use in settings plagued by CRE treatment option limitations.

The revelation that 90.2% of CREs in this quaternary hospital were positive for carbapenemase production is not surprising, given the fact that CPE rates differ greatly (20.7-97.4%) depending on epidemiological characteristics, frequency of molecular detection, and other influential variables.[7,21,34,40,49,52-54] The unexpected drop in total CPEs following COVID-19, together with the ensuing genotypic shift from *bla*$_{\text{NDM}}$ to *bla*$_{\text{OXA-48}}$ in this patient population, provides insight into the evolving dynamics of CPE dissemination. Our post-pandemic findings, which benchmarks the current situation, is in keeping with recent data showcasing *bla*$_{\text{OXA-48}}$ as the commonest carbapenemase-encoding gene circulating in SA, followed by *bla*$_{\text{NDM}}$,[34,37] but diverges from prior research undertaken at IALCH where *bla*$_{\text{NDM}}$ remained the predominant carbapenemase (86%) during 2013-2017.[25] This is probably due to antibiotic selection pressure of widespread carbapenem use, efficient plasmid spread, and diagnostic limitations that may result in earlier regional endemicity from sustained silent transmission.[8,37]

Enterobacter cloacae complex followed the reign of *Klebsiella pneumoniae* amongst all CPEs, with an almost tripled proportion of *bla*$_{\text{OXA-48}}$ observed in the latter during COVID-19 onwards. The rise elicited in individual isolates harbouring ≥2 carbapenemases is a cause for grave concern due to the potential complications that may arise in managing such CRE infections.[34] *Klebsiella pneumoniae* strains containing dual *bla*$_{\text{NDM and OXA-48}}$ was chiefly implicated in our study, which, depending on specimen type and regional distribution, demonstrates variable agreement to other findings pertaining to CPE samples with combination carbapenemases.[21,34,37,43,52,55-56] As reported by Bedenic et al, this typifies the pathogenic fitness of *Klebsiella pneumoniae* and its ability for progressive acquisition and horizontal spread of numerous resistance determinants owing to selection pressure from broad-spectrum antibiotic usage in the COVID-19 era.[16]

Irrespective of the COVID-19 period, majority (42.2%) of all-cause mortality specific to CPE-infected patients occurred ≤7 days from sample collection. However, an even more worrisome finding was the overall 30-day mortality rate (77%) which was conspicuously higher than some international norms (24-49%), courtesy a publication by Oka et al.[53] Pre-pandemic CPE-associated mortality (37.5%), which primarily occurred in the under 1-year age category, escalated to 43.4% subsequent to COVID-19, with an evolution towards adults aged 20-59 years old, underscoring the possible role played by the myriad of pandemic-related factors in this transition of clinical outcomes.[11,21,56] In relation to the contrasting time periods, this was further emphasised by the switch in gender propensity, with a greater proportion of deaths attributed to females. Demographic temporal shifts, analogous to ours or otherwise, have also been elucidated amid the limited comparative studies of this nature that reported on patient survival,[21,57] some notwithstanding COVID-19.[7,51]

Klebsiella pneumoniae was responsible for the overwhelming volume of deaths, particularly in the changeover of carbapenemase-encoding genes from bla_{NDM} to $bla_{\text{OXA-48}}$. Importantly, this also included the emergence of isolates bearing other uncommon or multiple carbapenemases not previously described locally, as indicated in a post-pandemic report by Thomas et al.[56] Our analysis that sterile site specimens, including intensive and high-care unit patient locations, were key contributory factors that increased the odds of inpatient mortality, concurred with a multicentre study incorporating public and private hospitals in the Western Cape province.[37] This, in itself, though supported by additional data, pays tribute to the fact that no matter the healthcare sector type, the burden of CPEs amongst CREs in SA, is prominent.[7,33-34,40,46,51]

In patients that died, the marked proportion of reduced susceptibility (<30%) of the predominating carbapenemase-specific CRE species against the wide array of antibiotics tested, is undoubtedly concerning. Compounding this situation within this specialised referral hospital is the unavailability of the newer β-lactam/β-lactamase inhibitor (BL/BLI) drug combinations, such as ceftazidime-avibactam, including agents like cefiderocol as an alternative treatment option, and aztreonam for metallo-β-lactamases. This unfortunate circumstance was also contextualised in a study by So-ngern et al in a tertiary healthcare centre in North-East Thailand where it was similarly deduced that aminoglycosides, tigecycline and colistin may serve as viable therapeutic options.[47]

It must be noted, however, that according to scientific reviews[14,58] and both local and global best practice management guidelines for CRE infections,[3,59-60] the newer generation BL/BLI antibiotics are preferred. Selection depends on organism identification, site of infection, phenotypic antimicrobial susceptibility testing, and resistance mechanism determination, with genotypic characterisation for carbapenemase producers. Studies illustrating the excellent susceptibility of ceftazidime-avibactam (87-100%) against CRE strains positive for *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{KPC}}$ show promise for use in our setting, given that *bla*$_{\text{OXA-48}}$ denotes the most rampant carbapenemase circulating in IALCH and SA.[37,54]

As outlined by Finlayson et al, the unprecedented alteration in the landscape of antibiotic resistance underpins the imperative need for multisectoral, concerted approaches to streamline the accessibility and responsible use of the newer agents.[61] Consequently, our research findings will be presented in detail to the KZN DoH for their consideration to address this need with great urgency. The CRE trends within this clinical setting correlated to poor patient outcomes provides comprehensive baseline data to impart policy reforms that advocate for these newer, effective antibiotics.

Limitations

Owing to laboratory-dependent factors – such as staff awareness, pandemic-related shift constraints, pathologist decision, and reasons unknown – not every CRE isolate was stored. The resultant potential sampling bias may reflect an underestimation of true CRE burden during that particular period. Although the pre- and post-pandemic timeframes analysed comprised an unequal number of months, results were standardly depicted as a proportion of total CREs for that period, thereby allowing for accurate comparisons. The study location – being a single-centre quaternary hospital – may be representative of a sicker population, thereby preventing generalisation of the findings. Samples were submitted at the discretion of the treating physician, with data pertaining to differentiating clinical significance from site colonisation not always available. Similarly, depending on the comprehensiveness of clinical record documentation, it was not possible to attribute patient mortality solely to the microbiological result derived, as opposed to influence from confounders not examined. This study did not extensively correlate underlying comorbidities, risk factors for CRE infections, biochemical parameters, illness scoring systems, or pharmacological strategies employed when assessing clinical outcomes. Additionally, the distribution of antibiotic minimum inhibitory

concentration (MIC) trends over time, or the direct effect of IPC measures, were not evaluated. Further studies are therefore required to investigate the impact of the abovementioned aspects.

Conclusion

This novel study provides crucial insight into the comparative trend analysis of various aspects surrounding CRE infections relative to COVID-19 in a South African quaternary healthcare setting. The rising CRE burden, coupled by the clinical and molecular paradigm shift elicited within this referral public hospital, is unmistakable. The ascertainment of CRE-associated demographic, microbiological, and patient outcome data – in addition to delineating the pre- and post-pandemic distribution of carbapenemase-encoding genes – represents a first for this facility, thereby providing crucial baseline data in this regard. Our findings highlight the imperative need for ongoing epidemiological surveillance and advise conducting routine carbapenemase testing in selected CRE-infected patients – this impacts IPC strategies and influences treatment selection choices amid strengthening AMS protocols. However, of fundamental importance, this study advocates for the urgent necessity of multidisciplinary, collaborative efforts directed at promulgating accessibility to the newer BL/BLI drug combinations, such as ceftazidime-avibactam, including agents like aztreonam and cefiderocol, in providing directed therapy against CREs – such interventions epitomise the invaluable potential in significantly improving patient clinical outcomes.

Supporting information

Supplementary Table 1: Distribution of CREs according to type of specimen (n = 1,481)

Specimen type	Overall N = 1,481	pre-COVID-19 N = 425	post-COVID-19 N = 1,056	*p*-value[1]
	n (%)	n (%)	n (%)	
Sterile	**536 (36.2)**	**146 (34.3)**	**390 (36.9)**	**0.35**
Blood culture	344 (64.2)	82 (56.2)	262 (67.2)	0.018
Fluid/aspirate	150 (28.0)	51 (34.9)	99 (25.4)	0.028
Tissue	23 (4.3)	8 (5.5)	15 (3.8)	0.41
CSF	19 (3.5)	5 (3.4)	14 (3.6)	0.93
Non-sterile	**945 (63.8)**	**279 (65.7)**	**666 (63.1)**	**0.35**
Urine	392 (41.5)	125 (44.8)	267 (40.1)	0.18
ETA	289 (30.6)	74 (26.5)	215 (32.3)	0.080
IVC tip	112 (11.9)	29 (10.4)	83 (12.5)	0.37
Wound swab	77 (8.1)	24 (8.6)	53 (7.9)	0.74
Sputum/BAL	52 (5.5)	15 (5.4)	37 (5.6)	0.91
Other[2]	23 (2.4)	12 (4.3)	11 (1.6)	0.016

CSF, cerebrospinal fluid; ETA, endotracheal aspirate; IVC, intravascular catheter; BAL, bronchoalveolar lavage

[1]Pearson's Chi-squared test

[2]stool (n = 12), ventricular catheter tip (n = 11); overall period

Individual samples types are represented as a proportion of their corresponding sterile or non-sterile categorical total

Supplementary Table 2: Comparison of CRE species isolated (n = 1,481)

