Investigation of in vitro susceptibility of OXA48 carbapenemase-producing Enterobacterales to ceftazidime-avibactam over a 4-year period: a longitudinal-retrospective study

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Declaration

I "Nomagugu Ndumo" declare that:

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ii. This thesis has not been submitted for any degree or examination at any other university.

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Mrs Nomagugu Ndumo Date

Approved for Final Submission

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Abstract

Background

One of the many health issues plaguing our society is the emergence of infectious agents that are resistant to treatment. This has resulted in multiple deaths over the years. Among multi-drug resistant pathogens of note are carbapenemase-producing Enterobacterales (CPE). As one of the measures to treat these super-bugs, the ceftazidime-avibactam (CZA) drug was formulated, and approved by the Food and Drug Administration (FDA) in 2015. Though not yet registered in South Africa at that time, susceptibility testing for CZA commenced at Lancet Laboratories in 2020. The drug was registered in South Africa in 2021. In the never-ending struggle to prevent the development of resistance to new drugs, surveillance measures need to be put in place to facilitate early detection of the beginning of a resistance pattern. In this way, early action can be taken to prevent further development of resistance.

Material and Methods

In this study, susceptibility patterns of the OXA48 subtype of CPE were measured and compared over 24 months to detect any resistance trend. In addition, isolates stored prior to 2020 were tested for CZA susceptibility, and a comparison made to the post-CZA-testing group. The demographic distribution of the OXA48 infections was also analysed. The different species of OXA48 CPE were compared to determine which of the enterobacterales exhibits more resistance to CZA. The PCR method was used to determine the carbapenemase type. The K-B method was used to determine CZA susceptibility. Bacterial identification was obtained using the MALDI-MS method.

Results

All eight OXA48 isolates from 2018 and 2019 were susceptible to CZA, yielding 0% resistance. 6% of the OXA48 isolates from 2020 were resistant, while 9% from 2021 were resistant. 81% of all the OXA48 isolates from 2020/2021 were

Klebsiella pneumoniae, while the same species constituted 86% of the CZA resistant population. 58% of the OXA48 isolates from 2020/2021 were isolated from the Durban area. There was 100% CZA susceptibility in the 2018/2019 period, compared to the 92.5% in the 2020/2021 period.

Conclusion

The beginning of a resistance trend was observed between the years 2020 and 2021. *Klebsiella pneumoniae* was the predominant species of OXA48 CPE isolated. Most OXA48 isolates cultured between January 2020 and December 2021 were from the Durban area. No statistical significance was discovered in the difference in CZA susceptibility between the 2018/2019 and the 2020/2021 periods.

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List of Acronyms

AMR	Antimicrobial resistance
ATCC	American type culture collection
BSL	Biosafety level
CPE	Carbapenemase-producing Enterobacterales
CRE	Carbapenem resistant Enterobacterales
CZA	Ceftazidime-avibactam
DUT	Durban University of Technology
ESBL	Extended-spectrum beta lactamase
FDA	Food and Drug Administration
GES	Guiana extended-spectrum
IMP	Imipenemase
IREC	Institutional Research Ethics Committee
K-B	Kirby-Bauer
KPC	*Klebsiella pneumoniae* carbapenemase
KZN	KwaZulu-Natal
MALDI-MS	Matrix-assisted laser desorption/ionisation mass spectrometry
MBL	Metallo-beta-lactamase
MDR	Multi-drug resistant
NDM	New Dehli Metallo-beta-lactamase
PCR	Polymerase chain reaction
PPE	Personal protective equipment
SAHPRA	South African Health Products Regulatory Authority
UTI	Urinary tract infection
VIM	Verona integron-mediated metallo-beta-lactamase
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

1.1 Introduction

This chapter of the thesis introduces the research title, outlines the summary of the study and describes the aims, objectives and rationale behind the research.

1.2 Background and rationale

The term Enterobacterales refers to a group of non-spore forming Gram-negative bacilli that ferment glucose. Enterobacterales are typically found inhabiting the human gastrointestinal tract as commensals. However, these bacteria can cause a wide range of infections in other areas of the human body. If not treated, these infections may lead to morbidity; and in advanced cases, even mortality. Depending on the species and antimicrobial susceptibility pattern of the isolated pathogen, different classes of antibiotics can be used for treatment.

The beta-lactam group of antibiotics is one example of a class of antibiotics that are used for the treatment of infections. Beta-lactam antibiotics are antibiotics that contain a beta-lactam ring in their structure. They include penicillins, cephalosporins, monobactams and carbapenems. Their mode of action includes binding to, and inhibiting, penicillin binding proteins (PBPs) on the bacterial structure. This results in digestion and rupture of the bacterial cell membrane. Successful combating of infection using antibiotics depends on bacterial susceptibility or resistance to the antibiotic used.

Over the years, Enterobacterales have developed resistance to these antibiotics. They achieved this by producing beta lactamase enzymes and extended spectrum beta lactamases (ESBL), which are enzymes that inactivate beta-lactam antibiotics. As more drugs were introduced in an attempt to fight infections caused by these resistant organisms, further resistance developed as more mutations occurred. This eventually led to an emergence of carbapenem-resistant Enterobacterales (CRE). These are Enterobacterales that are resistant to some or all carbapenem antibiotics (i.e. imipenem, meropenem, doripenem and ertapenem). Carbapenemase-producing Enterobacterales (CPE) on the other hand, are CRE that mediate carbapenem resistance by production of one or more carbapenemase enzymes. These enzymes hydrolyse and inactivate antimicrobials such as penicillins, cephalosporins, monobactams and carbapenems, rendering them ineffective against the respective pathogens. According to Bowers and Huang (2016), carbapenemases can be differentiated into:

1. Serine enzymes
   - Group A: Serratia marcescens enzyme (SME), Guiana extended-spectrum (GES), Imipenemase (IMP), non-metallo carbapenemase-A (NMC) and *Klebsiella pneumoniae* carbapenemase (KPC)
   - Class D Oxacillinase (OXA) enzymes: most commonly OXA48
2. Metallo-carbapenemases
   - Verona integron-mediated metallo-beta-lactamase (VIM)
   - New Dehli metallo-beta-lactamase (NDM).

There are multiple contributing factors in the development of antibiotic resistance. The World Health Organisation (WHO) documented misuse and overuse of antimicrobials as the main drivers in the development of drug-resistant pathogens (Jenner, Bhagwandin and Kowalski 2017). As the global health system wages war to curb the rise of antimicrobial resistance (AMR), there is a simultaneous on-going battle to develop new drugs to fight these multi drug-resistant (MDR) organisms. One of the drugs is ceftazidime-avibactam (CZA) which was approved by the Food and Drug Administration (FDA) in 2015. Ceftazidime is a broad-spectrum, third-generation cephalosporin, combined with avibactam, a diazabicyclooctane (DBO) β-lactamase inhibitor (Spiliopoulou, Kazmierczak and Stone 2020). CZA was available in South Africa from the beginning of 2021, although only registered in September 2021. First use of the drug for the treatment of a persistent CRE infection in a 17-month old burns patient in South Africa was reported in the same year, yielding successful results (Tootla *et al.* 2021).

The increasing rate of resistance to antibiotics is associated with significant morbidity and mortality (Fröhlich *et al.* 2019). Once new antibiotics are developed and implemented, it becomes vital to keep a close eye on their susceptibility pattern by conducting regular surveys to monitor resistance levels of the drug. In this way, resistance development can be identified and curbed in its early stages. This aids in maintaining good antibiotic stewardship and preventing further development of resistance. Fröhlich *et al.* (2019) specifically mention the importance of monitoring the resistance of OXA48-producing Enterobacterales to the CZA drug. This is due to the fact that data collected in the respective study revealed that exposure to CZA can lead to changes in OXA48, making the pathogens able to hydrolyse ceftazidime and thus resist the inhibitory action of avibactam.

This current study was used as a surveillance tool to monitor the susceptibility of CPE to CZA over a period of four years (2018 to 2021) and to provide early identification of development of a resistance trend.

1.3 Summary of the study

This dissertation reports the findings of a study that was conducted in Durban, KwaZulu-Natal (KZN) South Africa. The research was conducted using bacterial isolates stored at Lancet Laboratories. Carbapenemase genotyping and antibiotic susceptibility data was also obtained from the same laboratory. Durban houses the main Lancet Laboratories laboratory in KZN. Microbiological specimens from peripheral laboratories located in most major cities of the province are processed at this laboratory. The study results therefore represent most of the KZN province demographic. No similar study involving CZA susceptibility has been conducted on a local and national level in South Africa. On a global scale, many studies have been conducted on the susceptibility patterns of CZA leading to, and even after, its FDA approval in 2015.

Even though no studies have been conducted in South Africa to validate the susceptibility of Enterobacterales to the drug, a study by Flamm *et al.* (2014) reported a global *in vitro* surveillance done in 2011 on CZA and its comparator agents. The study was performed against urinary tract infection (UTI) isolates collected from different regions of the world, including the Asia-Pacific regions and South Africa. The drug was tested on a wide range of Gram-negative (including *Pseudomonas aeruginosa*) and some Gram-positive (including S*taphylococcus aureus* and β-haemolytic *Streptococcus* species) isolates of different susceptibility patterns, and was not limited to CPE isolates.

In this current study, frozen isolates from 2018 and 2019 were thawed, cultured onto blood agar plates and incubated for 18-24 hours at 37°C. The Kirby-Bauer (K-B) method was then used to determine the isolates' susceptibility to CZA. A mixture of different species of OXA48-producing CPE isolates from 2020 and 2021 were selected from the CPE database and used for the data analysis. Comparison was made between these two groups in relation to the species, the CZA susceptibility results and the demographic location of the source specimen.

1.4 Context of the study

Lancet Laboratories in KZN commenced *in vitro* testing of CPE susceptibility to the CZA drug in 2020. After Lancet Laboratories commenced susceptibility testing of CZA on CPE isolates, it was noted that 94% of the OXA48 isolates were susceptible to the drug. Isolates producing the NDM carbapenemase were found to be resistant to CZA. Spiliopoulou, Kazmierczak and Stone (2020) documented a study conducted on globally-collected isolates, where CZA was demonstrated to be more effective against serine carbapenemases (KPC, OXA48 and GES) compared to their MBL (metallo-β-lactamase) positive counterparts (those producing IMP, VIM and/or NDM). Alatoom *et al.* (2017) conducted a study in the United Arab Emirates in 2017, which found that very few of the NDM isolates were susceptible to the CZA drug. For this reason, only

OXA48-producing isolates were used in this current study. Isolates stored in 2018 and 2019 were retrieved from storage and tested for susceptibility to CZA. The aim of the study was to compare CZA susceptibility patterns of OXA48 CPE isolates from 2020/2021 and establish whether there has been development of resistance to the CZA drug. The hypothesis was that there was a developing resistance of OXA48 CPE to the CZA drug. Furthermore, eight isolates from 2018/2019, before CZA was being tested, were compared to all the isolates from 2020/2021 to determine percentage susceptibility in the pre-CZA and post-CZA time.

