



**Ceftazidime-avibactam and Aztreonam susceptibility of Carbapenem Resistant
Enterobacterales and *Pseudomonas aeruginosa* isolates at Chris Hani
Baragwanath Academic Hospital**

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degree of

Master of Medicine in Medical Microbiology

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Declaration

I, Bontle Bessie Phooko, declare that this Research Report is my own work. It is being submitted for

the Degree of Master of Medicine in Medical Microbiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Signature:

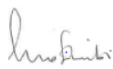
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Format of report

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Abstract

Background: The rising resistance of multidrug resistant organisms (MDROs) to currently available last resort antibiotics accompanied by increased mortality, has led to organisms such as Carbapenem Resistant Enterobacterales (CRE) and drug resistant *P. aeruginosa* becoming a global threat. New antibiotics such as Ceftazidime-Avibactam (CZA) and its use in combination with Aztreonam (ATM), has presented a glimmer of hope to this threat. There is a lack of susceptibility data of these MDROs to CZA and ATM in low to middle income countries such as South Africa. Finding out such information, may assist in motivating for acquisition and usage of these lifesaving antibiotics.

Objectives: The aim of the study was to determine the in vitro susceptibility data of CZA and ATM against CRE, Drug resistant *P. aeruginosa* at Chris Hani Baragwaneth Academic Hospital (CHBAH). Secondly, we aimed to investigate the invitro synergism of combined CZA and ATM to the CRE and *P. aeruginosa* isolates that were both CZA and ATM resistant.

Methods: This was a prospective laboratory-based study conducted at the National Health Laboratory Services (NHLS), Department of Microbiology, CHBAH. We tested 101 CRE isolates and 32 drug-resistant *P. aeruginosa* isolates, including multidrug-resistant (MDR) and extremely drug resistant (XDR) strains, against CZA and ATM. Those that were resistant to both CZA and ATM were then tested for synergy using the combination of CZA and ATM.

Results: Majority of the CRE isolates were *Klebsiella species* (84%) with OXA-48 & its variants being the predominant carbapenemase gene detected (67%). The *P. aeruginosa* isolates were predominantly carbapenem-resistant (88%), with 53% harbouring metallo- β -lactamases (VIM and NDM), and 78% classified as XDR. The overall CZA in vitro susceptibility amongst the serine active (OXA-48 positive) CRE in the study was 100%. The ATM susceptibility was 6% for all the CRE isolates tested. Synergism was seen in 96% of the CRE isolates that were resistant to both CZA and ATM. The activity of CZA and ATM against *P. aeruginosa* was 34% and 44% respective. Furthermore, the synergistic effect of combined CZA and ATM was seen in only 64 % of the *P. aeruginosa* isolates qualifying for testing.

Conclusion: Local evaluation of CZA and CZA-ATM synergy is important in this era of antimicrobial resistance where there is great need of new treatment options. The high number

of CZA susceptible organisms amongst the OXA-48 isolates is encouraging. Synergistic activity of CZA and ATM presents as a good alternative for the MBL producers. More rigorous studies are needed to determine the clinical impact of usage of these antimicrobials.

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List of abbreviations

ATM:	Aztreonam
BL-BLI:	β -lactam β -lactamase inhibitors
CHBAH:	Chris Hani Baragwanath Academic Hospital
CLSI:	Clinical and Laboratory Standards Institute
CPE:	carbapenemase producing <i>Enterobacterales</i>
CR:	carbapenem-resistant
CRE:	carbapenem-resistant Enterobacterales
CZA:	Ceftazidime- Avibactam
DTR:	Difficult-to-treat
ESBLs:	extended spectrum β lactamases
ESKAPE:	Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species.
E-test:	Epsilometer test
GERMS-SA:	Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa
HAIs:	Hospital acquired infections
IMP:	active-on-imipenem carbapenemase
KPC:	Klebsiella pneumoniae carbapenamases
MBLs:	metallo- β -lactamases
MDR:	Multidrug resistant
MDRO:	Multi drug resistant organisms
MIC:	Minimum inhibitory concentration
NDM:	New Delhi Metallo- β -lactamase

NHLS: National Health Laboratory Services

OXA-48 and its variants/OXA-48: Oxacillinase β -lactamases

P. aeruginosa: *Pseudomonas aeruginosa*

SA: South Africa

VIM: Verona integron-encoded Metallo- β -lactamase

WHO: World Health Organisation

XDR: Extremely drug resistant

1. RESEARCH REPORT

1.1.Introduction

The World Health Organisation (WHO) reports antimicrobial resistance as an important threat to global health (1). As such carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant (CR) *Pseudomonas aeruginosa* (*P. aeruginosa*) form part of the ESKAPE pathogens classified by the WHO as critical priority pathogens needing novel antibiotic intervention due to their resistance patterns (1). The prevalence of these multidrug resistant organisms (MDRO) as a cause of hospital acquired infections (HAIs) has been observed to be significantly increasing in South Africa (SA), where there are limited antibiotic options particularly in the public sector (2,3). This situation is further aggravated by the rising resistance to currently available last resort antibiotics such as colistin and tigecycline (2,4). This rising resistance, more reported in the Gauteng province, is accompanied by increased mortality (2,4). Furthermore, the unfavourable side effect profile of colistin and the limited utility of tigecycline, combined with the high in-hospital mortality rates despite the availability of these antimicrobials, underscore the critical need for better treatment options (2).