CRE species	Overall N = 1,481	pre-COVID-19 N = 425	post-COVID-19 N = 1,056	*p*-value[1]
	n (%)	n (%)	n (%)	
Klebsiella pneumoniae	1,269 (85.7)	340 (80.0)	929 (88.0)	<0.001
Enterobacter cloacae	95 (6.4)	35 (8.2)	60 (5.7)	0.070
Serratia marcescens	40 (2.7)	12 (2.8)	28 (2.6)	0.85
Escherichia coli	24 (1.6)	10 (2.4)	14 (1.3)	0.16
Other[2]	53 (3.6)	28 (6.6)	25 (2.4)	<0.001

[1]Pearson's Chi-squared test

[2]*Enterobacter* species, (n = 16); *Klebsiella* species, (n = 9); *Citrobacter* species, (n = 8); *Proteus* species, (n = 6); *Morganella* species, (n = 5); *Serratia* species, (n = 4); *Providencia* species, (n = 3); *Raoultella* species, (n = 1); *Salmonella* species, (n = 1)

Supplementary Table 3: Comparative resistance profile trends of CREs against various antibiotics tested *in vitro*

Antibiotic	pre-COVID-19	post-COVID-19	*p*-value[1]
	n/N (%)[2]	n/N (%)[2]	
AMC	400/424 (94.3%)	1,032/1,044 (98.9%)	<0.001
PTZ	392/414 (94.7%)	996/1,016 (98.0%)	<0.001
CTX	399/421 (94.8%)	1,012/1,032 (98.1%)	<0.001
CAZ	396/420 (94.3%)	974/992 (98.2%)	<0.001
ERT	139/211 (65.9%)	776/941 (82.5%)	<0.001
IMI	360/417 (86.3%)	850/1,029 (82.6%)	0.082
MER	387/425 (91.1%)	920/1,056 (87.1%)	0.033
GEN	349/416 (83.9%)	709/1,038 (68.3%)	<0.001
AMI	249/423 (58.9%)	342/1,042 (32.8%)	<0.001
CIP	344/425 (80.9%)	926/1,049 (88.3%)	<0.001
TIG	15/376 (4.0%)	100/979 (10.2%)	<0.001
SXT	376/423 (88.9%)	924/1,022 (90.4%)	0.38

AMC, amoxicillin-clavulanic acid; PTZ, piperacillin-tazobactam; CTX, ceftriaxone/cefotaxime; CAZ, ceftazidime; ERT, ertapenem; IMI, imipenem; MER, meropenem; GEN, gentamicin; AMI, amikacin; CIP, ciprofloxacin; TIG, tigecycline; SXT, trimethoprim-sulfamethoxazole

[1]Pearson's Chi-squared test

[2]n = isolates resulted as resistant; N = total isolates that obtained susceptibility testing results

Isolates with missing data, such as blank/no result available or where an organism was not tested against a particular antibiotic, were omitted from the denominator count

Acknowledgements

The authors are grateful to the Department of Medical Microbiology laboratory staff at Inkosi Albert Luthuli Central Hospital for storing of CRE isolates and the National Institute for Communicable Diseases for the provision of control strains. The authors also wish to thank the National Health Laboratory Service and study site for the data, Dr Ravesh Singh and Mr Diyothan Pillay for assistance with conducting molecular laboratory work, Mr Brian Brummer for aiding with statistical analyses and data visualisations, and Mr Malcolm Ellappen for guidance on the funding application process.

Author contributions

NB conceptualised the study, developed the methodology, wrote the protocol, submitted the applications for ethical approval and required consents, applied for funding, conducted the laboratory work, analysed the results, designed the visual representations, and wrote the original manuscript. KSSH was the project supervisor who evaluated and approved the proposed study idea, as well as reviewed and edited the protocol and manuscript. SM was the

co-supervisor who provided project design guidance, including appraisal and editing of the protocol and manuscript.

Financial disclosure

This project was supported by the National Health Laboratory Service K-Project Funding (K-Project Number PR2448733).

Availability of data and materials

Data is available upon reasonable request from the author, NB, following permission from the National Health Laboratory Service and study site, Inkosi Albert Luthuli Central Hospital.

Competing interests

The authors declare that no financial, professional, personal or other competing interests exist.

References

1. Cui X, Zhang H, Du H. Carbapenemases in Enterobacteriaceae: detection and antimicrobial therapy. Frontiers in microbiology. 2019 Aug 20;10:1823.

2. Gatya Al-Mayahie SM, Al-Guranie DR, Hussein AA, Bachai ZA. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic *Escherichia coli* phylogroup B2 isolates from outpatients in Wasit Province/Iraq. PLOS One. 2022 Jan 25;17(1):e0262984.

3. Brink AJ, Coetzee J, Richards GA, Feldman C, Lowman W, Tootla HD, Miller MG, Niehaus AJ, Wasserman S, Perovic O, Govind CN. Best practices: Appropriate use of the new β-lactam/β-lactamase inhibitor combinations, ceftazidime-avibactam and ceftolozane-tazobactam in South Africa. Southern African Journal of Infectious Diseases. 2022 Oct 20;37(1):453.

4. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases. 2017 Feb 15;215(suppl_1):S28-36.

5. Asokan GV, Ramadhan T, Ahmed E, Sanad H. WHO global priority pathogens list: a bibliometric analysis of Medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahrain. Oman medical journal. 2019 May;34(3):184.

6. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of clinical microbiology and antimicrobials. 2016 Dec;15(1):1-1.

7. Perovic O, Ismail H, Quan V, Bamford C, Nana T, Chibabhai V, Bhola P, Ramjathan P, Swe Swe-Han K, Wadula J, Whitelaw A. Carbapenem-resistant Enterobacteriaceae in patients with bacteraemia at tertiary hospitals in South Africa, 2015 to 2018. European Journal of Clinical Microbiology & Infectious Diseases. 2020 Jul;39:1287-94.

8. Madni O, Amoako DG, Abia AL, Rout J, Essack SY. Genomic investigation of carbapenem-resistant *Klebsiella pneumonia* colonization in an intensive care unit in South Africa. Genes. 2021 Jun 22;12(7):951.

9. Suay-García B, Pérez-Gracia MT. Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. Antibiotics. 2019 Aug 19;8(3):122.

10. Hansen GT. Continuous evolution: perspective on the epidemiology of carbapenemase resistance among Enterobacterales and other Gram-negative bacteria. Infectious Diseases and Therapy. 2021 Mar;10:75-92.

11. Wong SC, Chau PH, So SY, Chiu KH, Yuen LL, AuYeung CH, Lam GK, Chan VW, Chen JH, Chen H, Li X. Epidemiology of multidrug-resistant organisms before and during COVID-19 in Hong Kong. Infection Prevention in Practice. 2023 Jun 1;5(2):100286.

12. Rehman S. ReviewA parallel and silent emerging pandemic: Antimicrobial resistance (AMR) amid COVID-19 pandemic. Journal of Infection and Public Health. 2023 Feb 21.

13. Farfour E, Lecuru M, Dortet L, Le Guen M, Cerf C, Karnycheff F, Bonnin RA, Vasse M, Lesprit P. Carbapenemase-producing Enterobacterales outbreak: another dark side of COVID-19. American Journal of Infection Control. 2020 Dec 1;48(12):1533-6.

14. Coppola N, Maraolo AE, Onorato L, Scotto R, Calò F, Atripaldi L, Borrelli A, Corcione A, De Cristofaro MG, Durante-Mangoni E, Filippelli A. Epidemiology, Mechanisms of Resistance and Treatment Algorithm for Infections Due to Carbapenem-Resistant Gram-Negative Bacteria: An Expert Panel Opinion. Antibiotics. 2022 Sep 17;11(9):1263.

15. Ansari S, Hays JP, Kemp A, Okechukwu R, Murugaiyan J, Ekwanzala MD, Ruiz Alvarez MJ, Paul-Satyaseela M, Iwu CD, Balleste-Delpierre C, Septimus E. The potential impact of the COVID-19 pandemic on global antimicrobial and biocide resistance: an AMR Insights global perspective. JAC-antimicrobial resistance. 2021 Jun 1;3(2):dlab038.

16. Bedenić B, Luxner J, Car H, Sardelić S, Bogdan M, Varda-Brkić D, Šuto S, Grisold A, Beader N, Zarfel G. Emergence and Spread of Enterobacterales with Multiple Carbapenemases after COVID-19 Pandemic. Pathogens. 2023 May 3;12(5):677.

17. Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, Zhang W, Zhang Y, Shen A, Sun H, Li L. Global spread of carbapenem-resistant Enterobacteriaceae: epidemiological features, resistance mechanisms, detection and therapy. Microbiological Research. 2023 Jan 1;266:127249.

18. Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, Dever LL, Kreiswirth BN. Colistin-and carbapenem-resistant *Escherichia coli* harboring mcr-1 and bla NDM-5, causing a complicated urinary tract infection in a patient from the United States. mBio. 2016 Sep 7;7(4):e01191-16.

19. Yu H, González Molina MK, Carmona Cartaya Y, Hart Casares M, Aung MS, Kobayashi N, Quiñones Pérez D. Multicenter Study of Carbapenemase-Producing Enterobacterales in Havana, Cuba, 2016–2021. Antibiotics. 2022 Apr 12;11(4):514.

20. Shbaklo N, Corcione S, Vicentini C, Giordano S, Fiorentino D, Bianco G, Cattel F, Cavallo R, Zotti CM, De Rosa FG. An observational study of MDR hospital-acquired infections and antibiotic use during COVID-19 pandemic: a call for antimicrobial stewardship programs. Antibiotics. 2022 May 20;11(5):695.

21. Miftode IL, Leca D, Miftode RS, Roşu F, Plesca C, Loghin I, Timpau AS, Mitu I, Mititiuc I, Dorneanu O, Miftode E. The clash of the titans: COVID-19, carbapenem-resistant enterobacterales, and first mcr-1-mediated colistin resistance in humans in Romania. Antibiotics. 2023 Feb 3;12(2):324.