The aim and objectives of the study are laid out below.

1.4.1 Aim of the study

Based on the described background, study rationale and context provided, the aim of the study was to compare susceptibility patterns of OXA48 CPE isolates from January 2020 to December 2021 and establish whether there was development of resistance to the CZA drug.

1.4.2 Objectives

Objective 1 – To determine if there was a developing resistance over 24 months (using OXA48 isolates from January 2020 to December 2021) through surveillance of CZA susceptibility

Objective 2 – To determine which Enterobacterale species was the most resistant to CZA over the 24 months (provided there was any resistance observed)

Objective 3 – To determine the demographic distribution (source location) of the OXA48 infections in KZN over the 24 months

Objective 4 – To determine the difference in CZA susceptibility in the pre-CZA (2018/2019) versus post-CZA (2020/2021) era.

1.5 Limitations

This research study covered OXA48 CPE isolated from different parts (hospitals) of KZN. Since the Durban Laboratory cultures specimens from all parts of the province, the entire demographic area of KZN was represented in the results. It must, however, be noted that the number of isolates that were successfully revived and tested from the 2018/2019 storage was quite low. This was due to the low prevalence of CPE during that period. Also, some of the stored isolates were found to be non-viable due to prolonged storage. Because the main microbiology laboratory in Durban is the only laboratory within the Lancet Laboratories organisation which stores bacterial isolates in KZN, the study was limited to only those isolates. This is another reason why there was a limited number available for isolates stored in 2018/2019.

1.6 Assumptions

Isolates were transported under appropriate temperature-controlled conditions. All isolates were cultured using aseptic techniques to avoid cross-contamination between samples. Known quality control isolates were processed together with the samples to ensure that the antibiotic discs, culture media and environment were all optimal for the isolation, culture and susceptibility testing of the test population. All laboratory health and safety protocols were observed during the culture process.

1.7 Outline of the chapters in the dissertation

Chapter 1 provides the introduction to the thesis and provides a brief description of the research study context, background and study objectives. This chapter concludes with a brief outline of the limitations and assumptions of the study. Chapter 2 details all the literature relevant to the study topic that has been reviewed. Chapter 3 gives a detailed description of study design, tests performed, and principles and statistical methods used to report results. Chapter 4 is a detailed presentation of the collected data results and the use of statistical techniques to make meaning from the raw data. Chapter 5 includes an in-depth discussion of the results obtained from the study and presents the conclusions reached.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter provides details of all the literature related to the study topic that was reviewed. It starts by defining and categorising the research subjects, i.e. CPE, and their epidemiology globally and nationally. The section goes on to describe the OXA48 type CPE and the reasons why this subtype has been selected for the study. Literature that relates to antimicrobial resistance with its causes and implications is discussed. CZA, as the antibiotic of interest in this study, is described in detail, highlighting its importance and the role it has played in the treatment of CPE. Finally, the chapter covers antimicrobial surveillance systems that can be adapted to aid in the stewardship of this precious antibiotic.

2.2 Enterobacterales, carbapenem resistant Enterobacterales (CRE) and carbapenemase-producing Enterobacterales (CPE)

The term Enterobacterales refers to a group of non-spore forming Gram-negative bacilli that ferment glucose. According to Lin *et al.* (2020), Enterobacterales and *Pseudomonas aeruginosa* are the most common Gram-negative bacteria causing serious bacterial infection with high mortality and morbidity. In 2016, Kumar (2016) mentioned that until recently, carbapenems had been successfully used in the treatment of Enterobacterales.

CRE are defined by the Centers for Disease Control and Prevention as those Enterobacterales that are resistant *in vitro* to any carbapenem antimicrobial (Van Duin 2017). Depending on the mechanism of resistance, CRE can be divided into carbapenemase-producing CRE (CP-CRE/CPE) and non-CP-CRE (Vrancianu *et al.* 2021). Spera, Esposito and Pagliano (2019) define CPE as Enterobacterales that are resistant to any carbapenem and are documented to produce a carbapenemase. De Waele *et al.* (2018) list these organisms as one of the most urgent and serious threats for the intensive care unit (ICU). CPE infections are associated with significant morbidity and mortality and are therefore a serious threat to public health (Logan and Weinstein 2017). Antibiotic treatment options for CRE isolates are quickly becoming limited (Kumar 2016). In fact, in 2017 the WHO listed CRE as critical priority bacteria in a list of resistant bacteria against which there is an urgent need to develop new antibiotics (Vrancianu *et al.* 2021). Similarly, Huwaitat *et al.* (2016) note the drastic need for the development of innovative therapeutic solutions that selectively target multi-drug resistant Gram-negative infections.

According to Bowers and Huang (2016), carbapenemases can be differentiated into serine enzymes (subtypes include group A and Class D) and metallo-carbapenemases (subtypes include VIM and NDM). The OXA48 carbapenemase is the most common of the Class D Oxacillinase enzymes.

Surveillance studies have shown that OXA48-like carbapenemases are the most common in certain regions of the world (Pitout *et al.* 2019). According to Lee *et al.* (2022), carbapenemases were not commonly produced by bacteria a decade ago.

2.3 CPE in South Africa

An article by Brink *et al.* (2012) reported the emergence of New Delhi metallo-beta-lactamase (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC-2) in South Africa in 2011. Three years later, Chibabhai and Perovic (2014) reported on an epidemiology study conducted between 2012 and 2013 in Johannesburg. The aim of this study was to describe the characteristics of CPE isolated from patients at the Charlotte Maxeke Academic Hospital. The results revealed isolation of a total of 12 PCR-confirmed CPE isolates over a period of ten months. The genotypes included IMP, NDM, OXA48 and VIM. Furthermore, Thomas and Duse (2018) documented results of yet another study conducted in Johannesburg between 2015 and 2016, to establish epidemiology and compare screening tests for CPEs. The results revealed *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli* to be the predominant CPEs from the

Enterobacterales family. In the same study, the NDM, OXA48, GES and VIM were the identified carbapenemase genes. Vasaikar, Hanise and Abaver (2020) identified OXA48 and NDM as the common CPE genotypes in Umtata, and *Klebsiella pneumoniae* as the most common CPE-producing Enterobacterales. A study conducted by Perovic *et al.* (2020) in four South African provinces between the years 2017 and 2018 supported the latter statement as OXA-48 and NDM were found to be the most common carbapenemases (52% and 34% respectively), with *Klebsiella pneumoniae* being the predominant species. Lowe, Shuping and Perovic (2022a) reported on a study conducted on bacteraemia-causing CPE isolates from academic hospitals in South Africa between 2019 and 2020. The results yielded OXA48 *Klebsiella pneumoniae* as being the most common CPE; and KZN was among the top three provinces with the most CPE pathogens isolated (Lowe, Shuping and Perovic 2022a). The authors further stated that *Klebsiella pneumoniae* remains the dominant Enterobacterale species among patients with CPE infections globally.

2.4 OXA48

Lin *et al.* (2020) describe OXA48 as a unique carbapenemase with low hydrolytic activity against cephalosporins. This carbapenemase is also readily inhibited by ceftazidime as it does not significantly hydrolyse the drug (Lin *et al.* 2020). However, the authors go on to state that ceftazidime can lose its activity in the presence of extended-spectrum beta lactamase (ESBL). Surveillance studies have shown that OXA48-like carbapenemases are the most common in certain regions of the world (Pitout *et al.* 2019). In the same article, Pitout *et al.* (2019) state that most OXA-48 positive isolates were of the *Klebsiella pneumoniae* species. The first reported OXA48-producing Enterobacterale was a *Klebsiella pneumoniae* strain isolated in Turkey in 2001 (Van Duin and Doi 2017). OXA48-producing CPE are the most common CPE pathogens isolated in South Africa (Lowe, Shuping and Perovic 2022a).

2.5 Anti-microbial resistance: causes and implications

One of the most pressing current AMR concerns is the international spread of CRE, especially those producing carbapenemases (Pitout *et al.* 2019). In 2014, the WHO recognised AMR as a global health security threat requiring action across government sectors and society as a whole (Jenner, Bhagwandin and Kowalski 2017). Since resistant pathogens travel from one place to another, localised resistance can fast become a global problem (Jenner, Bhagwandin and Kowalski 2017).

As stated by Spera, Esposito and Pagliano (2019), the overuse of antimicrobials has led to the development of resistance. Siachalinga and Mufwambi (2022) highlight the lack of surveillance systems as one of the contributing factors towards the development of AMR. Jenner, Bhagwandin and Kowalski (2017) voiced a concern regarding some countries allowing pharmacies to sell antibiotics without prescription, and practitioners prescribing antibiotics for diseases where they are not indicated, for example, viral infections. For this reason, newly developed antibiotics should be used prudently to avoid development of resistance due to them being widely used (De Waele *et al.* 2018). Logan and Weinstein (2017) mention antimicrobial stewardship as one of the most successful solutions in controlling the spread of CPE in healthcare settings. Spiliopoulou, Kazmierczak and Stone (2020) recognise the need for continued surveillance of antimicrobial activity.

In 2016, Kumar (2016) reported colistin and tigecycline as the remaining hope for the treatment of CRE infections. These two drugs have been used as first-line therapy in the treatment of infections caused by CRE and CPE (Swaminathan, Routray and Mane 2022). However, the authors continue to highlight the limited clinical use of colistin due to its nephrotoxicity and neurotoxicity. Over the years, CPE have developed resistance to such drugs. Regrettably, resistance to these antibiotics is becoming increasingly alarming (Vrancianu *et al.* 2021). Kumar (2016) reported on a study that was conducted in the USA between the years of 2013 and 2015 to analyse the susceptibility patterns of CRE isolates to tigecycline and colistin. In the study, 100% of the CRE *Klebsiella pneumoniae* isolates were resistant to both drugs. Vrancianu *et al.* (2021) suggest that tigecycline resistance is due to its low serum concentration and low penetration into the epithelial lining fluid. Increased tigecycline concentrations are often associated with gastrointestinal side effects and decreased fibrinogen levels (Vrancianu *et al.* 2021). Furthermore, Lee *et al.* (2022) state that some of the antibiotics used in the treatment of CPE (amikacin, colistin, tigecycline and fosfomycin) have multiple limitations in clinical use. These include issues such as renal toxicity, ineffective concentration at affected sites and unreliable *in vivo* response (Lee *et al.* 2022).