The epidemiology of CRE in SA is largely made up of *K. pneumoniae*, with the most predominant carbapenemase producing Enterobacterales (CPE) genes being the Oxacillinase β -lactamases (OXA-48 and its variants/OXA-48) followed New Delhi Metallo- β -lactamase (NDM) (5). This is data from the Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South (GERMS-SA) CRE surveillance system for 2019-2020 and is consistent with what is seen at Chris Hani Baragwanath Academic Hospital (CHBAH) where the most common carbapenemase amongst the CRE is the OXA-48 and its variants (5)(unpublished data).

P. aeruginosa is an important pathogen, commonly acquired in the hospital setting, where it can present as multidrug-resistant (MDR) or extensively drug-resistant (XDR) (2,6). MDR and XDR is defined by Magiorakos et al. as “non-susceptibility to at least one agent in three or more antimicrobial categories and non-susceptibility to at least one agent in all but two or fewer antimicrobial categories” where susceptibility is expected, respectively (7). In *P. aeruginosa*, antimicrobial resistance mechanisms include the overexpression of efflux pumps combined with decreased permeability, target modification, and the acquisition of β -lactamases, including carbapenemases (2). Among these, the Verona integron-encoded

metallo- β -lactamase (VIM) is noted as the prevalent carbapenemase in South Africa(2). These resistance patterns, in their full extent can lead to what have recently been termed difficult-to-treat (DTR) *P. aeruginosa* where non susceptibility is seen in all the following antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, fluoroquinolones and the inclusion of carbapenems (8). The type of resistance reported in South Africa surveillance data for this organism, is 15% resistance to piperacillin-tazobactam, and 23% resistance to carbapenems (3). Although there is no mention of DTR *P. aeruginosa* in the fore mentioned surveillance data, emerging colistin resistance has been noted in certain publications, giving some idea of its existence (2). This emphasises the need for an expanded range of antimicrobial options to combat such infections.

Ceftazidime-avibactam (CZA), is classified as a new generation β -lactam/ β -lactamase inhibitors (BL-BLI) (2). It is a combination of a third-generation cephalosporin ceftazidime and the β -lactamase inhibitor avibactam, approved by the US Food and Drug Administration (FDA) for treating complicated intra-abdominal infections, complicated urinary tract infections (including pyelonephritis) and hospital-acquired bacterial pneumonia in patients as young as 3 months and older (9–11). CZA has demonstrated good efficacy against multidrug resistant (MDR) *P. aeruginosa* and CRE Ambler class A (e.g. *Klebsiella pneumoniae* carbapenemases (KPC)), C, and D (OXA-48 and its variants) β -lactamase-producing isolates, however, it is hydrolysed by metallo- β -lactamases (MBLs) thus rendered ineffective for these (2,6,10). It is currently registered in SA and its use is recommended in the guidelines for treatment of the above organisms albeit mostly limited to private institutions (2). Furthermore, CZA has been shown to have good tolerability and safety profiles as well as fair cost implications (2,11). CZA is presently the recommended agent for the treatment of patients with severe infections because of OXA-48-producing Enterobacterales failing on first line therapy (2).

Aztreonam (ATM) on the other hand, is a monobactam effective against MBLs, however, it is easily inactivated by extended spectrum β lactamases (ESBLs) (Amber class A), KPCs (Amber class A), and AmpC β lactamases (Amber class C) (12,13) . Because MBLs-carrying Gram negative organisms may frequently harbour additional ATM-inactivating β -lactamases such as ESBL, the use of ATM against these isolates is only recommended when being used in combination with other drugs, namely CZA (2,12,13). Combination treatment with ATM and CZA has demonstrated reasonable efficacy with a lower mortality rate in MBL producing CRE and in some MDR-*P. aeruginosa* infections albeit less pronounced in the latter (12,13).

Moreover, use of this combination treatment has been shown to be associated with a good safety profile (12,13).

Different methods testing the synergy between CZA and ATM have been described. Disk elution and gradient strip crossing are the two low complexity methods found to have reasonable accuracy and precision when using modified broth microdilution as a gold standard (14). Disk elution combines disk diffusion with use of broth, and gradient strip crossing involves placement of CZA and ATM gradient strips on Mueller-Hinton agar (14). These two methods are low complexity methods that would be accessible to a clinical microbiology laboratory(14).