22. Montrucchio G, Corcione S, Sales G, Curtoni A, De Rosa FG, Brazzi L. Carbapenem-resistant *Klebsiella pneumoniae* in ICU-admitted COVID-19 patients: Keep an eye on the ball. Journal of Global Antimicrobial Resistance. 2020 Dec 1;23:398-400.

23. Howard-Jones AR, Sandaradura I, Robinson R, Orde SR, Iredell J, Ginn A, van Hal S, Branley J. Multidrug-resistant OXA-48/CTX-M-15 *Klebsiella pneumoniae* cluster in a COVID-19 intensive care unit: salient lessons for infection prevention and control during the COVID-19 pandemic. Journal of Hospital Infection. 2022 Aug 1;126:64-9.

24. Elliott TM, Hurst C, Doidge M, Hurst T, Harris PN, Gordon LG. Unexpected benefit of COVID-19 hospital restrictions: Reduction in patients isolating with multidrug resistant organisms after restrictions were lifted. Infection, Disease & Health. 2022 Feb 1;27(1):14.

25. Maphumulo SL, Mlisana K, Singh, R, Swe Swe-Han K. Laboratory Screening and prevalence of Carbapenemase Producing Enterobacteriaceae isolates from Inkosi Albert Luthuli Central Hospital (IALCH). Unpublished data. MMed dissertation. University of KwaZulu-Natal, 2019.

26. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.

27. Watahiki M, Kawahara R, Suzuki M, Aoki M, Uchida K, Matsumoto Y, Kumagai Y, Noda M, Masuda K, Fukuda C, Harada S. Single-tube multiplex polymerase chain reaction for

the detection of genes encoding Enterobacteriaceae carbapenemase. Japanese journal of infectious diseases. 2020;73(2):166-72.

28. Diaz T, Strong KL, Cao B, Guthold R, Moran AC, Moller AB, Requejo J, Sadana R, Thiyagarajan JA, Adebayo E, Akwara E. A call for standardised age-disaggregated health data. The lancet Healthy longevity. 2021 Jul 1;2(7):e436-43.

29. Wise MG, Karlowsky JA, Mohamed N, Hermsen ED, Kamat S, Townsend A, Brink A, Soriano A, Paterson DL, Moore LS, Sahm DF. Global trends in carbapenem-and difficult-to-treat-resistance among World Health Organization priority bacterial pathogens: ATLAS surveillance program 2018–2022. Journal of Global Antimicrobial Resistance. 2024 Jun 1;37:168-75.

30. Sader HS, Carvalhaes CG, Kimbrough JH, Mendes RE, Castanheira M. Activity of aztreonam-avibactam against Enterobacterales resistant to recently approved beta-lactamase inhibitor combinations collected in Europe, Latin America, and the Asia-Pacific Region (2020–2022). International Journal of Antimicrobial Agents. 2024 Apr 1;63(4):107113.

31. Kolbe-Busch S, Djouela Djoulako PD, Stingu CS. Trends in healthcare-acquired infections due to multidrug-resistant organisms at a German university medical center before and during the COVID-19 pandemic. Microorganisms. 2025 Jan 25;13(2):274.

32. Linn KZ, Sutjipto S, Ng OT, Teo J, Cherng BP, Tan TY, Pada SK, Ooi ST, Smitasin N, Thoon KC, Huan X. Impact of COVID-19 pandemic on carbapenem-resistant Enterobacterales incidence in the South-East Asia region: an observational study. Antimicrobial Stewardship & Healthcare Epidemiology. 2023 Jan;3(1):e208.

33. Mashigo B, Parker A, Lalla U, Allwood BW, Moolla MS, Lovelock T, Koegelenberg CF. An outbreak within an outbreak: The impact of infection prevention and control strategies on hospital-acquired infections and the occurrence of multidrug-resistant organisms during the COVID-19 pandemic. South African Medical Journal. 2023 Dec 1;113(12):1544-7.

34. Ismail H, Zwane TB, Du Toit E, da Costa RM, Franceschi F, Perovic O. Carbapenem-resistant Enterobacterales among patients with bloodstream infections in South Africa: Consolidated surveillance data, 2015–2021. PLOS One. 2025 Jul 2;20(7):e0324262.

35. Moloto K, Newton-Foot M, Whitelaw A, Dramowski A. Emergence and expansion of carbapenem resistant enterobacterales in the Western Cape Province, South Africa. PLOS One. 2024 Aug 26;19(8):e0309315.

36. Shibabaw A, Sahle Z, Metaferia Y, Atlaw A, Adenew B, Gedefie A, Tilahun M, Ebrahim E, Kassa Y, Debash H, Wang SH. Epidemiology and prevention of hospital-acquired carbapenem-resistant Enterobacterales infection in hospitalized patients, Northeast Ethiopia. IJID regions. 2023 Jun 1;7:77-83.

37. Tootla HD, Prentice E, Moodley C, Marais G, Nyakutira N, Reddy K, Bamford C, Niehaus A, Whitelaw A, Brink A. Carbapenem-resistant enterobacterales among hospitalized patients in Cape Town, South Africa: clinical and microbiological epidemiology. JAC-Antimicrobial Resistance. 2024 Apr;6(2):dlae051.

38. Wielders CC, Schouls LM, Woudt SH, Notermans DW, Hendrickx AP, Bakker J, Kuijper EJ, Schoffelen AF, de Greeff SC. Epidemiology of carbapenem-resistant and carbapenemase-producing Enterobacterales in the Netherlands 2017–2019. Antimicrobial Resistance & Infection Control. 2022 Apr 9;11(1):57.

39. Greybe L, Nuttall JJ, Brink AJ, Tootla HD. Descriptive analysis of infections due to New Delhi metallo-β-lactamase-producing Enterobacterales in children at Red Cross War Memorial Children's Hospital. Southern African Journal of Infectious Diseases. 2022 Jul 27;37(1):440.

40. Perovic O, Britz E, Chetty V, Singh-Moodley A. Molecular detection of carbapenemase-producing genes in referral Enterobacteriaceae in South Africa: a short report: clinical update. South African Medical Journal. 2016 Oct 1;106(10):975-7.

41. Zhou R, Fang X, Zhang J, Zheng X, Shangguan S, Chen S, Shen Y, Liu Z, Li J, Zhang R, Shen J. Impact of carbapenem resistance on mortality in patients infected with Enterobacteriaceae: a systematic review and meta-analysis. BMJ open. 2021 Dec 1;11(12):e054971.

42. Yu H, Gonzalez Molina MK, Carmona Cartaya Y, Hart Casares M, Aung MS, Kobayashi N, Quiñones Pérez D. Multicenter Study of Carbapenemase-Producing Enterobacterales in Havana, Cuba, 2016–2021. Antibiotics. 2022 Apr 12;11(4):514.

43. Abdelaziz NA. Phenotype-genotype correlations among carbapenem-resistant Enterobacterales recovered from four Egyptian hospitals with the report of SPM carbapenemase. Antimicrobial Resistance & Infection Control. 2022 Jan 21;11(1):13.

44. Fisher M, Komarow L, Kahn J, Patel G, Revolinski S, Huskins WC, van Duin D, Banerjee R, Fries BC. Carbapenem-resistant Enterobacterales in children at 18 US health care system study sites: Clinical and molecular epidemiology from a prospective multicenter cohort study. Open Forum Infectious Diseases. 2024 Feb (Vol. 11, No. 2, p. ofad688). US: Oxford University Press.

45. Jing N, Yan W, Zhang Q, Yuan Y, Wei X, Zhao W, Guo S, Guo L, Gao Y, Zhao L, Shi C. Epidemiology and genotypic characteristics of carbapenem resistant Enterobacterales in Henan, China: A multicentre study. Journal of Global Antimicrobial Resistance. 2022 Jun 1;29:68-73.

46. Mzimela BW, Nkwanyana NM, Singh R. Clinical outcome of neonates with Carbapenem-resistant Enterobacteriaceae infections at the King Edward VIII Hospital's neonatal unit, Durban, South Africa. Southern African Journal of Infectious Diseases. 2021 Jan 21;36(1):223.

47. So-Ngern A, Osaithai N, Meesing A, Chumpangern W. Mortality rate and factors associated with mortality of carbapenem-resistant Enterobacteriaceae infection. Drug Target Insights. 2023 Oct 27;17:120.

48. Avcu G, Erci E, Bilen NM, Ersayoglu I, Ozek G, Celtik U, Terek D, Cilli F, Bal ZS. Clinical outcomes and the impact of treatment modalities in children with carbapenem-resistant Enterobacteriaceae bloodstream infections: a retrospective cohort study from a tertiary university hospital. Journal of Antimicrobial Chemotherapy. 2025 Jan;80(1):147-53.

49. Kedišaletše M, Phumuzile D, Angela D, Andrew W, Mae NF. Epidemiology, risk factors, and clinical outcomes of carbapenem-resistant enterobacterales in Africa: a systematic review. Journal of Global Antimicrobial Resistance. 2023 Dec 1;35:297-306.

50. Jeon K, Jeong S, Lee N, Park MJ, Song W, Kim HS, Kim HS, Kim JS. Impact of COVID-19 on antimicrobial consumption and spread of multidrug-resistance in bacterial infections. Antibiotics. 2022 Apr 18;11(4):535.

51. Lowe M, Shuping L, Perovic O. Carbapenem-resistant Enterobacterales in patients with bacteraemia at tertiary academic hospitals in South Africa, 2019-2020: An update. South African Medical Journal. 2022 Aug 1;112(8):55-62.

52. Dilagui I, Loqman S, Hanchi AL, Soraa N. Antibiotic resistance patterns of carbapenemase-producing Enterobacterales in Mohammed VI University Hospital of Marrakech, Morocco. Infectious Diseases Now. 2022 Sep 1;52(6):334-40.