Drug resistance accounts for 1.2 million deaths globally (Chetty *et al.* 2022). Papavarnavas and Mendelson (2022) provide an alarming statistic that in 2019, 4.95 million people died worldwide from antimicrobial resistant infections. This is more than HIV and malaria combined (Papavarnavas and Mendelson 2022). Chetty *et al.* (2022) highlight how infection with MDR bacteria has increased cost implications for the patient and the health system as a whole. This is due to the fact that the treatment of these pathogens requires more extensive therapeutic regimens and additional diagnostic testing (Chetty *et al.* 2022). According to Gajdács and Albericio (2019), AMR may result in the delay of appropriate treatment, the need to use older and more toxic antibiotics, longer hospital stays, a burdened healthcare infrastructure, increased mortality and an overall decrease in the quality of life. The authors also quote the misuse and overuse of drugs as one of the main causes of AMR development. In 2015, Mendelson and Matsoso (2015) predicted that 10 million people could die annually in the next 35 years if antibiotic misuse and overuse was not halted. According to Jamrozik and Heriot (2022), recent data suggests that annually, over one million deaths can still be attributed to antimicrobial resistance. The researcher's observation is that resistant pathogens, particularly CPE, continue to be isolated from patient specimens in the diagnostic microbiology laboratory.

2.6 CZA

CZA was approved by the Food and Drug Administration (FDA) in 2015. According to Xiong *et al.* (2022), it was the first drug approved for the treatment of CRE. The Infectious Diseases Society of America has recommended CZA as a first-line treatment against infections caused by OXA48 and KPC-producing CPE (Swaminathan, Routray and Mane 2022). This drug is a combination of ceftazidime (a third-generation cephalosporin) and avibactam (a novel non-β lactam β-lactamase inhibitor) (Mazer-Amirshahi, Pourmand and May 2017). According to Peri *et al.* (2019), CZA demonstrates good tolerability in clinical trials and represents a potential carbapenem-sparing option in the treatment of CPE. A review of treatment of severe GNB infections revealed that CZA has a good safety and tolerability profile (Shirley 2018). (Van Duin and Bonomo 2016) support this by stating that CZA is an important addition to currently available antibiotics. The drug represents a good treatment option for OXA48 CPE, with good susceptibility results (Van Duin and Bonomo 2016). This could be due to the fact that OXA-48 carbapenemases weakly hydrolyse carbapenems and have limited activity against broad-spectrum cephalosporins, especially ceftazidime (Pitout *et al.* 2019).

As stated by Jiang *et al.* (2022), the application of CZA has significantly improved the clinical cure rate and survival rate of patients with CRE infections. Ceftazidime-avibactam may be a reasonable alternative to colistin in the treatment of *Klebsiella pneumoniae* CPE infections, as noted by Van Duin *et al.* (2018). CZA demonstrated an excellent safety profile, with a low nephrotoxicity rate (Van Duin *et al.* 2018). In a study of colistin versus CZA conducted in the USA, CZA yielded better clinical outcomes than colistin (Van Duin *et al.* 2018). Lin *et al.* (2020) remark on how this drug has been used to successfully treat infections caused by Gram-negative pathogens that produce ESBL, AmpC, OXA48 or a combination of all inhibitors. In a similar study, Almangour *et al.* (2022) concluded that treatment with CZA is associated with a higher cure rate and a lower acute kidney injury rate compared to a colistin-based regimen.

Bowers and Huang (2016) mention combination therapy as the most effective approach in the treatment of MDR CPE. Vrancianu *et al.* (2021) deem CZA to be one of the most promising formulations recently revealed to fight against MDR CPE. Van Duin and Bonomo (2016) state that CZA is the preferred agent for the treatment of OXA48 carbapenemase producers. The other CPE genotypes are intrinsically resistant to the drug due to the enzymes they produce. According to Lee *et al.* (2022), common Enterobacterales exhibit a high susceptibility to CZA, including those that produce the KPC and OXA48 carbapenemases. In a clinical trial conducted in the USA, patients infected with OXA48-producing pathogens demonstrated successful clinical outcomes when treated with CZA (Lin *et al.* 2020). However, susceptibility is low in the presence of MBL carbapenemases (Lee *et al.* 2022).

In a study conducted by Spiliopoulou, Kazmierczak and Stone (2020) on globally-collected isolates, CZA was demonstrated to be more effective against serine carbapenemases (KPC, OXA48 and GES) compared to their MBL (metallo-β-lactamase) positive counterparts (those producing IMP, VIM and/or NDM). The MBL-negative isolates (those producing serine carbapenemases) yielded 99.8% susceptibility to CZA, whereas there was no activity against the MBL-positive isolates. Alatoom *et al.* (2017) conducted a study in the United Arab Emirates in 2017 which concluded that very few of the NDM isolates were susceptible to the CZA drug. Additionally, Karlowsky *et al.* (2016) documented a study outcome of 30 000 gram negative isolates tested against CZA, of which only 0.61% turned out to be resistant. One third of the 185 resistant isolates were MBL-producers (Karlowsky *et al.* 2016). Ongoing antimicrobial surveillance of the CZA drug by Lancet Laboratories has also produced data in keeping with this susceptibility trend.

The hypothesis is that resistance can develop over time with increased usage, as in any other setting. For example, Saudi Arabia reported the first case of OXA-48 carbapenemase-mediated CZA resistance in 2019 (Al Dabbagh *et al.* 2019). Tootla *et al.* (2021) commend CZA, among other antibiotics, as having been shown to be superior to aminoglycosides and colistin for certain CPE infections. There is, however, a concern that like with any other antibiotic, following the widespread clinical use of CZA, the percentage of Enterobacterales that are resistant to this antibiotic is likely to increase dramatically (Van Duin and Bonomo 2016). Spiliopoulou, Kazmierczak and Stone (2020) recommend continued surveillance of CZA antimicrobial activity.

Considering the above points, it is imperative that such a surveillance study be conducted for CZA in South Africa. This study will serve as a surveillance tool to monitor the susceptibility of CPE to CZA over 12 months in order to pick up any early signs of the beginning of resistance, or a resistance trend.

2.6.1 CZA in South Africa

Studies for the validation of the drug have not been recorded in South Africa. However, an article by Flamm *et al.* (2014) reported a global *in vitro* surveillance study conducted in 2011 on CZA and its comparator agents. The study was performed against urinary tract infection (UTI) isolates collected from different regions of the world, including the Asia-Pacific and South Africa (APAC) regions. The drug was tested on a wide range of Gram-negative (including *Pseudomonas aeruginosa*) and some Gram-positive (including S*taphylococcus aureus* and β-haemolytic *Streptococcus* species) isolates of different susceptibility patterns, and not limited to CPE isolates. Tootla *et al.* (2021) reported the first use of ceftazidime avibactam (CZA) for the treatment of a persistent CRE infection in a 17-month old burns patient in South Africa. However, the author also highlighted a few challenges encountered with acquiring the drug since at that time it was prescribed under Section 21. This meant that at the time, ceftazidime avibactam was available but not yet registered in South Africa. However, one year later, (Brink *et al.* 2022) reported that the two new β-lactam/β-lactamase inhibitor combinations, ceftazidime-avibactam and ceftolozane-tazobactam were registered in South Africa by the end of 2021.

2.6.2 CZA resistance

Though many studies reveal positive results associated with CPE treatment using CZA, Vrancianu *et al.* (2021) note that the enthusiasm generated by these studies is tempered by the observations of CZA resistance in certain CRE isolates. Despite highlighting positive notes regarding CZA use, Van Duin *et al.* (2018) reported concerns of CZA resistance observed in 3 out of 10 patients. Echoing the same view, Xiong *et al.* (2022) state that the increased clinical application of CZA has resulted in the development of resistant strains.

Several studies have revealed molecular mechanisms that may be associated with CZA resistance (Vrancianu *et al.* 2021). Xiong *et al.* (2022) suggest that Pro68Ala and Tyr211Ser amino acid substitutions in OXA-48-producing strains have resulted in decreased susceptibility to CZA. However, most of the resistance mechanisms involve MBLs and mutations in KPC enzymes (Vrancianu *et al.* 2021). A study conducted in 2015 at the University of Texas reported results of Enterobacterales susceptibility to CZA. Of the CPE isolates, 64% were resistant to CZA, 55% of which were NDM-producing (Aitken *et al.* 2016). Göttig *et al.* (2019) reported an emergence of CZA-resistant CPE, further stating that it is especially linked to KPC mutations. The first reported case of CZA resistance was in Los Angeles in October of 2015; a KPC-producing strain of *Klebsiella pneumoniae* isolated from a 62-year old woman's blood culture (Humphries *et al.* 2015; Xiong *et al.* 2022). In South Africa, Brink *et al.* (2022) reported an alarming rise in CZA resistance not only in *Klebsiella pneumoniae*, but also in other common Enterobacterales species.

2.7 Antimicrobial stewardship and surveillance

Gajdács and Albericio (2019) describe antimicrobial stewardship as the implementation of policies, guidelines, drug utilisation reports and point prevalence surveys (both locally and internationally) aimed at optimising the use of antimicrobial agents. The suggested definition by Dyar *et al.* (2017) is that antimicrobial stewardship is a coherent set of actions that promote the responsible use of antimicrobials.

Jenner, Bhagwandin and Kowalski (2017) reported on a study that conducted a literature review done to assess the differences in the way countries use antibiotics. The review revealed that countries that use relatively few antibiotics, like Europe, have very low levels of AMR(Jenner, Bhagwandin and Kowalski 2017). Overuse of antibiotics in animals also remains a key problem in the AMR battle (Jenner, Bhagwandin and Kowalski 2017). The authors noted that the high agricultural use of colistin by China has resulted in resistance to the antibiotic. They also highlighted that hospital overuse of antibiotics in China also results from the fact that hospitals and clinics receive incentives for prescribing drugs. The lack of surveillance systems is one of the contributing factors towards the development of AMR (Siachalinga and Mufwambi 2022).

According to a global survey carried out in 2015, very few data were available for the implementation of antimicrobial systems in Africa (Howard *et al.* 2015). Brink *et al.* (2016) document an antimicrobial stewardship implementation programme across 47 hospitals in South Africa. The programme resulted in an 18.1% reduction in antibiotic prescription. Prior to the implementation of the programme, no antibiotic stewardship activity had been performed in 41 of the 47 hospitals (Brink *et al.* 2016). One of the steps taken by South Africa in the fight against AMR was the endorsement of the Antimicrobial National Strategy Framework in 2014 (Chetty *et al.* 2022). The authors listed optimising surveillance and early detection of antimicrobial resistance as one of the objectives of the strategy.