The use of CZA as well as a combination of CZA and ATM against the above mentioned MDR organisms is mostly limited to private facilities in South Africa (2). Furthermore, there is a lack of susceptibility data of CZA and ATM against CRE and *P. aeruginosa* isolates in public facilities including our centre, CHBAH. Knowledge of the susceptibility profile of CRE and *P. aeruginosa* isolates in a large public tertiary referral centre found in Gauteng province in SA, would be a steppingstone in beginning to use these lifesaving drugs for a community that is in dire need for new therapeutic options. Therefore, the aim of the study was to demonstrate the in vitro susceptibility of CRE, and drug-resistant *P. aeruginosa* to CZA and ATM at CHBAH.

Secondarily, we aimed to determine the invitro synergism of combined CZA and ATM to the CRE and *P. aeruginosa* isolates that were both CZA and ATM resistant.

1.2. Methods

Study Design and Setting

This was a prospective laboratory-based study conducted over a period of 8 months, between the months of September 2022 to May 2023. The study was conducted at the National Health Laboratory Services (NHLS), Department of Microbiology, CHBAH. CHBAH is a 3200-bed hospital in Soweto, Johannesburg, SA serving as a referral centre for the surrounding smaller hospitals (15). It consists of an adult, paediatric and neonatal intensive care unit as well as a specialised burn's unit. This is an academic hospital with functional specialist multidisciplinary teams comprising of microbiologists, pharmacists, infectious disease physicians and other clinical specialists responsible for making patient treatment decisions,

particularly for those with difficult to treat infections. An antimicrobial stewardship programme is in place, to ensure the appropriate use and conservation of expensive last resort antimicrobials such as CZA and ATM.

Sample and Sample Size

Samples tested were known CRE and *P. aeruginosa* isolates cultured from different body sites (blood, cerebrospinal fluid, pus, tissue, fluid, urine, and respiratory samples) submitted as part of the routine laboratory hospital procedure for microscopy, culture, and susceptibility testing at CHBAH NHLS microbiology laboratory. All swabs (including surveillance swabs) were excluded. Deduplication of the samples was ensured, meaning with samples of the same organism from the same patient including those from different sites, only one was used. Convenience sampling was used for sample collection.

One hundred and one CRE isolates were used for this study, which amounts to 33.7% of Enterobacterales isolates seen at CHBAH per month. A sample size of 32 *P. aeruginosa* were used which amounts to the average number of *P. aeruginosa* isolates seen in our institution a month. The CRE and *P. aeruginosa* isolate numbers were chosen to minimise cost and are not representative of a statistically significant estimation. The current CRE rate at CHBAH ranges between 17- 44% and the drug-resistant *P. aeruginosa* rate is between 0- 33% monthly (unpublished data).

CRE and drug-resistant *Pseudomonas aeruginosa* detection

The known CRE and *P. aeruginosa* isolates that were used, were those that have been cultured and identified according to the CHBAH Microbiology Laboratory Standard Operating Procedures for the respective sample types. The specimens were inoculated on 5% blood agar, chocolate agar and MacConkey agar plates and incubated aerobically overnight at 35°C–37°C. Bacterial species identification was achieved using the API® 20E kit (bioMérieux SA, France) or the MicroScan WalkAway system. Routine antimicrobial susceptibility testing was performed using the disk diffusion method or the MicroScan WalkAway system as prescribed by Clinical and Laboratory Standards Institute (CLSI) M100 33rd edition (16). At the time when this study was performed, CHBAH microbiology laboratory used mostly manual methods (API® 20E kit (bioMérieux SA, France) and disk diffusion) for identification and antimicrobial susceptibility testing. The automated MicroScan WalkAway system with use of the NM44 panel would only be employed in certain situation, such as difficulty with identification of an organisms using manual methods.

Enterobacterales resistant to any of the carbapenems (ertapenem, imipenem or meropenem) were identified as CRE. Those that were intrinsically resistant to imipenem without acquired resistance to carbapenems such as those in the Proteaeae family were excluded. Further CRE characterisation was detected using the RESIST-4 O.K.N. V[®] (Coris Bioconcept, Belgium) and then later RESIST-5 O.K.N.V. I[®] (Coris Bioconcept, Belgium) which are both invitro immunochromatographic rapid diagnostic tests for the detection of OXA-48 & its variants, KPC, NDM, VIM with the addition of active-on-imipenem (IMP) carbapenemases for RESIST-5 O.K.N.V.I[®], in bacterial culture (17). RESIST-4 O.K.N.V[®] was used for 82% of the CRE isolates as RESIST-5 had not yet been introduced at CHBAH at the time of testing. RESIST-5 O.K.N.V.I[®] was later used for the rest of the (18 %) of CRE isolates and all the *P. aeruginosa* isolates. The reason for this was due to the discontinuation of RESIST-4 O.K.N.V[®] by the supplier and introduction of RESIST-5 O.K.N. V. I[®]. The antibiotics that are routinely tested at NHLS CHBAH for *P. aeruginosa* are piperacillin-tazobactam, ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin, and aminoglycosides (tobramycin, amikacin, gentamicin). The *P. aeruginosa* isolates used were those that were drug resistant, either carbapenem resistant, or resistance to first line antibiotics (piperacillin-tazobactam and ceftazidime) but susceptible to the carbapenems. Additionally, these drug-resistant *P. aeruginosa* isolates also met the criteria of either being MDR or XDR according to established definitions. DTR classification could not be applied to the *P. aeruginosa* isolates in this study because Aztreonam was not routinely tested at the time of the research.