53. Oka K, Matsumoto A, Tetsuka N, Morioka H, Iguchi M, Ishiguro N, Nagamori T, Takahashi S, Saito N, Tokuda K, Igari H. Clinical characteristics and treatment outcomes of carbapenem-resistant Enterobacterales infections in Japan. Journal of Global Antimicrobial Resistance. 2022 Jun 1;29:247-52.

54. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, Zheng Y, Guo Y, Zhang R, Hu F, China Antimicrobial Surveillance Network (CHINET) Study Group. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant Enterobacteriaceae isolated from adult and children patients in China. Frontiers in cellular and infection microbiology. 2020 Jul 3;10:314.

55. Pascale R, Bussini L, Gaibani P, Bovo F, Fornaro G, Lombardo D, Ambretti S, Pensalfine G, Appolloni L, Bartoletti M, Tedeschi S. Carbapenem-resistant bacteria in an intensive care unit during the coronavirus disease 2019 (COVID-19) pandemic: a multicenter before-and-after cross-sectional study. Infection Control & Hospital Epidemiology. 2022 Apr;43(4):461-6.

56. Thomas GR, Corso A, Pasterán F, Shal J, Sosa A, Pillonetto M, de Souza Peral RT, Hormazábal JC, Araya P, Saavedra SY, Ovalle MV. Increased detection of carbapenemase-producing Enterobacterales bacteria in Latin America and the Caribbean during the COVID-19 pandemic. Emerging infectious diseases. 2022 Nov;28(11):e220415.

57. Sahebi L, Dalili H, Shariat M, Kosari NS, Omidi Z. Impact of the COVID-19 pandemic on neonatal sepsis: epidemiological shifts, antibiotic resistance patterns, and pathogen profiles in a tertiary referral hospital. Annals of Clinical Microbiology and Antimicrobials. 2025 Aug 31;24(1):49.

58. Cruz-Lopez F, Martinez-Melendez A, Morfin-Otero R, Rodriguez-Noriega E, Maldonado-Garza HJ, Garza-Gonzalez E. Efficacy and in vitro activity of novel antibiotics for infections with carbapenem-resistant gram-negative pathogens. Frontiers in cellular and infection microbiology. 2022 May 20;12:884365.

59. Tamma PD, Hsu AJ. Defining the role of novel β-lactam agents that target carbapenem-resistant gram-negative organisms. Journal of the Pediatric Infectious Diseases Society. 2019 Jul;8(3):251-60.

60. Tamma PD, Heil EL, Justo JA, Mathers AJ, Satlin MJ, Bonomo RA. Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections. Clinical infectious diseases. 2024 Aug 7:ciae403.

61. Finlayson H, Chibabhai V, Jeena P, Kolman S, Lowman W, Manzini TC, Nana T, Nuttall J, Parker A, Skosana P, Ueckermann V. The changing landscape of antimicrobial resistance and use in South Africa: The need for access to new antibiotics: A position paper. South African Medical Journal. 2024 Oct 1;114(10):26-32.

Appendices

Appendix A - Protocol

University of KwaZulu-Natal

School of Laboratory Medicine and Medical Science

Department of Medical Microbiology

Master of Medicine (MMed) in Medical Microbiology

Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19

Principal investigator : Nitesh Brijlal

Student number: 207500990

Supervisor: Dr K Swe Swe/Han

Co-Supervisor: Dr Sandra Maphumulo

June 2023

Contents

1. Abbreviations

AMR: Antimicrobial resistance

AMS: Antimicrobial stewardship

β: Beta

BL/BLI: β-lactam/β-lactamase inhibitor

bla: Beta-lactamase

CLSI: Clinical and Laboratory Standards Institute

CPE: Carbapenemase-producing Enterobacterales

CRE: Carbapenem-resistant Enterobacterales

DTR-GNB: Difficult-to-treat resistant gram-negative bacteria

ECDC: European Centre for Disease Prevention and Control

HAI: Hospital-acquired infection

IALCH: Inkosi Albert Luthuli Central Hospital

ICU: Intensive care unit

IMI: Active-on-imipenem/Imipenemase

IMP: Imipenem metallo-β-lactamase

IPC: Infection prevention and control

GES: Guiana extended-spectrum β-lactamase

KPC: *Klebsiella pneumoniae* carbapenemase

KZN: KwaZulu-Natal

MBL: Metallo-β-lactamase

MDRO: Multidrug-resistant organism

MGE: Mobile genetic elements

NDM: New Delhi metallo-β-lactamase

OXA: Oxa-type carbapenemase/Oxacillinase

PBP: Penicillin binding protein

PPE: Personal protective equipment

SA: South Africa

SME: *Serratia marcescens* enzyme

SOP: Standard operating procedure

VIM: Verona integron-encoded metallo-β-lactamase

WHO: World Health Organization

2. Background

Carbapenems are often considered one of the most effective and reliable last-resort antibiotics in the treatment of many drug-resistant organisms.[1-3] Their widespread use, however, has resulted in the rapidly increasing occurrence and distribution of difficult-to-treat resistant gram-negative bacteria (DTR-GNBs), such as carbapenem-resistant Enterobacterales (CREs), worldwide.[1-2,4] Infections caused by CREs pose a serious public health threat and are associated with significant patient morbidity and mortality.[5] A matter of extreme concern for more than the past decade, they have been categorised as "critical" according to the first published global priority pathogen list in 2017 by the World Health Organization (WHO).[6,7]

Enterobacterales are among the commonest gram-negative bacteria isolated from clinical specimens and cause both community- and hospital-acquired infections, often associated with poor clinical outcomes.[8-9] The production of beta(β)-lactamase (*bla*) enzymes called carbapenemases is the most frequently mediated mechanism by which Enterobacterales develop carbapenem resistance.[2,8,10-11] Carbapenemases belong to the Ambler structural classification, being serine β-lactamases (Class A and D) and metallo-β-lactamases (Class B), which differ in their response to antimicrobial agents, impacting selection choices in patient management.[5,10,12]

The global prevalence of CRE infection, faecal carriage, and outbreaks over time is well-described.[5,13-14,15] From a South African context, studies in tertiary hospitals revealed the commonest carbapenemase genes in patients with bacteraemia to be $bla_{\text{OXA-48-like}}$ (Oxa-type carbapenemase/oxacillinase-48-like) and bla_{NDM} (New Delhi metallo-β-lactamase),[8-9] while screening and genomic investigation at a public hospital in the uMgungundlovu District found $bla_{\text{OXA-48}}$-producing *Klebsiella pneumoniae* endemic in its intensive care unit (ICU), colonising the patients.[10]

The landscape of antimicrobial resistance (AMR) was greatly challenged during the outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), owing to the worldwide mass consumption of broad-spectrum antibiotics.[16-18] Whatever the rationale for use – be it empirically or directed therapy for suspected or confirmed bacterial co-infection – this practise had mostly elevated the prevalence of multidrug-resistant organisms (MDROs), including CRE colonisation and infections

harbouring multiple carbapenemase-encoding genes.[6,17-20] However, some studies report a reduced MDRO prevalence during the pandemic due to the strict implementation of policies related to hand hygiene, personal protective equipment (PPE) use, isolation precautions, and heightened infection control awareness.[21-22] An epidemiologic analysis of CRE infections and outcomes comparing pre- and post-COVID-19 data in a KwaZulu-Natal (KZN) healthcare setting has, to date, not been conducted.

Since colonisation with resistant strains often precedes infection, patients with asymptomatic gastrointestinal carriage of carbapenemase-producing Enterobacterales (CPEs) attain a significantly higher risk of developing infections from these pathogens.[15,22] Upon concurrent carbapenem use, a four-fold increased risk for bacteraemia exists, highlighting the importance of CPE/CRE surveillance and its beneficial implications in directing antimicrobial stewardship (AMS) and infection prevention and control (IPC) strategies.[1]

The development of therapeutic agents such as the new β-lactam/β-lactamase inhibitor (BL/BLI) combinations and cefiderocol have afforded much relief in providing combative strategies to treat DTR-GNBs, including CREs.[1,3-4,13] However, drug selection is influenced by knowledge of the carbapenemase gene(s) present (if any), as well as accessibility to these newer antibiotics.[1,3-4,13]

3. Problem statement

Antimicrobial resistance to carbapenem antibiotics represents a substantial burden to health care, contributing greatly to patient morbidity and mortality.[5,15] The global epidemiology of CRE-related infections, gut colonisation, molecular characterisation of carbapenemase genes, including patient clinical outcomes, is well-documented.[5,13-15] Surveillance of high-risk patients for CRE carriage is imperative to facilitate the timely implementation of appropriate protective measures, thereby reducing spread of bacterial resistance.[1,10,15] Although many recent studies have reported a substantial increase in MDROs, including CREs, following mass usage of antibiotics during the COVID-19 pandemic,[6,17-20] some research demonstrate the opposite effect owing to the practise of rigid IPC protocols.[21-22]

Previous CRE data in SA studies have isolated the carbapenemases *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{OXA-181}}$ or *bla*$_{\text{NDM}}$ in either retrospective or screening studies.[8-9,23-24] Maphumulo *et al.* demonstrated a

prevalence of *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$ carbapenemase genes in a retrospective study comprising 194 clinical CRE isolates from Inkosi Albert Luthuli Central Hospital (IALCH) during 2013 to 2017, which was prior to COVID-19.[25] However, whether the paradigm has now shifted to encompass other or multiple carbapenemase-encoding genes in this adult and paediatric population, especially following amplified antibiotic prescribing practises during the COVID-19 pandemic, remains unknown.