Morel *et al.* (2021) reported on a study that was conducted to determine the requirements of antimicrobial surveillance for new drugs. The authors reported on the shortfall of current antibiotic surveillance systems in that they only monitor drugs that have been on the market for a long time. A new, public system should be set up that focuses on detecting the emergence of resistance to new antibiotics (Morel *et al.* 2021). The authors recommended implementation of a two-phase system. The first phase would involve an immediate early-warning surveillance system to be implemented as soon as new drugs are introduced locally. This was highlighted as especially essential for drugs that are critically important, such as those used in the treatment of MDR pathogens (Morel *et al.* 2021). A surveillance phase such as this is crucial in enabling quick action with regard to infection prevention and control measures. The authors concluded by highlighting the essential role played by AMR surveillance in the preservation of effective antibiotics through early detection of resistance emergence (Morel *et al.* 2021). Brink *et al.* (2022) reported on the alarmingly increasing rate of CZA resistance in South Africa, and recommended that if these vital antibiotics are to be preserved, they should be well stewarded. Specifically, the authors mentioned that withholding the use of antibiotics like CZA for resistant infections that can be treated with currently available antibiotics is a critical part of stewardship (Brink *et al.* 2022).

CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter provides an outline of the research methods that were followed in this study. It provides information on the study subjects (CPE isolates) and the inclusion and exclusion criteria used to sample the study population. The research design that was chosen, and the reason/s for the choices, are discussed. The instruments, materials and procedures that were used to analyse the isolates are described in detail. Data analysis methods will also be highlighted, as will be the challenges encountered during testing and data collection; and the ethical and safety considerations that were taken during this study.

3.2 Study design

This was a longitudinal and retrospective quantitative study. It used laboratory-based surveillance to determine the pattern of OXA48 susceptibility to CZA over 24 months. Furthermore, the study aimed to compare CZA susceptibility over two time periods: the period prior to CZA susceptibility testing commencement in South Africa, and the current period where CZA susceptibility testing is available in the country. So, the study was divided into two parts as highlighted in sections 3.6.1 and 3.6.2.

3.3 Study setting

The study was carried out in Durban in KZN on isolates and data collected from different areas around the KZN province and analysed at the main Lancet Laboratories laboratory in Durban. This laboratory keeps data related to all the CPE bacteria isolated in the laboratory as a result of testing, including their respective carbapenemase genotype and (CZA) susceptibility results.

3.4 Sample population

3.4.1 Sample size and sample selection

Lancet Laboratories Durban currently keeps data of all the CPE bacteria isolated in the laboratory, including their respective genotype and (CZA) susceptibility results from the time they started testing for the drug (2020 onwards). The laboratory also keeps storage of CPE isolates from the years prior to 2020. These have not been tested for susceptibility to CZA.

In total, 934 OXA48 isolates were tested for CZA susceptibility at the Durban Lancet Laboratories laboratory between January 2020 and December 2021. Part 1 of the study included all the 934 isolates from 2020/2021. As determined using the Raosoft® 2011 sample size calculator, this sample size carried a 3.13% margin of error and a confidence level of 99%. Part 2 of the study consisted of eight isolates from 2018/2019, which will be compared to the 924 isolates from the 2020/2021 population. The eight isolates from 2018/2019 were assessed for CZA susceptibility as the drug was not being tested during this time period, and thus these isolates didn't have CZA susceptibility results prior to this study. Due to the low prevalence of CPE in the previous years, there was a low number of stored viable OXA48 isolates available for testing. As a result of this, the sample size for 2018 and 2019 was very low (see section 5.3. Therefore, Part 2 of the study offered a 94.6% confidence level and a 24.49% margin of error (Raosoft® 2011).

In summary:

- 2018/2019 – 08 OXA48 isolates
- 2020/2021 – 934 OXA48 isolates

3.4.2 Inclusion criteria

Enterobacterales were included in this study if they were carbapenem resistant, produced the OXA48 carbapenemase, and were isolated in the years 2018, 2019, 2020 and 2021.

3.4.3 Exclusion criteria

Isolates were excluded from this study if they were not Enterobacterales and if they were carbapenem resistant Enterobacterales producing the other carbapenemases, e.g. VIM, NDM, and GES.

3.5 Ethical considerations

Ethical approval was obtained (Ethics clearance number IREC 098/22) from the Durban University of Technology (DUT) Institutional Research Ethics Committee (IREC). Gate-keeper permission was obtained from the Lancet Laboratories Publications Board to use the isolates and the data. As property of Lancet Laboratories, the isolates were labelled with the name of the bacteria and the carbapenemase genotype. No source patient information was made available for the purposes of this study. Only isolate-related information such as the name of the bacteria, CZA susceptibility, carbapenemase genotype and demographic location of the source patient were retrieved from the database. No patient identification-related data, for example the requisition number, was used or quoted. This guarantees that data cannot be traced back to the respective source patients and thus maintains patient confidentiality and eliminated the need for patient informed consent.

3.6 Data collection

There were two parts to the data collection.

3.6.1 Part 1 (2020/2021 isolates)

Nine hundred and thirty four samples from 2020 and 2021 were used to address Objectives 1 to 3. These samples already had CZA susceptibility results as tested at Lancet Laboratories.

3.6.1.1 Analysis of current isolates

A mixture of different species (e.g. *Klebsiella pneumoniae, Serratia marcescens, Escherichia coli* Enterobacter species, and *Morganella morganii*) of OXA48-producing CPE isolates (495 from 2020 and 439 from 2021) were retrieved from the Lancet Laboratories database and used for the data analysis. The CZA susceptibility, source location, and species identification of the isolates were analysed.

3.6.2 Part 2 (2018/2019 isolates)

Eight OXA48 isolates from 2018/2019 were retrieved from storage and the K-B susceptibility testing method used to establish their susceptibility to CZA. For comparison, all 934 OXA48 isolates from the 2020/2021 population were used to compare CZA susceptibility. This was addressing Objective 4.

3.6.2.1 Retrieval and culture of 2018/2019 isolates

CPE isolates, previously identified and then genotyped for carbapenemase production, are stored (frozen in Microbank™ beads) at the Lancet Laboratories microbiology laboratory in Durban. The type of carbapenemase produced by each isolate was established using the Cepheid® GeneXpert® polymerase chain reaction (PCR) assay. The bacterial identification of each isolate was determined using either matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) or the Vitek® 2 system. These are all established laboratory methods with standard operating procedures (SOPs) in place for them.

The retrieval process of the isolates was as follows:

- The micro-banking vials were removed from the freezer and allowed to thaw at room temperature.
- The vials and blood agar plates were labelled with respective numbers from 1 to 8.
- Using a sterilised straight wire, beads were removed from the vial and inoculated (by rolling) onto the respective blood agar plate (Figure 3.1).

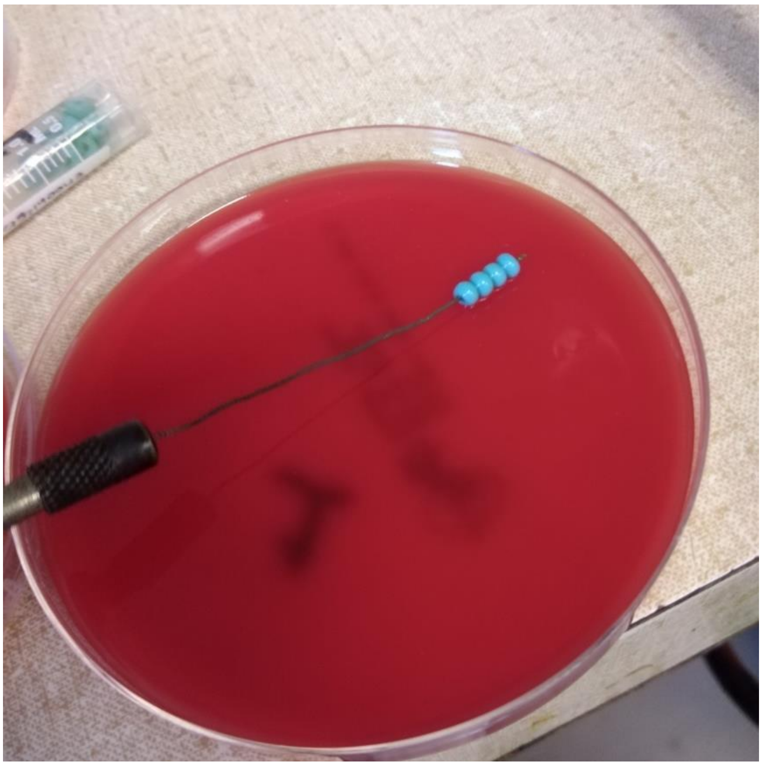

Figure 3.1: Culture of beads onto blood agar

- The agar plates were streaked for isolated colonies and then incubated at 37°C.
- After 24 hours of incubation, the agar plates were checked for growth. Plates without growth were re-incubated, and the respective isolate re-cultured from the Microbank™ vial. Any culture growth that was too scanty and insufficient for susceptibility testing (not enough colonies to make up a 0.5 McFarland concentration) was plated out onto sterile blood agar plates and streaked out for isolated colonies.
- Once sufficient growth was obtained, the isolated colonies were then used for CZA susceptibility testing as in section 3.6.2.2.

3.6.2.2 Kirby-Bauer susceptibility testing

- Saline vials were labelled with the respective blood agar plate numbers.
- Single, isolated colonies of the bacteria were selected and emulsified in saline to give a concentration/density (turbidity) equivalent to a 0.5 McFarland standard. McFarland standards are suspensions of either barium

sulphate or latex particles that allow visual comparison of bacterial density (Hudzicki 2009).

- A sterile swab was then immersed into the solution; drained off of excess liquid and seeded onto a Mueller Hinton agar plate (the swab is rolled side-to-side onto the agar from the top of the plate all the way to the bottom. The process is repeated three times, rotating the plate at a 45° angle each time). This type of agar has all the basic ingredients needed by non-fastidious organisms, allows for better reading of susceptibility results due to its see-through nature, and was suggested by the WHO because it was regarded as a relatively simple medium (Biemer 1973).
- The plates were allowed to dry, then (CZA) antibiotic-impregnated discs placed onto the inoculum. The discs were gently pressed down to avoid them falling off.
- The plates were incubated at 37°C in an aerobic incubator.
- After 24 hours, the plates were examined for the presence of a zone of growth inhibition around the antibiotic disc. The Clinical Laboratory Standards Institute susceptibility guideline (2020 Edition) was used to interpret CZA susceptibility . Resistant/non-susceptible refers to a zone size diameter of less than or equal to 20mm, whereas sensitive/susceptible refers to a zone size diameter of greater than or equal to 21mm (Figure 3.2).