Ceftazidime-avibactam and Aztreonam antimicrobial susceptibility testing

The disk diffusion method was used to test susceptibility of both CZA and ATM against the CRE and *P. aeruginosa* isolates. Disk concentrations 30/20µg for CZA and that of 30µg for ATM were used. Interpretation of susceptibility was according to the CLSI guidelines, with *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 being used as quality control strains. In vitro susceptibility for CZA was interpreted as a disk zone diameter of ≥ 21 mm as according to the CSLI (16) for all the isolates tested. For Enterobacterales, the CLSI recommends confirmatory minimum inhibitory concentration (MIC) testing for isolates that have a zone diameter of between 20 - 22mm for CZA to avoid reporting false-susceptible or false-resistant results (16). CZA with an MIC of $\leq 8/4\mu\text{g/mL}$ was reported as susceptible as per CLSI guidelines (16). The Epsilometer test (E-test) gradient test strip from Liofilchem[®] was used to confirm the MIC. For ATM, a disk zone of ≥ 21 mm and ≥ 22 mm was read as susceptible for CRE and *P. aeruginosa* isolates respectively (16).

Ceftazidime-avibactam and Aztreonam synergy testing.

Isolates that were resistant to both CZA and ATM were further tested using CZA and ATM in combination. The strip cross method described by Khan et.al was used (14). For this method, Muller Hinton agar plates were inoculated with 0.5 McFarland inoculum of the isolate (14). The ATM E-test strip was then placed directly on the agar followed by the CZA E-test strip crossing perpendicular to the ATM strip at the intermediate breakpoint of 8µg/ml for CRE and 16µg/ml for the *P. aeruginosa* isolate (14). These were then incubated aerobically at 37°C over 24 hours.

A strain that was considered synergy positive was one that was resistant to both CZA and ATM, but the ATM MIC became susceptible ($\leq 4\mu\text{g/mL}$ & $\leq 8\mu\text{g/mL}$ for CRE and *P. aeruginosa* isolates respectively) or intermediate ($8\mu\text{g/mL}$ & $16\mu\text{g/mL}$ for CRE and *P. aeruginosa* isolates respectively) in the presence of the CZA strip (14). The choice of method was primarily driven by funding constraints, to minimize expenses on additional consumables.

1.3. Data Analysis

The isolates tested were reported as number and percentage susceptible or resistant as described above. STATA, version 18 (StataCorp, USA) was used to analyse and formulate tables of the data generated from the samples tested. The assistance of a biostatistician was utilised.

1.4. Ethics

The study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Clearance certificate number: M220556. All samples were anonymised, furthermore, there was no patient information nor patient participation included in this study.

1.5. Funding

Funding for the study was through NHLS K-Funding Project (REF PR2227016).

1.6. Results

Distribution of isolates

The distribution of the CRE isolates used in this study are depicted in Table 1. Of the 101 isolates, 85 (84%) of them were *Klebsiella species* (i.e. *K. pneumoniae*, *K. oxytoca* and *K. species*) which made up the majority of the CRE tested, followed by 8 (7%) *E. coli* and 4 (4%) *E. cloacae*. The isolates were mostly from blood cultures (Table 2), followed by respiratory samples (sputum and tracheal aspirates) then urine. The predominant carbapenemase gene detected was OXA-48 & variants (OXA-48), detected in 68 (67%) of the isolates, with the second commonest being a combination of OXA-48 and NDM genes in 19 isolates (19%) (Table 3).

The *P. aeruginosa* isolates used in this study consisted of 88% (n=28) carbapenem resistant isolates, and 22% (n= 4) showed resistance to first line antibiotics (piperacillin-tazobactam and ceftazidime) but susceptibility to the carbapenems. Seventy eight percent (n=25) were classified as XDR, while 22% (n=7) were MDR. Majority of these were from blood cultures 11 (34%) (Table 2) and more than half of the isolates had metallo- β -lactamase genes (VIM and NDM) (Table 4). No carbapenemase genes were detected in the other 15 (47%) *P. aeruginosa* isolates (Table 4).

Table 1: Distribution of Carbapenemase Resistant Enterobacterales used in the study.