To date, from a South African public health sector perspective in the KZN academic complex, an analysis of CRE infection demographics and the correlation of its management to patient clinical outcomes, is lacking. Additionally, optimal management of patients with CRE colonisation or infection is greatly hindered by the absence of standard operating procedures (SOPs) to (1) ascertain CRE colonisation in high risk patients via surveillance, and (2) routinely identify carbapenemase genes from clinical CRE specimens. The paucity of the above data at IALCH, a quaternary referral health facility in Durban, KZN, impedes the timely adjustment of empirical treatment and implementation of swift IPC measures as per colonisation status, as well as advocating for the currently unavailable new therapeutic agents within this clinical setting.

4. Research question

How have antibiotic prescribing practices during the COVID-19 pandemic influenced the prevalence, molecular characterisation, and patient clinical outcomes of CRE infection at a quaternary public hospital in KZN, South Africa?

5. Study rationale

There is a need to quantify the local prevalence of CREs, in conjunction to correlating patient risk factors and treatment strategies to different clinical outcomes. Molecular characterisation will provide valuable information into the resistance mechanisms (being a CPE or not), including identification of the carbapenemase gene(s) present in our clinical setting. Ascertaining such data will strengthen IPC interventions directed at curtailing the spread of drug-resistant organisms whilst simultaneously aiding in the selection of appropriate directed therapy, where available.

6. Study impact/clinical relevance

This novel study in our setting will afford insight into the pre- and post-COVID-19 demographic profile of patients at IALCH who are infected with CREs, including their clinical outcome. Organism profiles will be described and carbapenemase genes identified, so as to delineate the molecular characteristics of currently circulating carbapenemases in this health institution. In essence, scientific evidence will be provided to determine the epidemiology and distribution of CPEs at IALCH. The study results will also serve as a baseline in providing recommendations on the need for establishing SOPs for introducing a rapid test for carbapenemase identification, or initiating the practice of performing routine CRE surveillance in select patients. In addition to building a repository of CRE isolates for use during further research purposes, the study will, rather importantly, inform antimicrobial prescribing practises and assist in advocating for the new appropriate drugs that target CREs, which are currently unavailable in the KZN public health sector.

7. Aim

To determine the prevalence, carbapenemase genes, and clinical outcome of CRE-associated infection in a quaternary public sector hospital in KZN, South Africa, both prior to and following the COVID-19 pandemic.

8. Objectives

8.1 To determine the prevalence of CRE infections at IALCH, both prior to and following the COVID-19 pandemic onset.

8.2 To describe the demographic characteristics and clinical outcome of patients in whom CREs are isolated, inclusive of any specimen site.

8.3 To analyse the antimicrobial susceptibility profiles and molecularly identify the carbapenemase gene(s) present (if any) from both stored and prospectively-collected CRE isolates.

8.4 To utilise the study findings to advocate for the availability of novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations effective against CREs in this clinical setting.

9. Literature review

9.1 Search strategy

The following electronic search engines were used for identification of relevant literature:

- EBSCOhost
- PubMed
- Google Scholar
- Science Direct
- Frontiers
- Nature
- BioMed Central (BMC)

Full-text English articles published within the past 5 years were filtered for inclusion. The following keywords were utilised:

- Carbapenem-resistant Enterobacterales (CRE)
- Carbapenemase-producing Enterobacterales (CPE)
- COVID-19
- Carbapenemase genes
- Colonisation and surveillance
- β-lactam/β-lactamase inhibitor combination (BL/BLI)

9.2 Introduction

Enterobacterales are gram-negative bacteria that colonise the human intestinal tract.[26-27] They are currently recognised as the leading cause of serious community-acquired and nosocomial infections, and are among the most frequently isolated bacteria from clinical specimens.[8,26] These microorganisms are responsible for a broad range of clinical conditions; such as bacteraemia, pneumonia, meningitis, as well as intra-abdominal, skin and soft tissue, and urinary tract infections.[26-27]

Carbapenems (i.e. doripenem, ertapenem, imipenem and meropenem) are a group of potent, broad-spectrum β-lactam antibiotics commonly utilised to treat MDROs or DTR-GNBs, except CREs.[2-3,9] Its widespread use, however, has given rise to the emergence and increased spread of carbapenem-resistant Enterobacterales (CREs) – a serious and significant concern for healthcare systems worldwide, with adverse impacts in medical expenditure, human and economic loss.[9,26-27] Predominant risk factors for CRE infections include recent exposure to healthcare facilities or broad-spectrum antibiotics, extensive invasive procedures with medical device usage, severe comorbid conditions, among others.[8,23]

9.3 Carbapenem resistance in Enterobacterales

According to the Clinical and Laboratory Institute (CLSI) guidelines (33rd edition), a CRE is defined as a bacterium belonging to the order *Enterobacterales* that is resistant to one or more carbapenems.[9,28] Mechanisms of such resistance entail efflux pump overactivity, porin loss, altered target penicillin-binding proteins (PBPs), and most commonly, production of carbapenemases – β-lactamase enzymes that hydrolyse a broad range of β-lactam antibiotics, rendering them ineffective.[2,10] CREs rank among the top three on the World Health Organization (WHO) global priority pathogen list, highlighting their importance from a public health perspective.[29,43]

9.4 Carbapenemases and their therapeutic implications

Nomenclature for classifying β-lactamases can be based on amino acid sequence (structural) – defined as the Ambler classification – which comprises 4 major classes (A-D), or based on functional characteristics – the Bush-Jacoby-Medeiros classification system.[5,10] Carbapenemases belong to the Ambler class A [e.g. *Klebsiella pneumoniae* carbapenemase (KPC), *Serratia marcescens* enzyme (SME), active-on-imipenem/Imipenemase (IMI), and Guiana extended-spectrum β-lactamase (GES)]; class B [e.g. New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), and Imipenem metallo-β-lactamase (IMP); and class D [e.g. Oxa-type carbapenemase/Oxacillinase (OXA)].[10] Class A and D are serine β-lactamases, while class B are metallo-β-lactamases (MBLs).[10] Since the resistance profile to β-lactam agents differs among the carbapenemase classes, identification and knowledge of the carbapenemase(s) present in clinical settings is fundamental to selecting appropriate antibiotic regimens.[3,5,10,29]

Carbapenemase-encoding genes are located on mobile genetic elements (MGEs), including but not limited to transposons, integrons, and plasmids.[10] Acquisition of this can occur via horizontal gene transfer, thus facilitating their dissemination within and between different bacteria.[30] Carbapenemase-producing bacterial strains are also often resistant to multiple drugs and attain a high ability to spread in healthcare settings, directly affecting IPC measures.[2,29] With limited treatment options, particularly in resource-deprived settings, morbidity and mortality rates of CRE-associated infections have significantly escalated, supporting the urgent need and accessibility of new appropriate therapeutic agents [e.g. the new β-lactam/β-lactamase inhibitor (BL/BLI) combinations].[4,8,26,31]

9.5 Epidemiological burden of CPE and CRE infections

Although the different carbapenemases were previously typically associated with specific countries or regions, the geographical distributions are increasingly converging.[14] Such evolving epidemiology attains the potential to progressively complicate management, including selection choices of the novel BL/BLIs.[14]

9.5.1 Global

KPC is the most commonly encountered CRE worldwide, primarily due to clonal expansion of *K. pneumoniae* strains, and is responsible for a dramatic increase in disease burden and major outbreaks.[5,14] In the United States, CRE infections result in an estimated 26% mortality, coupled with exceedingly high hospital costs.[5,32] Other KPC-endemic regions include Greece, Israel and Latin America.[5,33] IMP-type MBL-containing Enterobacterales are endemic only in Japan and Taiwan, with sporadic outbreaks or single reports described in other countries.[5] Currently, VIM-2 is the commonest VIM-type MBL worldwide, with at least 46 bla_{VIM} variants now catalogued.[5] Following its discovery in 2008, there has been a global dissemination of NDM-type MBLs with rapid gene transfer between species, including their spread via environmental sources in community settings of lower-income countries.[5] Regarding the class D oxacillinase-type carbapenem-hydrolysing β-lactamases, the $bla_{\text{OXA-48}}$ element was discovered in Turkey in 2001, with OXA-producing bacteria now endemic in that country.[5,26] In the Indian subcontinent, however, $bla_{\text{OXA-181}}$ is predominant.[5]

In terms of demographics, CRE prevalence in Europe occurs mostly in the 19-64 year age group, affecting more males than females.[34] The European Centre for Disease Prevention and Control (ECDC) documented in 2019 that 43% of countries reported regional or inter-regional spread of CREs, a large population of which were invasive CRE *E. coli* infections.[13] According to a study by Han *et al.* in 2020, CPE in paediatric patients was dominated by NDM, followed by KPC.[34-35] There was also a notable absence of multi-enzyme genes in this population – a comparison that significantly differed from adults.[34-35]

The WHO 2021 Global Antimicrobial Resistance and Use Surveillance System (GLASS) study showed that, among African countries, Egypt, Uganda and Madagascar attain prominently high resistance rates to carbapenems.[34,36] While the prevalence of CRE in China has increased in recent years, CRE dissemination in Southeast Asian countries were much lower.[31,34] A study in Thailand encompassing CRE strains from urine, sputum and blood revealed 80% of samples

to be carbapenemase-producers, with 17% producing more than one enzyme.[34] Similarly, a phenotype-genotype correlation among CREs received from four Egyptian hospitals elicited the co-harbouring of carbapenemases in over half the isolates.[37]

A surveillance study from the ATLAS programme (2018-2020) describing CRE distribution in Africa and the Middle East identified NDM-1 as the commonest MBL.[38] During the same time period, over 80% of CREs cultured in a cross-sectional prospective study in a Moroccan hospital were carbapenemase-producers, majority of which had been isolated from the neonatal unit.[27] In Northeast Ethiopia, CREs were predominantly found to be due to hospital-acquired infections (HAIs), with *Escherichia coli* and *Klebsiella pneumoniae* accounting for the commonest microorganisms. [39]