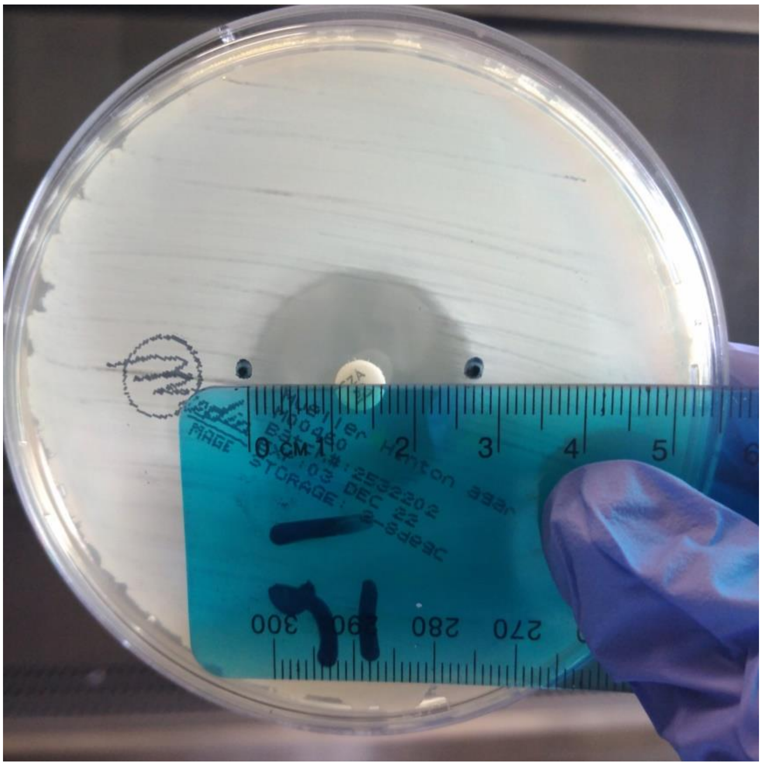

Figure 3.2: Measurement of inhibition zone around antibiotic disc

- Strains of *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control (QC) of CZA susceptibility.

3.7 Reliability and validity of laboratory processes

All laboratory methods and instrumentation used in this study have previously been validated by Lancet Laboratories before being implemented for use in the laboratory. These methods and instruments are carried out on a daily basis, and are operated using established standard operating procedures. The instruments undergo daily, weekly and monthly maintenance processes to ensure that they remain in the best condition for sample processing. Both external and internal quality control samples are processed periodically using these instruments and methods to check for accuracy and precision of results. During the processing of the 2018/2019 isolates, two control organisms were used. These are the *Escherichia coli* ATCC25922 and the *Pseudomonas aeruginosa* ATCC27853 strains. This was a control check to ensure that all the materials used in the K-B testing method were in good condition, and that all the techniques employed were correct. The CZA susceptibility results for both strains were within acceptable values. Purity agar plates were set up with every K-B test to ensure that the isolate culture was not contaminated.

3.8 Health, safety and sterility

The following aseptic techniques were adhered to during the culture process:

- Disinfecting the bench-top before and after processing.
- Sterilising the pick-off straight-wire and loop between uses.

The following health and safety protocols were observed, as shown in Figure 3.3:

- A surgical mask and goggles were used during processing to protect from any splashes and aerosols.
- Full personal protective equipment (PPE) was used, i.e. fully buttoned laboratory coat, gloves and closed-toe shoes.
- The samples were processed inside a biosafety level 2 (BSL-2) cabinet. Biosafety levels refer to a combination of laboratory practices, techniques, safety equipment and laboratory facilities. This combination of practices must be specifically appropriate for the operations performed, the suspected routes of transmission of the infectious agent, and the laboratory function (Meechan and Potts 2020). Biosafety Level 2 (BSL-2) standard practices, safety equipment, and facility specifications are applicable to laboratories in which work is performed using a broad-spectrum of biological agents and toxins that are associated with causing disease in humans of varying severity (Meechan and Potts 2020).
- All culture waste was disposed of in biohazard boxes.
- All laboratory and environmental health and safety protocols were adhered to.

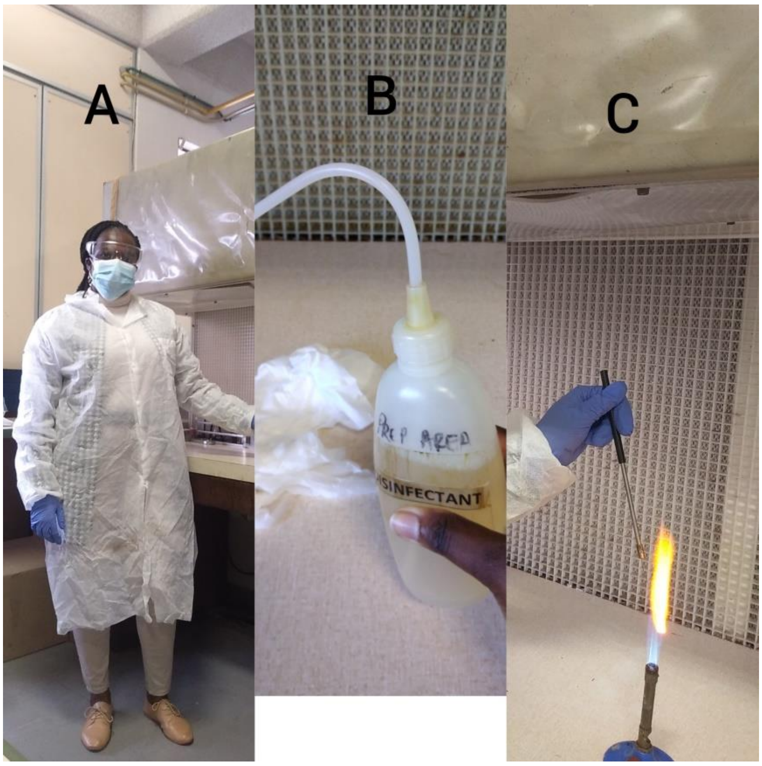

Figure 3.3: Health, safety and aseptic techniques in the microbiology laboratory. A: full PPE; B: well-disinfected surface; C: sterilised culture equipment

3.9 Statistical analysis

Data was captured into Microsoft Office and then imported into SPSS, version 27 for analysis. Simple descriptive statistics (means and frequency distribution tables) were used to summarise the demographic characteristics of the study isolates. Trend analysis and patterns were used to establish whether there is a developing trend of Enterobacterales resistance to CZA from January 2020 to December 2021 (Objective 1).

Percentage resistance = number of resistant OXA48 isolates per year ÷ total number of OXA48 isolates per year X 100).

These values were then graphically compared for the four years to establish if there is a positive gradient.

Paired samples t-test and the Wilcoxon sign rank test were used to compare the resistance of CPE isolates from the period before CZA was tested for, and the period after CZA was being tested.

3.10 Challenges during testing

Due to the long-term storage of the isolates, some yielded scanty growth which had to be sub-cultured to get sufficient colonies for K-B testing. This prolonged the testing process. Even more challenging was the isolates that did not grow at all even after re-culture and prolonged incubation. This led to a further reduction in isolate numbers for 2018/2019 due to non-viability of the selected isolates.

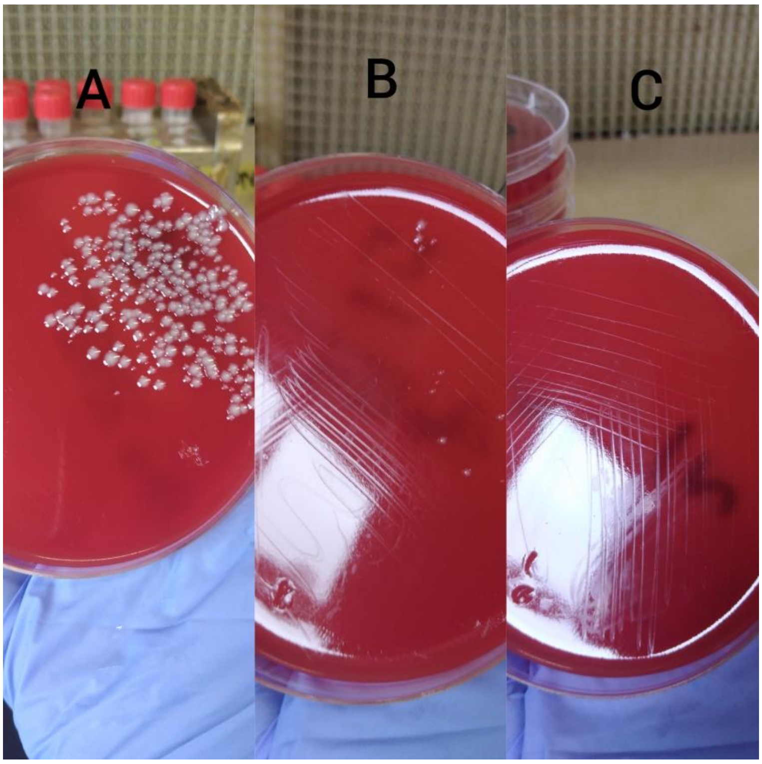

Figure 3.4: Comparison in culture growth. A: confluent and sufficient growth; B: very scanty, insufficient growth requiring subculture; C: no growth

CHAPTER 4: RESULTS

4.1 Introduction

In this chapter of the dissertation, the results obtained in this research study are presented in detail according to each objective. The first objective was to compare CZA susceptibility results and determine if a trend of resistance is developing. Secondly, the study examined the difference in the number of OXA48 isolates according to species, and the CZA resistance thereof. Furthermore, the demographic distribution of OXA48 infections in KZN was analysed. Lastly, a comparison was made regarding CZA susceptibility between the period prior to the commencement of CZA susceptibility testing in South Africa, compared to the period after the testing commenced, to deduce the statistical significance of the difference, if any.

4.2 CZA resistance between 2020 and 2021

In 2020, the Lancet Laboratories database documented a total of 902 CPE isolates, of which 502 were OXA48. This is 56% of all the CPE isolates for the year. In the year 2021, they documented a total of 1 473, of which 522 were OXA48, which translates to 35% of the total CPE in the year. However, due to some missing data for other isolates such as, location and CZA susceptibility, only 934 OXA48 isolates were selected over the two years (495 from 2020 and 439 from 2021).