Distribution of CRE per organism	
Organism	Number
<i>K. pneumoniae</i>	81 (80%)
<i>K. oxytoca</i>	1 (1%)
<i>K. species</i> *	3 (3%)
<i>E. coli</i>	8 (7%)
<i>E. cloacae</i>	4 (4%)
<i>C. freundii</i>	1 (1%)
<i>M. morgani</i>	1 (1%)
<i>S. liquefaciens</i>	1 (1%)
<i>S. marcescens</i>	1 (1%)

*Refers to isolates that could not be speciated that belong to the genus *Klebsiella*.

Table 2: Culture source for isolates of Carbapenemase Resistant Enterobacterales and *Pseudomonas aeruginosa* collected for the study.

Specimen type	Number of CRE isolates	Number of <i>P. aeruginosa</i> isolates
Total number of isolates	101 (100%)	32 (100%)
Blood culture	47 (46%)	11 (34%)
Urine	12 (12%)	1 (3%)
Sputum	8 (8%)	3 (9%)
Tracheal aspirate	6 (6%)	4 (13%)
Catheter tip	6 (6%)	4 (13%)
Pus	6 (6%)	3 (9%)
Tissue	9 (9%)	1 (3%)
Fluid	6 (6%)	5 (16%)
CSF*	1 (1%)	0 (0%)

*CSF Cerebrospinal fluid

The CRE distribution per patient population of the tested isolates, was made up largely of isolates from the adult medical wards which comprised mainly of OXA-48 producing CRE (Figure 1).

The other common areas that were represented are the neonatal wards with only OXA-48 positive isolates, intensive care unit (ICU), general surgery and orthopaedic wards with CRE harbouring largely OXA-48 genes, followed by the combination of OXA- 48 and NDM. (Figure 1).

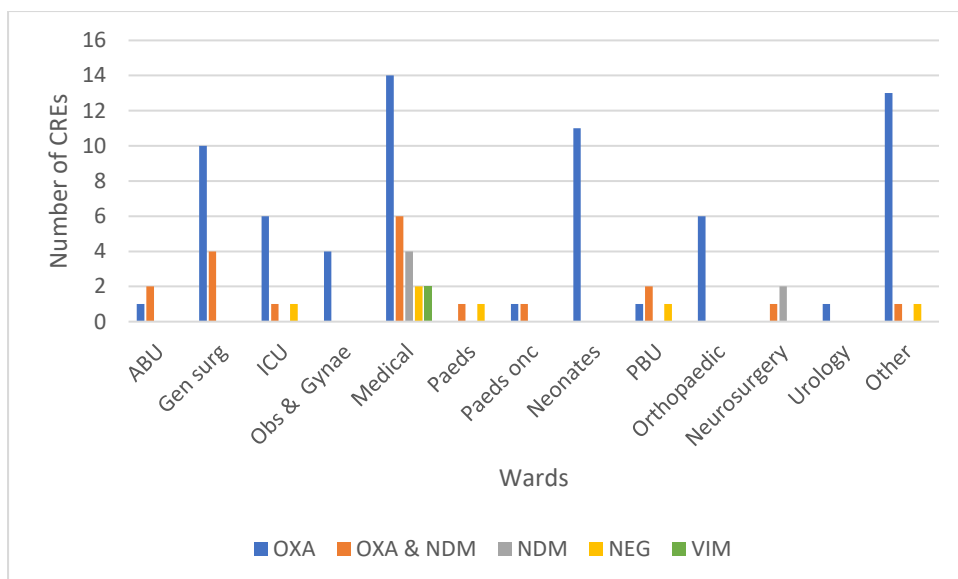


Figure 1: Ward distribution of CREs.

ABU= adult burns unit; Gen surg = general surgery; ICU= intensive care unit; Obs & Gynae = obstetrics and gynaecology; Paeds= Paediatrics; Paeds onc=Paediatric oncology; PBU= paediatric burns unit; Other= other wards

The *P. aeruginosa* isolates were isolated largely from the general surgery wards, adult burns unit (ABU) and intensive care unit (ICU) respectively (Figure 2). Isolates from the general surgery wards were predominantly ATM susceptible and VIM positive. However, ATM was active in only half from ICU with majority being carbapenemase negative isolates. ABU largely had ATM resistant, VIM positive isolates. CZA-ATM Synergy was observed in these isolates that qualified for testing (see supplementary data).