9.5.2 SA and KZN

In the limited studies evaluating CRE infection, screening and carbapenemase gene characterisation in South Africa (SA), Perovic *et al.* demonstrated the commonest carbapenemases to be $bla_{\text{OXA-48}}$ (52%) and bla_{NDM} (32%) in hospital-acquired CRE bacteraemia patients during 2015-2020.[8-9] Of all the countries included in the ATLAS programme study, VIM-1 (5 isolates in total) was only detected in SA.[38] A point prevalence study in 2019 in Western Cape's Tygerberg Hospital assessing CPE colonisation revealed only 12 samples (2.73%) to be carbapenemase-producers, concluding not to recommend routine screening at the time.[23] An increase in isolation of NDM-producing CRE (26 isolates) was described among 22 children in a retrospective descriptive analysis at the Red Cross War Memorial Children's Hospital in 2022, with *Klebsiella pneumoniae* and *Serratia marcescens* being the only bacteria implicated.[24]

In the uMgungundlovu District of KZN, a prospective screening study using rectal swabs showed a 14.4% prevalence of CPEs among ICU patients receiving carbapenems, with $bla_{\text{OXA-181}}$ being endemic to that setting.[10] A retrospective study evaluating CREs in the neonatal unit of King Edward VIII hospital in Durban established 32 cases over a 2-year period – the prevalence of which was 5 per 1000 admissions, with majority of patients having been exposed to prior antimicrobials for approximately 4 days.[40]

9.6 CRE and COVID-19

It has been proposed that COVID-19 may accelerate CPE spread via viral promotion of bacterial attachment and respiratory tract colonisation, leading to increased rates of CRE infection.[34] Additionally, high-risk patients with COVID-19 and CRE dual infections may attain a poorer prognosis and increased mortality.[34] Data from six countries revealed KPC, OXA-48 and NDM to be the dominant genes during such infections, comprising predominantly of respiratory and bloodstream specimen types.[34,41] According to the ECDC, the COVID-19 pandemic has impacted negatively on the implementation of antimicrobial stewardship programmes, escalating the prevalence of MDROs secondary to greater antimicrobial use (and misuse).[13] In a multicentre study conducted in Croatia during COVID-19, 80% of CRE isolates were found to harbour multiple carbapenemases, demonstrating the ability of microorganisms to acquire various resistance determinants under the selection pressure of antibiotics widely used in the pandemic.[6] Although COVID-19 has been associated with an increased incidence in hospital-acquired resistant bacterial co-infections, [19-20,42] some global data depict that prolonged PPE use and well-enforced IPC policies have been beneficial in minimising the spread of drug resistant pathogens in some healthcare environments.[21-22]

9.7 Surveillance and controlling CRE spread in healthcare facilities

In order to curtail the spread of CRE within health institutions, interventions comprising bundled IPC measures are often employed; such as patient isolation or cohorting, contact precautions, education and training of staff, improving antimicrobial stewardship, limiting invasive device usage, and enhancing environmental cleaning.[5] Regional surveillance programmes and policies in high-prevalence areas can also allow for the early identification of CRE carriage in high-risk patients.[5] Together with molecular characterisation and epidemiological studies, the subsequent implementation of an appropriate public health response can be initiated.[5]

9.8 Conclusion

With the ever-increasing global dispersal of CREs, it is imperative that our understanding also keep abreast of its epidemiology, resistance mechanisms, clinical outcomes, and timely therapeutic and preventative approaches.[5,12] Determining the local prevalence, especially following escalated antimicrobial usage in COVID-19, will provide clinically relevant data to guide future CRE diagnostic, surveillance and management strategies.[16,19]

10. Methodology

10.1 Study design, type and method

- Retrospective, descriptive, quantitative, single-centre chart review of electronically-stored microbiological and patient clinical data/records.
- Prospective, laboratory-based, molecular characterisation of carbapenemase genes using:
  (i) stored CRE clinical isolates in the IALCH Department of Medical Microbiology from 2018, and
  (ii) prospectively collected CRE isolates till 31 May 2023.

10.2 Study location/site

- Inkosi Albert Luthuli Central Hospital (IALCH) – a quaternary public sector hospital in Durban, KwaZulu-Natal, South Africa.

10.3 Study period

- For the retrospective chart review, the pre-COVID-19 study period will be defined from 01 January 2018 to 05 March 2020 (first COVID-19 case in SA). The period onwards thereafter till 31 May 2023 will resemble the COVID-19/post COVID-19 period.
- Carbapenemase molecular characterisation will be done on all stored CRE isolates from the Medical Microbiology department for the period 01 January 2018 to 31 May 2023.

10.4 Study population and sampling strategy

- The study will comprise analysing data from both the adult and paediatric patients with CRE infections.

10.4.1 Inclusion criteria

- Only isolates or clinical specimens of CREs, as defined by the Clinical and Laboratory Standards Institute (CLSI) interpretative criteria for minimum inhibitory concentration (MIC) breakpoints for *Enterobacterales* against carbapenems, will be included.
- Continuous, de-duplicated patient data will be used (a CRE infection obtained after 21 days of the last CRE infection for the same patient will be regarded as a new CRE infection).

10.4.2 Exclusion criteria

- Patient data that is not attributed to CRE infections.

- Microbiological isolates with no resistance to any carbapenem antibiotic (including intermediate susceptibility).

10.5 Sample size

- All retrospectively stored and prospectively collected clinical isolates within the study period will be used.
- The estimated sample size will be a minimum of 100 CRE isolates/specimens.

10.6 Data collection and tools

- Retrospective microbiological and patient clinical data will be collected from the hospital's database (MediTech) and from the National Health Laboratory Service (NHLS) Laboratory Interface System (LIS) database (LabTrak/TrakCare).
- This will be transferred onto a data collection sheet *(Appendix A)* for analysis, which will be secured by being a password-protected document that is accessible only by myself or my research supervisors.
- Patient confidentiality will be maintained by using only clinical specimen or stored isolate episode numbers – these are devoid of patient name or patient identifiers.
- Collected data will include patient demographics, microorganism identification, antibiotic susceptibility profiles, and clinical outcome.

10.7 Laboratory methods

- Prospectively collected clinical specimens will be identified using either the VITEK 2® (bioMérieux, Marcy-l'Étoile, France) automated system, the VITEK® MS PRIME MALDI-TOF [Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (bioMérieux, Marcy-l'Étoile, France)] automated system, or a combination of the above.
- Antimicrobial susceptibility testing to determine the MIC will be done either via Kirby-Bauer disk diffusion, the VITEK 2® (bioMérieux, Marcy-l'Étoile, France) automated system, an Epsilometer test (E-test), or a combination of the above.
- The identification of carbapenemase genes (i.e. molecular characterisation) will be done either via singleplex or multiplex real-time reverse transcription polymerase chain reaction (RT-PCR), or an immunochromatographic method using a commercial lateral flow assay (LFA) kit as per the manufacturer's guidelines.

11. Statistical planning and analysis

Data will be entered in Microsoft (MS) Excel and analysed in either R Statistical Software (version 4.1.2 or later) or SPSS (version 28 or later). Frequencies and percentages will be calculated to summarise categorical variables. Central tendency and dispersion of numerical data will be measured using means and standard deviations if these variables are normally distributed, or medians and interquartile ranges if the variables are skewed. Pearson chi-squared test or Fisher's exact test will be used to test for association between patient outcomes and categorical demographic and clinical factors. Ordinal logistic regression analysis will be used to identify risk factors of patient mortality. A p-value less than 0.05 will be considered statistically significant.

12. Study limitations

The study will only include data and clinical isolates from the Medical Microbiology department at IALCH.

13. Ethical considerations

Ethical approval will be obtained from the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal (UKZN). Approval will be obtained from the KZN Department of Health (DoH) Ethics Committee. Gatekeeper permission will be obtained from the medical manager at IALCH. Departmental permission will be obtained from the Medical Microbiology Head of Department (HoD) at the IALCH/KZN Academic Complex. No consent will be required from patients as this is a retrospective, descriptive chart review and patient personal/identifying details from the electronic databases will not be shown, thus ensuring confidentiality.

14. Funding/budget

Application for funding will be made to NHLS, UKZN and other organisations.

15. Expected outcomes

The prevalence of CREs following the COVID-19 pandemic will be greater than the pre-COVID-19 study period, owing to the mass use of antibiotics during the pandemic. There will be a shift in paradigm in terms of carbapenemase types now found in IALCH patients, with

some isolates possibly harbouring multiple carbapenemase genes. The mechanism of resistance of majority of the CREs will be due to carbapenemase enzyme production, as opposed to other mechanisms.

16. Envisaged study outputs

- To obtain a Masters of Medicine (MMed) in Medical Microbiology.
- Presentation of study results at a local, national or international conference.
- Study publication in a peer-reviewed journal.
- Provision of results that will:

  (i) influence further studies on the topic, and

  (ii) be fundamental to directing future antibiotic choices, guidelines and policies in CRE infections and colonisation.

17. Dissemination

- The study results will be presented to the Department of Medical Microbiology and IALCH staff, including being made available to the KZN DoH.
- Conference presentation.
- Dissertation (thesis).
- Journal publication.

18. Timeline

- Collection of clinical CRE isolates: till May 2023.
- October 2023: Quotations from suppliers for equipment and reagents for molecular characterisation; funding applications.
- November 2023 – March 2024: Retrospective data analysis.
- March 2024 – June 2024: Molecular characterisation of carbapenemase genes.
- July 2024 – September 2024: Analysis of molecular data.
- October 2024 – August 2025: Dissertation and journal publication scientific writing.
- September 2025 – December 2025: Submission of dissertation and journal article.