The total number of CZA-resistant strains over the two years was 70. There were 30 resistant isolates in 2020 and 40 in 2021. A monthly break-down of the OXA48 numbers is presented in Table 4.1. A trend line graph is used to display the rise and fall in the numbers of OXA48 isolates over the 24 months (Figure 4.1). A sharp rise in numbers is noted at the beginning of each year, in contrast to the decline in isolate numbers toward the end of the year.

The numbers of CZA non-susceptible OXA48 for 2020 and 2021 are presented in Figure 4.2. A line graph is used to depict the trend of CZA resistance over the years. For the trend analysis, 2018 and 2019 numbers were added for reference (Figure 4.3).

Table 4.1: OXA48 numbers between January 2020 and December 2021

Month	Number of susceptible isolates
2020 Jan	1
2020 Feb	1
2020 Mar	6
2020 Apr	3
2020 May	4
2020 Jun	1
2020 Jul	4
2020 Aug	2
2020 Sep	2
2020 Oct	3
2020 Nov	3
2020 Dec	0
2021 Jan	4
2021 Feb	4
2021 Mar	8
2021 Apr	6
2021 May	3
2021 Jun	5
2021 Jul	2
2021 Aug	2
2021 Sep	0
2021 Oct	3
2021 Nov	2
2021 Dec	1

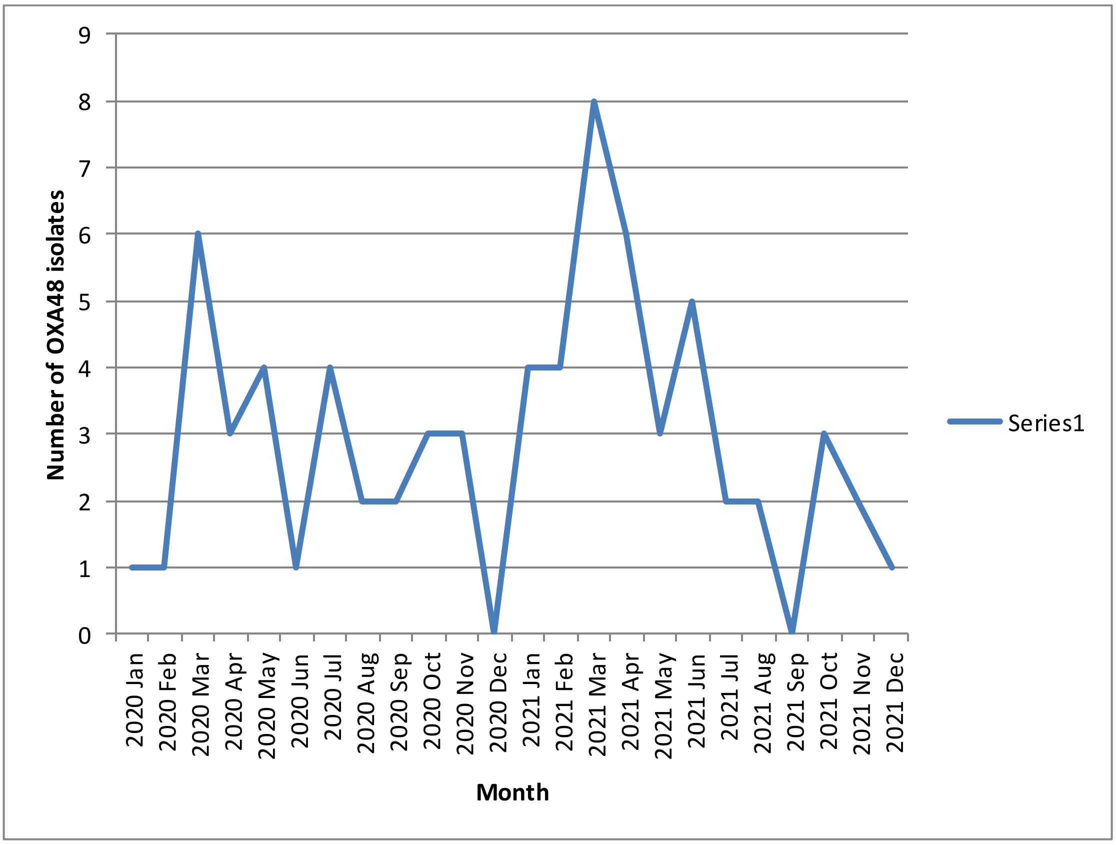

Figure 4.1: OXA48 isolates from January 2020 to December 2021

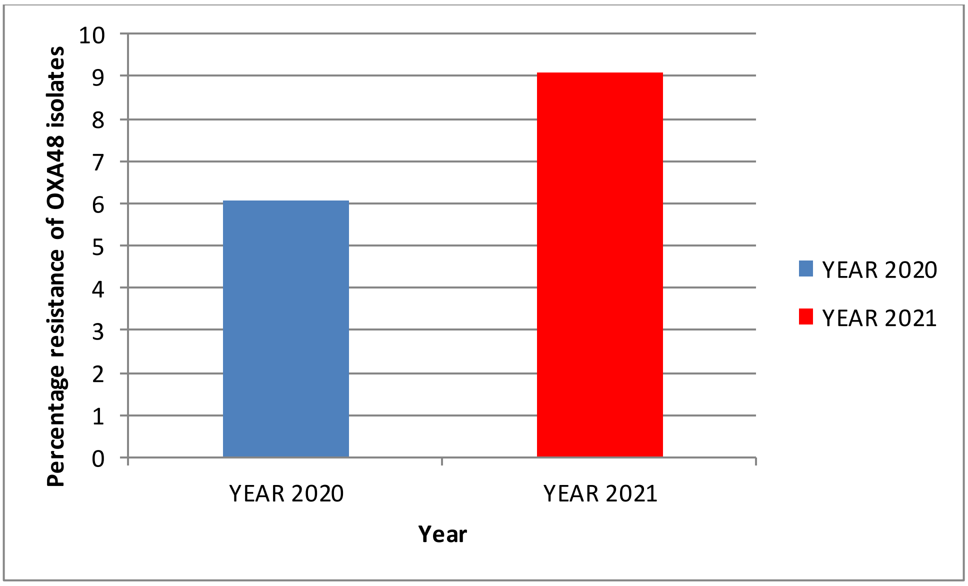

Figure 4.2: Percentage resistance of OXA48 to CZA between year 2020 and 2021

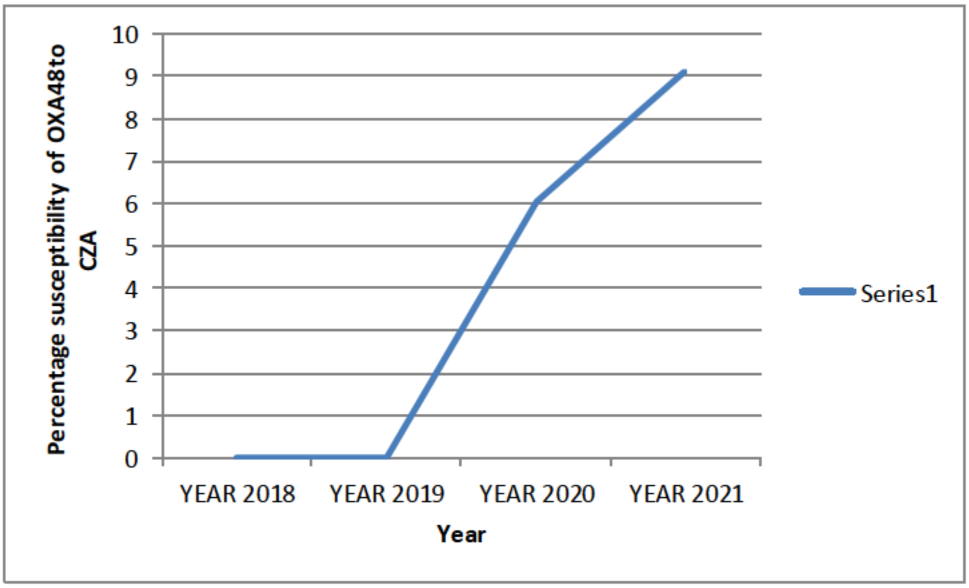

Figure 4.3: Trend of CZA resistance over four years

4.3 Species

4.3.1 OXA48 isolates by species

Enterobacterales can be broken down into 120 different species. Of the 934 OXA48 isolates analysed for the years 2020 and 2021, the species distribution was as per Table 4.2.

Table 4.2: OXA48 distribution by species

Species	Number
Citrobacter freundii	5
E coli	18
Enterobacter species	110
Klebsiella pneumoniae	752
Klebsiella oxytoca	1
Serratia marcescens	47
Morganella morganii	1

Klebsiella pneumoniae takes the lead in number, followed by Enterobacter species. *Serratia marcescens, E coli, Citrobacter freundii, Klebsiella oxytoca* and *Morganella morganii* were in the lower region of predominance.

4.3.2 CZA Resistance by species

Of the 70 resistant OXA48 strains, there were 60 *Klebsiella pneumoniae,* 5 *Serratia marcescens,* 4 *Enterobacter* species, *and* 1 *Klebsiella oxytoca.* The distribution is represented in Figure 4.4.