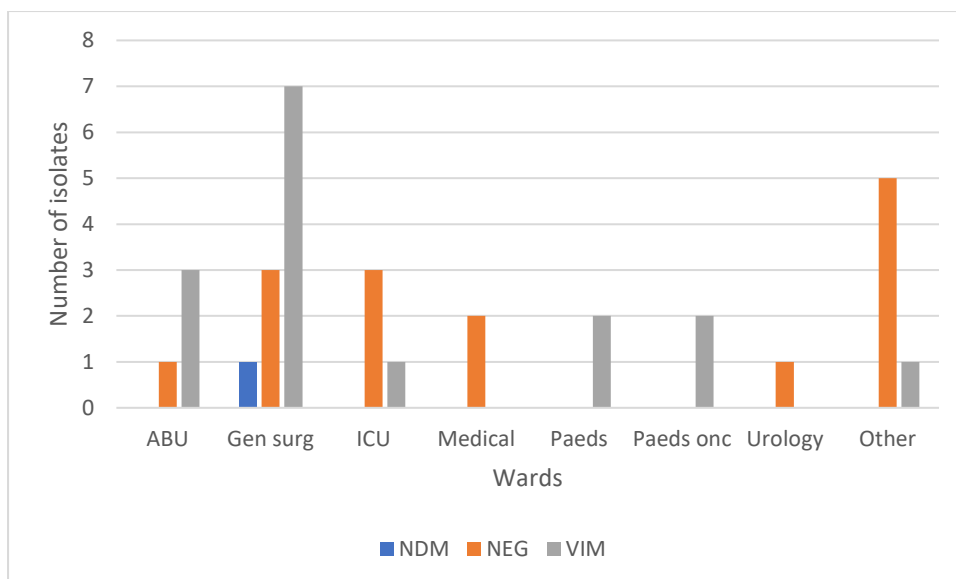


Figure 2: Ward distribution of *P. aeruginosa* isolates.

ABU= adult burns unit; Gen surg = General surgery; ICU= intensive care unit; Paeds= Paediatrics; Paeds onc=Paediatric oncology; Other= other wards

CZA and ATM susceptibility testing results for CRE isolates.

More than half of the CRE isolates were OXA-48 producers, with CZA demonstrating 100% (n=68) in vitro susceptibility. (Table 3). ATM susceptibility was only seen in 3% (n=2) of these isolates (Table 3). Synergy in these isolates was not tested as this was not required.

The overall activity of ATM against CRE was low, only 6% (n=6) isolates were susceptible, including those that harboured MBL genes with activity in 11% (3 of the 27 isolates).

However, the overall synergistic effect when combining CZA and ATM was good at 96% for the 26 isolates where testing was appropriate (Table 3).

The 6 CRE isolates that tested negative for carbapenemases, resulted in 50% (n= 3) of them showing CZA in vitro susceptibility and 17% (n=1) being ATM susceptible (Table 3). For the 2 carbapenemase negative isolates with dual resistance to CZA and ATM, synergism was seen when these drugs were tested in combination (Table 3).

Table 3: Susceptibility results of CRE

Distribution of CRE per carbapenemase enzyme	Number of isolates	CZA susceptible	CZA resistant	ATM susceptible	ATM resistant	*Synergy positive	*Synergy negative
OXA-48 & variants	68 (67%)	68 (100%)	0 (0%)	2 (3%)	66(97%)	N/A	N/A
NDM	6 (6%)	0 (0%)	6 (100%)	3 (50%)	3 (50%)	3 (100%)	0 (0%)
OXA-48 & variants and NDM	19 (19%)	0 (0%)	19 (100%)	0 (0%)	19 (100%)	18 (95%)	1 (5%)
VIM	2 (2%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)
Neg	6 (6%)	3 (50%)	3 (50%)	1 (17%)	5 (83%)	2 (100%)	0 (0%)
Total number CRE	101 (100%)	71 (70%)	30 (30%)	6 (6%)	95 (94%)	25 (96%)	1 (4%)

*Isolates tested for synergy, are those that are both CZA and ATM resistant.

CZA and ATM susceptibility results for *P. aeruginosa* isolates

In vitro susceptibility of *P. aeruginosa* isolates to CZA for the isolates that are non-MBL producers (n=15) was demonstrated in 73% (n=11) isolates (Table 4). ATM activity was shown in 44% (n=14) of the 32 isolates, with only 50% susceptibility amongst the MBL positive population (Table 4). Positive synergism was seen in 64% of the isolates that showed dual resistance to CZA and ATM (n=11) (Table 4). CZA susceptibility was only seen in the isolates where carbapenemases were undetected whereas majority of ATM susceptibility occurred in the isolates with the metallo- β -lactamases VIM and NDM, 50% (n=8) and 100% (n=1) respectively. Synergism was also highest amongst the MBLs at 87.5% (Table 4).

Table 4: Susceptibility results of *P. aeruginosa* isolates

Distribution of <i>P. aeruginosa</i> isolates per carbapenemase enzyme	Number of isolates	CZA susceptible	CZA resistant	ATM susceptible	ATM resistant	*Synergy positive	*Synergy negative
VIM	16 (50%)	0(0%)	16 (100%)	8 (50%)	8(50%)	7 (87.5%)	1 (12.5%)
NDM	1 (3%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	Not tested	Not tested
NEG	15(47%)	11 (73%)	4 (27%)	5 (33%)	10 (67%)	0 (0%)	3 (100%)
Total number of <i>P. aeruginosa</i> isolates	32 (100%)	11 (34%)	21 (66%)	14 (44%)	18 (56%)	7 (64%)	4 (36%)

*Isolates tested for synergy, are those that are both CZA and ATM resistant.