19. References

1. Cui X, Zhang H, Du H. Carbapenemases in Enterobacteriaceae: detection and antimicrobial therapy. Frontiers in microbiology. 2019 Aug 20;10:1823.

2. Gatya Al-Mayahie SM, Al-Guranie DR, Hussein AA, Bachai ZA. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic *Escherichia coli* phylogroup B2 isolates from outpatients in Wasit Province/Iraq. PLOS One. 2022 Jan 25;17(1):e0262984.

3. Peters BK, Reddy N, Shungube M, Girdhari L, Baijnath S, Mdanda S, Chetty L, Ntombela T, Arumugam T, Bester LA, Singh SD. In Vitro and In Vivo Development of a β-Lactam-Metallo-β-Lactamase Inhibitor: Targeting Carbapenem-Resistant Enterobacterales. ACS Infectious Diseases. 2023 Feb 14;9(3):486-96.

4. Brink AJ, Coetzee J, Richards GA, Feldman C, Lowman W, Tootla HD, Miller MG, Niehaus AJ, Wasserman S, Perovic O, Govind CN. Best practices: Appropriate use of the new β-lactam/β-lactamase inhibitor combinations, ceftazidime-avibactam and ceftolozane-tazobactam in South Africa. Southern African Journal of Infectious Diseases. 2022 Oct 20;37(1):10.

5. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases. 2017 Feb 15;215(suppl_1):S28-36.

6. Bedenić B, Luxner J, Car H, Sardelić S, Bogdan M, Varda-Brkić D, Šuto S, Grisold A, Beader N, Zarfel G. Emergence and Spread of Enterobacterales with Multiple Carbapenemases after COVID-19 Pandemic. Pathogens. 2023 May 3;12(5):677.

7. Asokan GV, Ramadhan T, Ahmed E, Sanad H. WHO global priority pathogens list: a bibliometric analysis of Medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahrain. Oman medical journal. 2019 May;34(3):184.

8. Perovic O, Ismail H, Quan V, Bamford C, Nana T, Chibabhai V, Bhola P, Ramjathan P, Swe Swe-Han K, Wadula J, Whitelaw A. Carbapenem-resistant Enterobacteriaceae in patients with bacteraemia at tertiary hospitals in South Africa, 2015 to 2018. European Journal of Clinical Microbiology & Infectious Diseases. 2020 Jul;39:1287-94.

9. Lowe M, Shuping L, Perovic O. Carbapenem-resistant Enterobacterales in patients with bacteraemia at tertiary academic hospitals in South Africa, 2019-2020: An update. South African Medical Journal. 2022 Aug 30;112(8):545-52.

10. Madni O, Amoako DG, Abia AL, Rout J, Essack SY. Genomic investigation of carbapenem-resistant *Klebsiella pneumonia* colonization in an intensive care unit in South Africa. Genes. 2021 Jun 22;12(7):951.

11. Suay-García B, Pérez-Gracia MT. Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. Antibiotics. 2019 Aug 19;8(3):122.

12. Hansen GT. Continuous evolution: perspective on the epidemiology of carbapenemase resistance among Enterobacterales and other Gram-negative bacteria. Infectious Diseases and Therapy. 2021 Mar;10:75-92.

13. Coppola N, Maraolo AE, Onorato L, Scotto R, Calò F, Atripaldi L, Borrelli A, Corcione A, De Cristofaro MG, Durante-Mangoni E, Filippelli A. Epidemiology, Mechanisms of Resistance and Treatment Algorithm for Infections Due to Carbapenem-Resistant Gram-Negative Bacteria: An Expert Panel Opinion. Antibiotics. 2022 Sep 17;11(9):1263.

14. Brink AJ. Epidemiology of carbapenem-resistant Gram-negative infections globally. Current opinion in infectious diseases. 2019 Dec 1;32(6):609-16.

15. Willems RP, van Dijk K, Vehreschild MJ, Biehl LM, Ket JC, Remmelzwaal S, Vandenbroucke-Grauls CM. Incidence of infection with multidrug-resistant Gram-negative bacteria and vancomycin-resistant enterococci in carriers: a systematic review and meta-regression analysis. The Lancet Infectious Diseases. 2023 Jan 30.

16. Wong SC, Chau PH, So SY, Chiu KH, Yuen LL, AuYeung CH, Lam GK, Chan VW, Chen JH, Chen H, Li X. Epidemiology of multidrug-resistant organisms before and during COVID-19 in Hong Kong. Infection Prevention in Practice. 2023 Jun 1;5(2):100286.

17. Rehman S. ReviewA parallel and silent emerging pandemic: Antimicrobial resistance (AMR) amid COVID-19 pandemic. Journal of Infection and Public Health. 2023 Feb 21.

18. Farfour E, Lecuru M, Dortet L, Le Guen M, Cerf C, Karnycheff F, Bonnin RA, Vasse M, Lesprit P. Carbapenemase-producing Enterobacterales outbreak: another dark side of COVID-19. American Journal of Infection Control. 2020 Dec 1;48(12):1533-6.

19. Shbaklo N, Corcione S, Vicentini C, Giordano S, Fiorentino D, Bianco G, Cattel F, Cavallo R, Zotti CM, De Rosa FG. An observational study of MDR hospital-acquired infections and antibiotic use during COVID-19 pandemic: a call for antimicrobial stewardship programs. Antibiotics. 2022 May 20;11(5):695.

20. Miftode IL, Leca D, Miftode RS, Roşu F, Plesca C, Loghin I, Timpau AS, Mitu I, Mititiuc I, Dorneanu O, Miftode E. The clash of the titans: COVID-19, carbapenem-resistant enterobacterales, and first mcr-1-mediated colistin resistance in humans in Romania. Antibiotics. 2023 Feb 3;12(2):324.

21. Howard-Jones AR, Sandaradura I, Robinson R, Orde SR, Iredell J, Ginn A, van Hal S, Branley J. Multidrug-resistant OXA-48/CTX-M-15 *Klebsiella pneumoniae* cluster in a COVID-19 intensive care unit: salient lessons for infection prevention and control during the COVID-19 pandemic. Journal of Hospital Infection. 2022 Aug 1;126:64-9.

22. Elliott TM, Hurst C, Doidge M, Hurst T, Harris PN, Gordon LG. Unexpected benefit of COVID-19 hospital restrictions: Reduction in patients isolating with multidrug resistant organisms after restrictions were lifted. Infection, Disease & Health. 2022 Feb 1;27(1):14.

23. Nel P, Roberts LA, Hoffmann R. Carbapenemase-producing Enterobacteriaceae colonisation in adult inpatients: A point prevalence study. Southern African Journal of Infectious Diseases. 2019 Mar 1;34(1):1-5.

24. Greybe L, Nuttall JJ, Brink AJ, Tootla HD. Descriptive analysis of infections due to New Delhi metallo-β-lactamase-producing Enterobacterales in children at Red Cross War Memorial Children's Hospital. Southern African Journal of Infectious Diseases. 2022 Jul 27;37(1):440.

25. Maphumulo SL, Swe Swe/Han K, Mlisana K, Singh, R. Laboratory Screening and prevalence of Carbapenemase Producing Enterobacteriaceae isolates from Inkosi Albert Luthuli Central Hospital (IALCH). MMed dissertation. University of KwaZulu-Natal, 2019.

26. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals of clinical microbiology and antimicrobials. 2016 Dec;15(1):1-1.

27. Dilagui I, Loqman S, Hanchi AL, Soraa N. Antibiotic resistance patterns of carbapenemase-producing enterobacterales in Mohammed VI University Hospital of Marrakech, Morocco. Infectious Diseases Now. 2022 Sep 1;52(6):334-40.

28. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.

29. Bianco G, Boattini M, Comini S, Casale R, Iannaccone M, Cavallo R, Costa C. Occurrence of multi-carbapenemases producers among carbapenemase-producing Enterobacterales and in vitro activity of combinations including cefiderocol, ceftazidime-avibactam, meropenem-vaborbactam, and aztreonam in the COVID-19 era. European Journal of Clinical Microbiology & Infectious Diseases. 2022 Apr;41(4):573-80.

30. Ramsamy Y, Mlisana KP, Amoako DG, Abia AL, Ismail A, Allam M, Mbanga J, Singh R, Essack SY. Mobile genetic elements-mediated Enterobacterales-associated carbapenemase antibiotic resistance genes propagation between the environment and humans: A One Health South African study. Science of the Total Environment. 2022 Feb 1;806:150641.

31. Jing N, Yan W, Zhang Q, Yuan Y, Wei X, Zhao W, Guo S, Guo L, Gao Y, Zhao L, Shi C. Epidemiology and genotypic characteristics of carbapenem resistant Enterobacterales in

Henan, China: a multicentre study. Journal of Global Antimicrobial Resistance. 2022 Jun 1;29:68-73.

32. Jean SS, Harnod D, Hsueh PR. Global threat of carbapenem-resistant gram-negative bacteria. Frontiers in Cellular and Infection Microbiology. 2022:236.

33. Tadese BK, Darkoh C, DeSantis SM, Mgbere O, Fujimoto K. Clinical epidemiology of carbapenem-resistant Enterobacterales in the Greater Houston region of Texas: a 6-year trend and surveillance analysis. Journal of Global Antimicrobial Resistance. 2022 Sep 1;30:222-7.

34. Ma J, Song X, Li M, Yu Z, Cheng W, Yu Z, Zhang W, Zhang Y, Shen A, Sun H, Li L. Global spread of carbapenem-resistant Enterobacteriaceae: Epidemiological features, resistance mechanisms, detection and therapy. Microbiological Research. 2022 Nov 4:127249.

35. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, Zheng Y, Guo Y, Zhang R, Hu F, China Antimicrobial Surveillance Network (CHINET) Study Group. Dissemination of carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) among carbapenem-resistant Enterobacteriaceae isolated from adult and children patients in China. Frontiers in cellular and infection microbiology. 2020 Jul 3;10:314.

36. Global antimicrobial resistance and use surveillance system (GLASS) report 2021. Geneva: World Health Organization (WHO); 2021. Licence: CC BY-NC-SA 3.0 IGO.

37. Abdelaziz NA. Phenotype-genotype correlations among carbapenem-resistant Enterobacterales recovered from four Egyptian hospitals with the report of SPM carbapenemase. Antimicrobial Resistance & Infection Control. 2022 Dec;11(1):1-0.

38. Karlowsky JA, Bouchillon SK, El Mahdy Kotb R, Mohamed N, Stone GG, Sahm DF. Carbapenem-resistant Enterobacterales and *Pseudomonas aeruginosa* causing infection in Africa and the Middle East: A surveillance study from the ATLAS programme (2018–20). JAC-Antimicrobial Resistance. 2022 Jun;4(3):dlac060.

39. Shibabaw A, Sahle Z, Metaferia Y, Atlaw A, Adenew B, Gedefie A, Tilahun M, Ebrahim E, Kassa Y, Debash H, Wang SH. Epidemiology and prevention of hospital-acquired carbapenem-resistant Enterobacterales infection in hospitalized patients, Northeast Ethiopia. IJID regions. 2023 Jun 1;7:77-83.

40. Mzimela BW, Nkwanyana NM, Singh R. Clinical outcome of neonates with Carbapenem-resistant Enterobacteriaceae infections at the King Edward VIII Hospital's neonatal unit, Durban, South Africa. Southern African Journal of Infectious Diseases. 2021 Jan 21;36(1):223.

41. Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, Dever LL, Kreiswirth BN. Colistin-and carbapenem-resistant *Escherichia coli* harboring mcr-1 and bla NDM-5, causing a complicated urinary tract infection in a patient from the United States. mBio. 2016 Sep 7;7(4):e01191-16.

42. Montrucchio G, Corcione S, Sales G, Curtoni A, De Rosa FG, Brazzi L. Carbapenem-resistant *Klebsiella pneumoniae* in ICU-admitted COVID-19 patients: Keep an eye on the ball. Journal of Global Antimicrobial Resistance. 2020 Dec 1;23:398-400.

43. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet infectious diseases. 2018 Mar 1;18(3):318-27.

20.Data collection tool

From date:															
To date:															
Region:						Kwa-Zulu Natal									
Branch:						KZN Academic Complex									
User site:	Inkosi Albert Luthuli Central Hospital														
Episode No.	Registration Date	Admission Date	Hospital Number	Sex	DOB	Age	Clinical History	Ward	Specimen Group	Specimen Type	Test Set	Organism	Growth Comment	Imipenem	Meropenem

Appendix B – Departmental approval (Medical Microbiology)

UNIVERSITY OF KWAZULU-NATAL INYUVESI YAKWAZULU-NATALI
COLLEGE OF HEALTH SCIENCES

ACADEMIC COMPLEX BUSINESS UNIT
DEPARTMENT OF MEDICAL MICROBIOLOGY
SCHOOL OF LMMS, CHS, UKZN & NHLS
Level 4, Pathology Laboratory Building
Inkosi Albert Luthuli Central Hospital
800 Vusi Mzimela (Bellair) Road, Mayville, 4091
Tel: +27 (0)31 240 2787
Reference:

NATIONAL HEALTH LABORATORY SERVICE
Practice No: 5200296

Permission letter from Medical Microbiology Department

IALCH Academic complex,
NHLS/ KZN

Date: 07 August 2023

RE: Title of the Project: Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19

Student name: Nitesh Brijlal

Student number: 207500990

School of Laboratory Medicine and Medical Sciences (LMMS)
College of Health Sciences
University of KwaZulu-Natal

Provisional BREC approval reference: BREC/00005907/2023

Supervisor: Prof Khine Swe Swe-Han; Co-Supervisor: Dr Sandra Maphumulo (Pathologist, Microbiology)

In my capacity as the Academic Head of Department, the Discipline of Medical Microbiology at the National Health Laboratory Services (NHLS)/ University of KwaZulu -Natal (UKZN), KZN, it gives me great pleasure to write this letter in support of your MMed Degree Research Project as per your protocol, objectives and methodology of the project. You as a UKZN/NHLS MMed student has permitted to work on the *Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19* during study period under supervisors after BREC final approval. That letter would support to get final approval of BREC.

Yours sincerely,

[redacted]

Prof. Khine Swe Swe/Han (MB,BS; DTMH; PDIC; FC Path; MMed; PhD(Med Micro)
Head of Academic Department (HOD)
Medical Microbiology Department- KZN
Inkosi Albert Luthuli Central Hospital Academic Complex
Univ of KwaZulu-Natal & National Health Laboratory Services
School of Laboratory Medicine & Medical Sciences
Phone: 031 2402784/2787/2793 ; Fax: 0312402786 ; Email: Sweswe-han@ukzn.ac.za;
www.nhls.ac.za; Practice Number: 5200296

Appendix C – Site approval (Inkosi Albert Luthuli Central Hospital)

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:
NKOSI ALBERT LUTHULI CENTRAL HOSPITAL
OFFICE OF THE MEDICAL MANAGER
Private Bag X03, Mayville, 4058
300 Vusi Mzimela (Bellair) Road, Mayville,4091
Tel: 031 240 1059 Fax: 031 240 1005 Email: Ursula.john@ialch.co.za

Reference: BREC 00005907/2023
Enquiries: Medical Management

8 August 2023

Dr N Brijlal (207500990)
School of Laboratory Medicine & Medical Science
Medical School

Dear Dr Brijlal

RE: PERMISSION TO CONDUCT RESEARCH AT IALCH

I have pleasure in informing you that permission has been granted to you by the Medical Manager to conduct research on: **Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19**

Kindly take note of the following information before you continue:

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

Your[redacted]
.....
Dr L P Mtshali
Medical Manager

GROWING KWAZULU-NATAL TOGETHER

Appendix D – KwaZulu-Natal Department of Health approval

KWAZULU-NATAL PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DIRECTORATE:

Physical Address: 330 Langalibalele Street, Pietermaritzburg
Postal Address: Private Bag X9051
Tel: 033 395 2805/ 3189/ 3123 Fax: 033 394 3782
Email:

Health Research & Knowledge Management

NHRD Ref: KZ_202307_012

Dear Dr N Brijlal
(UKZN)

Approval of research

1. The research proposal titled **'Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19 '** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

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

2. You are requested to take note of the following:
   a. ***Kindly liaise with the facility manager BEFORE your research begins.***
      This is to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.
   b. *All research conducted in KwaZulu-Natal must comply with government regulations relating to Covid-19. These include but are not limited to: regulations concerning social distancing, the wearing of personal protective equipment, and limitations on meetings and social gatherings.*
   c. *Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.*
   d. *Provide an interim progress report and final report (electronic and hard copies) when your research is complete to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za*
   e. *Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.*

For any additional information please contact Dr. G Shezi on 033-395 3189.

Yours Sincerely

[redacted]

Dr E Lutge
Chairperson, Provincial Health Research Committee
Date: 11/08/2023.

GROWING KWAZULU-NATAL TOGETHER

Appendix E – Biomedical Research Ethics Committee (BREC) approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

28 August 2023

Mr Nitesh Brijlal (207500990)
School of Laboratory Medicine & Medical Science
Medical School

Dear Mr Brijlal,

Protocol reference number: BREC/00005907/2023
Project title: Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19
Degree Purposes: MMed

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 28 August 2023. Please ensure that any outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 28 August 2023. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on RIG on the appropriate BREC form 2-3 months before the expiry date.

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

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2020) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

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

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 12 September 2023.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Appendix F – Turnitin report

turnitin

Digital Receipt

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

The first page of your submissions is displayed below.

Submission author:	Nitesh Brijlal
Assignment title:	Carbapenem-resistant Enterobacterales at a quaternary publi...
Submission title:	MMed 2025 - Dr Nitesh Brijlal - Carbapenem-resistant Enterob...
File name:	MMed_2025_-_Dr_Nitesh_Brijlal_-_Carbapenem-resistant_Ente...
File size:	6.42M
Page count:	107
Word count:	17,503
Character count:	109,648
Submission date:	06-Nov-2025 09:09AM (UTC+0200)
Submission ID:	2803162686

UNIVERSITY OF KWAZULU-NATAL
COLLEGE OF HEALTH SCIENCES
SCHOOL OF LABORATORY MEDICINE AND MEDICAL SCIENCES
DEPARTMENT OF MEDICAL MICROBIOLOGY

Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinical and molecular paradigm shift following COVID-19

Principal Investigator: Dr Nitesh Brijlal
Student number: 207580990

Supervisor: Prof Khine Swe Swe-Htet
Co-supervisor: Dr Sandra Maphumulo

Submitted in partial fulfilment of the academic requirements for the degree of Master of Medicine (MMed) in Medical Microbiology

November 2025

turnitin

Class Portfolio

NOW VIEWING: HOME > DR NITESH B_ MMED _MICRO 2025 > CARBAPENEM-RESISTANT ENTEROBACTERALES AT A QUATERNA...

About this page

This is your assignment dashboard. You can upload submissions for your assignment from here. When a submission has been processed you will be able to download a digital receipt, view any grades and similarity reports that have been made available by your instructor.

› Carbapenem-resistant Enterobacterales at a quaternary public hospital in Durban, KwaZulu-Natal: Ascertaining the clinica...

Paper Title	Uploaded	Grade	Similarity
MMed 2025 - Dr Nitesh Brijlal - Carbapenem-resistant Enterobacterales.docx	11/06/2025 9:09 AM	--	14%