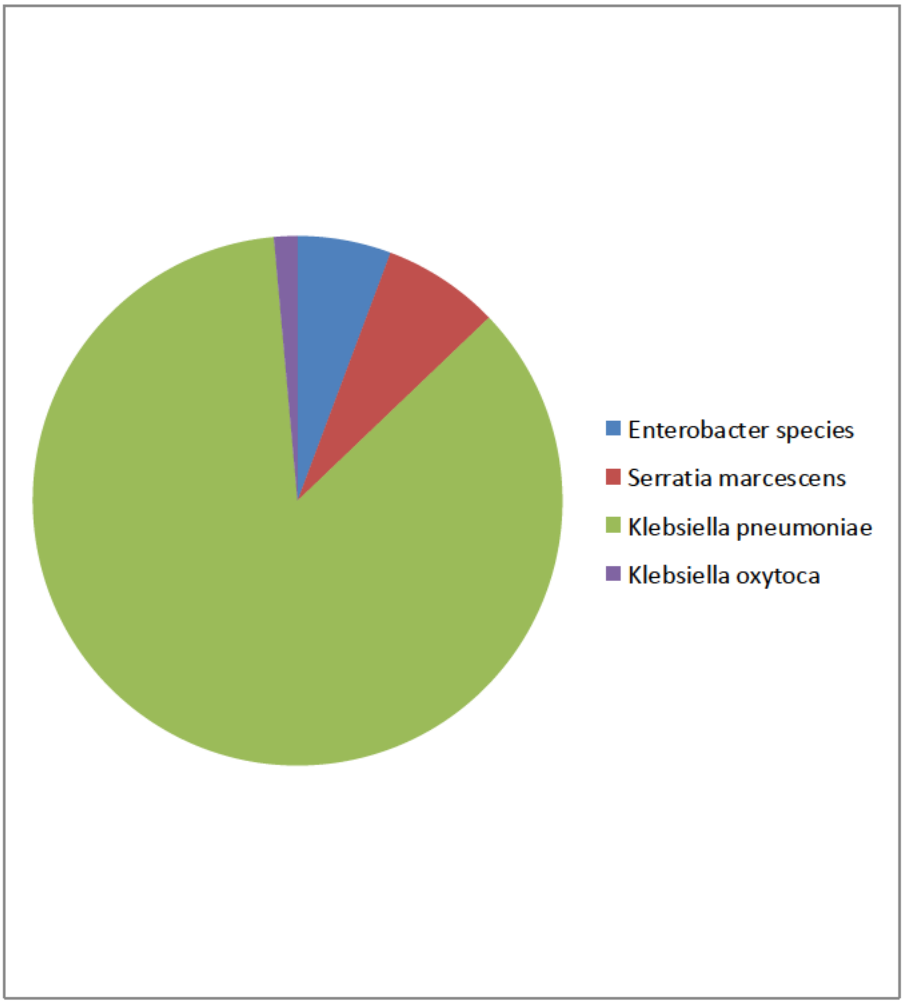

Figure 4.4: CZA resistance by species

4.4 Demographic distribution of OXA48 infections in KZN over 24 months

The distribution of OXA48 isolates is represented in Figure 4.5 according to the major towns and suburbs in KZN. This data was deduced from the location of the source hospital. However, for confidentiality reasons, the names of the source hospitals are not recorded in this study report.

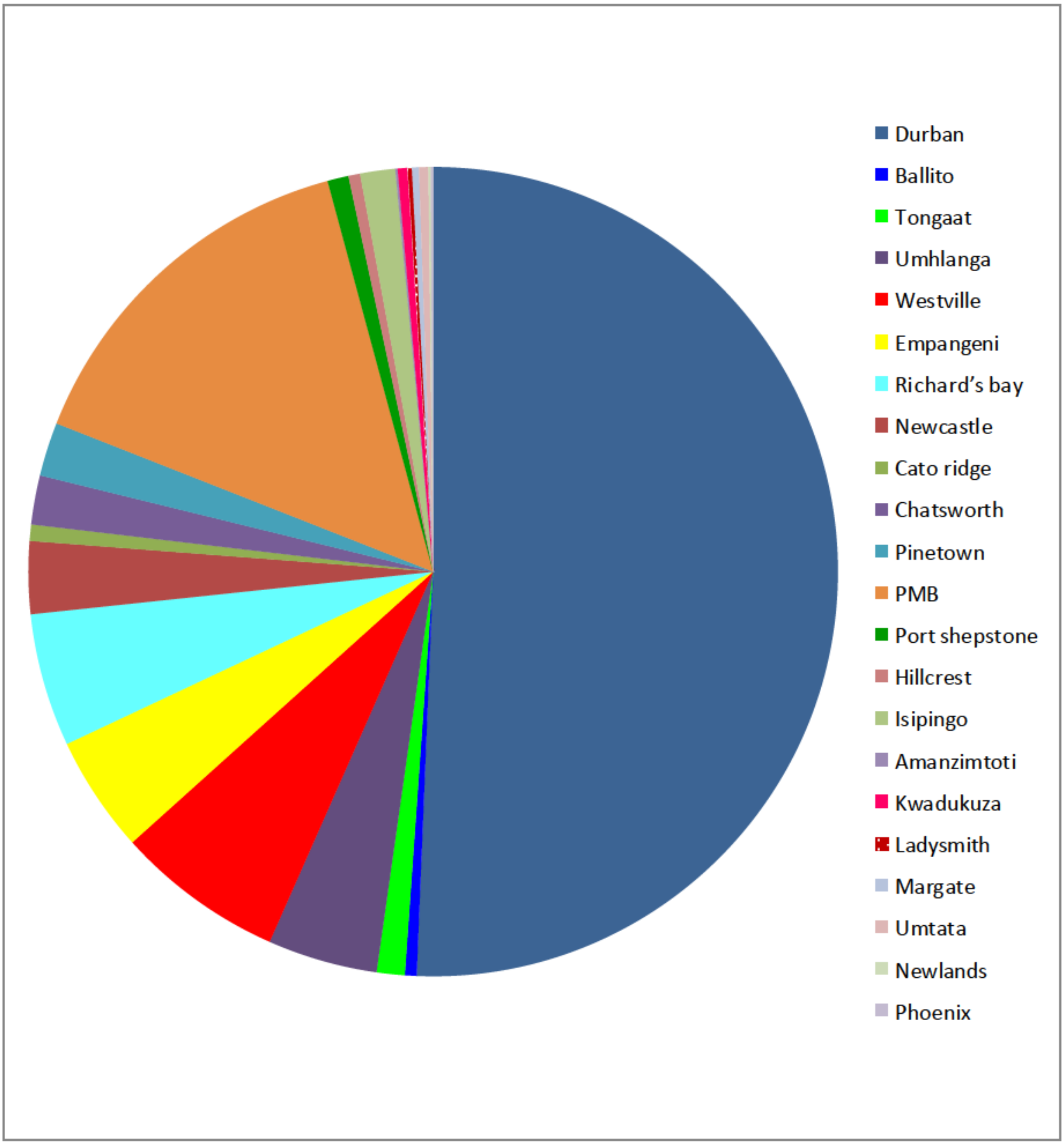

Figure 4.5: Distribution of OXA48 infections in the major KZN suburbs between 2020 and 2021

4.5 The difference in CZA susceptibility in the pre-CZA (2018/2019) versus post-CZA (2020/2021) period.

The p-value of ≤ 0.05 was used to determine if there was a statistical significance in the difference in percentage susceptibility of OXA48 isolates between the pre-CZA testing and the post-CZA testing periods. More isolates were CZA-susceptible in 2018/2019 than in 2020/2021. All eight OXA48 isolates tested from 2019/2020 were susceptible to CZA. From a total of 934 tested in 2020/2021, 864 were susceptible. Table 4.3 displays the percentage susceptibility for both years. Table 4.4 presents the proportion and the p-value to determine if there is any statistical significance in the difference between the two time periods. This is discussed in more detail in section 5.2.4.

Table 4.3: Percentage susceptibility of OXA48 to CZA in the pre-CZA-testing period compared to the post-CZA-testing period

Time-frame	Percentage susceptibility
2018/2019 (pre-CZA testing commencement)	100%
2020/2021 (post-CZA testing commencement)	92.50%

Table 4.4: Two-sample test of proportions

Group	N	Proportion	P-value
2018/2019	8	1	0.4208
2020/2021	934	0.925	

Chapter 5 provides a detailed discussion of the results and conclusions.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 Introduction

The aim of this study was to compare susceptibility patterns of OXA48 CPE isolates from January 2020 to December 2021 and establish whether there is development of resistance to the CZA drug. OXA48 isolates retrieved from 2018 and 2019 storage were also tested for CZA susceptibility and the results added to the susceptibility trend analysis. A breakdown of the demographic location of source patients was also made using the hospital location where the samples were sent from. In addition, analysis was done to establish which Enterobacterale species dominates the OXA48 group, and also which species was involved with the highest number of CZA-resistant isolates. Furthermore, a comparison was made between the 2018/2019 isolates and those from 2020/2021 to establish whether there is any statistical significance in the differences, if any, obtained between the two time periods.

In this chapter, the results of the study are discussed in detail. The results obtained are discussed in relation to the aims and objectives of the study, and the literature review. The chapter also brings to light the limitations and biases presented by the challenges encountered during the research process. Conclusions are drawn regarding what the results mean in relation to the study aims and objectives. Finally, recommendations are made as to how the information obtained in this study could be applied in the health sector to facilitate better patient care. The author also gives recommendations on how the challenges encountered during this study could have been overcome, and what can be done differently in similar studies in the future.

5.2 Discussion

5.2.1 OXA48 CPE and CZA resistance between 2020 and 2021

CPE infections are a serious threat to public health and are associated with high morbidity and mortality (Logan and Weinstein 2017). Brink *et al.* (2022) mention an increase in the proportion of CPE-mediated bacteraemia in South Africa. The current study showed that in 2020, 56% of CPE isolates documented in the Lancet Laboratories database were OXA48. This is higher than the 35% in 2021. Nevertheless, the OXA48 phenotype constituted a larger percentage of the total CPE population documented by the laboratory, among which were NDM, VIM and a combination of NDM and OXA48. These results concur with the findings of Lowe, Shuping and Perovic (2022a) that OXA48-producing CPE are the most common CPE pathogens isolated in South Africa.

The total number of CPE isolates documented for 2020 and 2021 were 902 and 1 473 respectively. This indicates a rise in the number of CPE isolated between the two years. Looking at Figure 4.2, a sharp rise in number of isolates is observed during the first trimester of the year for both 2020 and 2021. A decline in numbers is observed toward the last trimester of each year. This may not be statistically significant, but could instead be related to the overall decline in number of private hospitalisations observed toward every year end associated with unavailability of medical aid funds, and the subsequent rise early in the year as more funds become available.

Tootla *et al.* (2021) reported the first use of ceftazidime avibactam (CZA) for the successful treatment of a persistent CRE infection in a 17-month old burns patient in South Africa. In this current study, a total of 8 OXA48 isolates from the 2018/2019 period were available for analysis. Nine hundred and thirty four isolates were selected from the 2020/2021 period. No CZA resistance was observed in the isolates from the years 2018 and 2019. According to Van Duin and Bonomo (2016), CZA represents a good treatment option for OXA48 CPE, with good susceptibility results. This also proved true in this study because even though resistance to CZA was observed in the latter years, OXA48 susceptibility to the drug was over all very good, with 100% susceptibility observed in the 2018/2019 period, and 92.50% in the 2020/2021 period.

However, the country is already witnessing a rise in resistance to CZA due to its increased use in the private sector (Brink *et al.* 2022). The authors noted that the rise of resistance to CZA is alarming, not only in *K. pneumoniae*, but also recently among other common Enterobacterales species (Brink *et al.* 2022). Spera, Esposito and Pagliano (2019) highlighted the overuse of antibiotics as one of the major reasons for the development of resistance against these drugs. As stated by Van Duin and Bonomo (2016), there is concern that the widespread clinical use of CZA might be followed by a dramatic increase in resistance to the drug. In the current study, of the 495 isolates in 2020, 30 (6.06%) were resistant to CZA. Of the 439 isolates in 2021, 40 (9.11%) were resistant to CZA. Looking at Figure 4.3, it can be observed that the percentage resistance in 2021 was higher than that in 2020. If we are to include 2018 and 2019 in this observation, Figure 4.4 shows the beginning of an upward trend of resistance. This means that the KZN province has already begun to see a rise in CPE resistance to CZA.