1.7. Discussion

The findings of this study of a predominance of *K. pneumoniae* CRE together with the OXA-48 & variants (OXA-48) carbapenemase being dominant, is what is characteristic of the epidemiology of CRE in SA (5). In this study the incidence of *K. pneumoniae* and OXA-48 carbapenemase was noted to be 78% and 77% respectively. In SA, *K. pneumoniae* CRE has an estimated prevalence of 79.8% with the predominance of the OXA-48 carbapenemase being at 76.8 % (5). Our study identified a predominance of the OXA-48 carbapenemase, followed by a combination of OXA-48 and NDM, and then NDM alone among the tested Enterobacterales. This pattern differs slightly from findings in tertiary academic hospitals like ours, where the order of predominance was OXA-48, NDM, and VIM (5). The variation may be attributed to the larger sample sizes and the inclusion of multiple hospitals in those studies. Global surveillance data on the epidemiology of Meropenem-Nonsusceptible (MEM-NS) Enterobacterales, show *K. pneumoniae* constituting the majority at 71.5%, with MBLs represented in 37.1%, KPC in 25.5%, and OXA-48 carbapenemase in 24.1% of these (MEM-NS) carbapenemase-positive isolates (18). However, when focusing on region specific epidemiology of the leading carbapenemase between the year 2018-2019, in Africa it is NDM (subset of MBL) at 44.3% followed closely by OXA-48 carbapenemases at 42.7%, in Europe OXA-48 at 32.4% dominates, and lastly in the Americas KPC at just over 53% (18). Prior to this time period OXA-48 carbapenemases were leading in Africa and still are in SA which is consistent with our study (5,19).

The total CZA invitro susceptibility of the CRE isolates was observed in 71 (70%) of our isolates. However, when looking at the isolates where CZA is known to be active, that being those that are OXA-48 positive in our study, susceptibility was excellent and observed in all the 68 (100%) isolates. The high susceptibility seen in these is consistent with that of a recent study done in sub-Saharan Africa where all the serine active CRE (this included 98 % OXA-48 carriers and 2% KPC carriers) were CZA susceptible (19). This sub-Saharan study included data that had been collected from the years 2017-2021 (19). Notably, CZA was only registered for use by the South African Health Products Regulatory Authority (SAHPRA) in September 2019. Prior to this, it had been an unregistered drug requiring licencing through SAHPRA, used predominately in the private sector from 2018 (2) . When our study was conducted, only a handful of patients had received CZA for treatment at CHBAH. The limited use and, consequently, lack of exposure of CRE to this drug may explain the currently high susceptibility rates observed in our setting. The overall CZA susceptibility of CRE in the

above-mentioned sub-Saharan study was found to be 34.1% which is much lower compared to our study. This is because their CRE isolates consisted mainly of MBL producers 61% in comparison to ours which had 67% serine carbapenemase producing CRE. Similarly, a study done in the Arabian Peninsula showed a lower CZA susceptibility of 53% for CRE collected as their MBL producing isolates where much higher at 46.3% compared to those in our study 27% (20). Although CZA resistance has not yet been described in OXA-48 producing CREs, there is a rising concern for resistance most notable in *K. pneumoniae* harbouring mutations in the *blaKPC* gene (21). With the steady increase in KPC that is being seen in Gauteng province SA, this may become a problem we face soon in our setting (2). Other potential reasons that have been suggested for CZA resistance are use in the immunocompromised population with deep seated infection, as well as use in those receiving continuous renal replacement therapy due to dosing uncertainty (22). This patient population type does exist in our context, and this may therefore present as a potential threat for resistance.

ATM susceptibility of CRE as expected, was low with only 6 (6%) isolates being inhibited. Wise et al. showed a 9.6% ATM susceptibility of CRE that were non-MBL producers and an even lower 7.9% susceptibility in those that are serine based CRE, which was the majority of isolates in our study (19).

When looking at the distribution of *P. aeruginosa* isolates in our study, the MBL carbapenemase VIM was detected in half of the samples 16 (50%) together with 1 (3%) NDM isolate. No carbapenemase enzymes was detected in the rest of the isolates 15 (47%). Similarly, in surveillance programmes looking at global prevalence of *P. aeruginosa*, VIM-type MBL appears to be the most common carbapenemase amongst the carbapenemase positive isolates (21,23).

The overall CZA resistance of the *P. aeruginosa* isolates was high 21 (66%), however the isolates that are MBL negative had a resistance of 27% which is 4 of the 15 isolates. The high CZA resistance is expected as more than half of the isolates were MBL producers, and thus 100% resistance was seen for these. The resistance rate of the MBL negative isolates is comparable to a global study, looking at different regions, that has seen an 8.6-53.2 % resistance (21) . The ATM resistance was noted to be higher, 18 isolates (56%), than what has been recorded in the sub- Saharan African region (30.9%) but comparable to other parts of the world (48-97%) as seen in the data collected by the Antimicrobial Testing Leadership

and Surveillance (ATLAS) programme used in the above global study, and in a subset of it which was more specific to our region (19,21).