5.2.2 CZA resistance by species

A study conducted between 2019 and 2020 in South Africa and reported by Lowe, Shuping and Perovic (2022a) showed that OXA48 *Klebsiella pneumoniae* was the most common CPE in the country.

Similarly, in this study, *Klebsiella pneumoniae* was the predominant species of CPE isolated. The isolate made up 80% of the total number of the OXA48 isolates. This is also in keeping with the observation made by Lowe, Shuping and Perovic (2022b) that *Klebsiella pneumoniae* remains the dominant Enterobacterale species among patients with CPE infections. In fact, King, Schmidt and Essack (2020) reported the presence of *Klebsiella* species in hospital effluents and wastewater in the Pietermaritzburg (PMB) area in KZN. Some of the *Klebsiella* isolates tested positive for the OXA48 carbapenemase (King, Schmidt and Essack 2020).

In this current study, 86% of the resistant isolates were *Klebsiella pneumoniae.* One could argue that this is due to the fact that the species already constituted the majority of the CPE population. However, this cannot present a bias to the conclusion since the Enterobacter species was the second most common OXA48 group, yet none of the isolates demonstrated any resistance. In fact, the first CZA-resistant OXA48 isolate reported in Saudi Arabia was a *Klebsiella pneumoniae* (Al Dabbagh et al. 2019). Russo *et al.* (2022) mentioned how important the management of MDR *Klebsiella pneumoniae* is, as this organism is associated with a high rate of nosocomial infections and mortality. The authors continue to emphasise how combined actions are needed to address infection control and antibiotic stewardship; and to delay the emergence of resistance (Russo *et al.* 2022). Even more importantly, the authors conclude that it is important to pay attention to the emergence of resistance to novel drugs, as already experienced with ceftazidime/avibactam, thus molecular microbiological surveillance must be implemented (Russo *et al.* 2022).

5.2.3 Demographic distribution of OXA48 infections in KZN

In a study conducted among South African academic hospitals between the years 2019 and 2020, KZN was among the top three provinces with the most CPE pathogens isolated (Lowe, Shuping and Perovic 2022b).

According to the results of the current study, most of the isolates were collected from patients residing in the Durban central area. This could be expected due to the location of the laboratory itself. However, as much as the laboratory is located in Durban, it receives samples from all over the province. Therefore, this number could still be a true reflection of demographic prevalence. The second highest number of isolates was from the PMB area, which is not as proximal to the laboratory as the other suburbs such as Amanzimtoti, Newlands and Phoenix. Yet the latter, more proximal areas had the lowest number of isolates. This could be confirmation that proximity to the laboratory did not affect the numbers isolated from that area.

5.2.4 The difference in CZA susceptibility in the pre-CZA versus post-CZA-testing period

The p-value of ≤ 0.05 was used to determine if there was a statistical significance in the difference in percentage susceptibility of OXA48 isolates between the pre-CZA testing and the post-CZA testing periods. The p value in this case is 0.4208, which means that there was no statistically significant difference between the CZA susceptibility in the pre-CZA (2018/2019) versus post-CZA (2020/2021) era. However, it is worth mentioning that the population numbers in the 2018/2019 time period were much lower. This is discussed in more detail in section 5.3.

5.3 Limitations and bias

The analysis of demographic distribution presented a bias for two reasons. Firstly, most samples turned out to be from the Durban area. This may not be a true comparison with the other areas due to the fact that the laboratory is already located in the Durban city centre. Secondly, the distribution of OXA48 infections was derived from the hospital that submitted the sample rather than from the patients' residence. This does not take into consideration out-of-town patients and those who reside far from the hospitals that they use. Hence the location analysis may not be an accurate reflection of the demographic prevalence of the OXA48 infections.

The population size for 2018/2019 presented a challenge. During this period, the prevalence of CPE infections was not as high as in the present age. As a result of this, there was a limited number of isolates available for testing. Furthermore, due to the prolonged storage of isolates, most of them turned out to be non-viable for use. This further reduced the number of isolates available for analysis and created a bias when comparing a population of 8 to that of 934. In order to minimise having non-viable isolates for research purposes, better isolate storage should be made use of if the isolates are to be preserved for periods longer than 2 years. According to Tedeschi and Paoli (2011), temperatures at -20°C are only sufficient to keep most organisms for a period of one to two years. A temperature of -70°C would need to be considered if isolates are to be stored longer than that.

The short time-frame taken to perform the surveillance also presented a limitation. As much as the study established the beginning of a trend, it would be advisable to carry the observation on for two or three more years to establish if the trend continues. It would be even more ideal if an antimicrobial stewardship intervention were to be introduced for the drug sometime along the continuing surveillance to see if it alters the course of the trend.

5.4 Conclusions

There is a rise in OXA48 resistance to the CZA drug between 2020 and 2021. The drug was registered by the South African Health Product Regulatory Authority (SAHPRA) on the 21st of September 2021. Surveillance and stewardship measures need to be put in place to ensure that it does not lose its effectiveness in the country's health system. *Klebsiella pneumoniae* still remains the predominant infection-causing CPE species. Further studies may need to be carried out to determine whether Durban really is the epicentre of CPE infections in the KZN province. Although there is a difference in CZA susceptibility between the isolates from the period before CZA susceptibility was being tested in South Africa, and those from the period after CZA susceptibility testing commenced, the difference was not statistically significant.

5.1 Recommendations

Further surveillance studies for CZA susceptibility in South Africa, such as prolonging the surveillance period by another year or two, would give a clearer picture of the pattern of resistance and how this problem could be curbed. Brink *et al.* (2022) wrote a review focused on how two vital new antibiotics, which included CZA, could be stewarded in South Africa. The authors suggested withholding of antibiotics like CZA in the treatment of resistant infections that can otherwise be treated with currently available antibiotics.

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Appendices

Appendix 1: Two-sample test

Two-sample test of proportions in STATA

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. . prtesti 8 1 934 .925

Two-sample test of proportions                     x: Number of obs
>  =       8
                                                   y: Number of obs
>  =     934
─────────────┬────────────────────────────────────────────────────────
> ───────────
             │       Mean   Std. Err.      z    P>|z|     [95% Conf
> . Interval]
─────────────┼────────────────────────────────────────────────────────
> ───────────
           x │          1          0                             1
>          1
           y │       .925   .0086184                      .9081082
>    .9418918
─────────────┼────────────────────────────────────────────────────────
> ───────────
        diff │       .075   .0086184                      .0581082
>    .0918918
             │  under Ho:    .093155     0.81   0.421
─────────────┴────────────────────────────────────────────────────────
> ───────────
        diff = prop(x) - prop(y)                                     z
>  =   0.8051
    Ho: diff = 0

    Ha: diff < 0                 Ha: diff != 0                 Ha:
> diff > 0
 Pr(Z < z) = 0.7896         Pr(|Z| > |z|) = 0.4208          Pr(Z >
> z) = 0.2104

.
```

Appendix 2: Editing certificate

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EDITING CERTIFICATE

Re: **Nomagugu Ndumo**
Master's dissertation: **Investigation of in vitro susceptibility of OXA48 carbapenemase-producing Enterobacterales to ceftazidime-avibactam over a 4-year period: a longitudinal-retrospective study**

I confirm that I have edited this dissertation and the references for clarity and language. I returned the document to the author with track changes so correct implementation of the changes and clarifications requested in the text and references is the responsibility of the author. I am a freelance editor specialising in proofreading and editing academic documents. My original tertiary degree which I obtained at the University of Cape Town was a B.A. with English as a major and I went on to complete an H.D.E. (P.G.) Sec. with English as my teaching subject. I was a part-time lecturer in the Department of Homoeopathy at the Durban University of Technology for 13 years and supervised many master's degree dissertations during that period.

Dr Richard Steele
2023
per email

Appendix 3: Gatekeeper permission

LANCET LABORATORIES

Lancet Data Use Agreement Extension 12 months

Lancet Laboratories and Nomagugu Ndumo hereby agree as follows;

1. The recipient agrees to use Lancet generated data only for research, and for public health and direct health care purposes.
2. The recipient agrees to 12 months exclusive use of this data from date that this document is signed. If a renewal or further time is required for the use of the data, the recipient must apply in email to the Lancet Publications Committee (LPC).
3. All Lancet generated data use will be used ethically and with respect for the patients involved.
4. The data will not be shared with any third parties of any kind without prior written permission from the Lancet Publications Committee (LPC).
5. Abstracts, draft and approved manuscripts, presentations or any written document generated with Lancet data is to be forwarded to the Lancet Publications Committee (LPC) on submission or completion.
6. Acknowledgements of Lancet for data used must be included in any presentation, written document, abstract or manuscript.

Signature and Name Recipient Date [illegible]

Signature and Name Lancet Publications Committee (LPC) Representative Date 19 July 2022

Appendix 4: Ethics approval

Institutional Research Ethics Committee
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26 August 2022

Mrs N Ndumo
31 Sunbird Crescent
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4111

Dear Mrs Ndumo

Investigation of in vitro susceptibility of OXA48 carbapenemase-producing Enterobactelares to ceftazidime-avibactam over a 4-year period: a longitudinal-retrospective study.

I am pleased to inform you that Full Approval has been granted to your proposal.

The Proposal has been allocated the following Ethical Clearance number **IREC 098/22**. Please use this number in all communication with this office.

Approval has been granted for a period of **ONE YEAR**, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the DUT-IREC. This form must be submitted to the DUT-IREC at least 3 months before the ethics approval for the study expires.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the DUT-IREC according to the DUT-IREC SOP's.

Please note that any deviations from the approved proposal require the approval of the DUT-IREC as outlined in the DUT-IREC SOP's.

Yours Sincerely

Dr K Padayachy
Deputy Chairperson: DUT-IREC

Appendix 5: Approval of amendment to PG2a

Institutional Research Ethics Committee
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4 January 2023

Mrs N Ndumo
31 Sunbird Cresent
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Dear Mrs Ndumo

Application for Amendment of Approved Research Proposal

Investigation of in vitro susceptibility of OXA48 carbapenemase-producing Enterobacterales to ceftazidime-avibactam over a 4-year period: a longitudinal-retrospective study
Ethics Clearance Number: IREC 098/22

I am pleased to inform you that your application for amendment to the sample size, samp selection and objectives of your study has been approved.

Yours Sincerely

Prof J K Adam
Chairperson: DUT-IREC