The synergy rate between CZA and ATM for the CRE and *P. aeruginosa* isolates that had dual resistance, was 96% and 64% respectively. The synergy results for the CRE isolates in this study were consistent with previously reported in vitro synergy rates of 80-100% (20,23,24). However, the synergy rates for the *P. aeruginosa* isolates were found to be lower than the $\geq 80\%$ observed in other studies. (24,25). The low synergy results may be attributed to the small number of *P. aeruginosa* isolates tested.

On the other hand, a significantly higher synergistic effect of 87.5% was observed when focusing specifically on the MBL-positive isolates within the same *P. aeruginosa* group. This may be due to the enhanced mechanisms of action provided by the combination of these antimicrobials, which are particularly effective against this class of resistance (24). The conflicting evidence regarding CZA-ATM combination therapy for *P. aeruginosa* is also reflected in the literature, with some studies demonstrating synergistic effects, while others show none (13,24,25). This could potentially be attributed to the variability in testing methods employed.

Although there is evidence of in vitro synergism for combination CZA-ATM, there is a lack of good clinical evidence for it. There are currently no randomised control trials, only observational and cohort studies exist. Though individual studies suggest improved clinical outcomes with this combination treatment for multidrug-resistant organisms, systematic reviews have highlighted significant heterogeneity in definitions and outcomes, resulting in an unclear overall benefit (13,24).

Studies with a context similar to what we have in South Africa, has shown promising results. Taha et al. stated that the combination of CZA and ATM is effective especially against isolates producing more than one carbapenemase genes (26). Prayag and colleagues in a study based in India where there is a predominance of NDM and OXA-48 producing CRE, compared polymyxin based combination therapy and CZA based therapy (with or without ATM). The results of this retrospective study demonstrated that CZA-based therapy was associated with better outcomes compared to polymyxin-based combination therapy, including lower 14 and 28-day mortality rates as well as fewer adverse effects (27). These two studies reflect a context similar to that of CHBAH, South Africa, in terms of the type of CRE resistance and the current reliance on polymyxin-based drugs for treatment, driven by

drug availability and usage patterns. Thus, this combination would present a potentially effective therapeutic option, however, clinical efficacy, the optimal dosing strategy and safety of this regimen must be evaluated locally.

The limitations of this study include the fact that molecular detection of the carbapenemase genes was not done, instead RESIST-4 O.K.N.V and then later RESIST-5 O.K.N.V.I, both immunochromatographic lateral flow assay, were used for this purpose. This assay and its predecessors, although highly sensitive and specific, has had problems with detection of false negative NDM in isolates with NDM and combination OXA 48/NDM carbapenemases (28–30). This was demonstrated when some of the isolates that were resulted as purely OXA-48 positive, were CZA resistant. When these isolates were retested, both OXA-48 and NDM genes were detected.

Due to cost, the isolate numbers chosen were minimised and are not representative of a statistically accurate estimation. Furthermore, by virtue of being a single centre study, its findings cannot be generalised to other institutions in SA with different patient populations. A larger dataset, which includes data from other public and private hospitals, would offer further insight. Albeit this statistical limitation, this gives a general idea of the resistance pattern and carbapenemase distribution that may be representative of what is seen at CHBAH, a hospital that serves a large population in Gauteng, SA. Lastly, the study focused only on the in vitro susceptibility rates of isolates and was not designed to assess the clinical and microbiological outcomes.

Overall, the findings of this study provide a context for the possible utility of CZA monotherapy and dual use of CZA and ATM for the treatment of MDROs. The observed resistance patterns and demonstrated in vitro susceptibility of CRE and drug-resistant *P. aeruginosa* to these drugs, either as monotherapy or in combination, provide reassurance of their potential for successful future use. The study also proves that testing in a routine diagnostic laboratory is feasible, even with the limited resources available in the South African context. More robust clinical studies are needed to evaluate the real-world impact and benefits of using these drugs in our setting, where there is a pressing need for improved therapeutic options. Nevertheless, this study can serve as a strong foundation for further research on this topic.

1. 8. Conclusion

The importance of this study lies in that it is the first to locally evaluate the susceptibility of CZA and ATM, as well their synergy against CRE and MDR *P. aeruginosa*. The excellent CRE susceptibility to CZA shown by the study is very encouraging. The low susceptibility of ATM to these organisms is as expected. Studies with a better statistical sample representation as well as in vivo studies are required to see a more accurate picture. Furthermore, the combination use of CZA and ATM does paint a hopeful picture for the isolates where CZA inactivity is observed. More rigorous studies are required to look at this combination's beneficial use clinically in this setting.

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Appendix

1. Protocol



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DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: Master of Medicine (MMED)			
PART-TIME OR FULL-TIME: Full time			
FIRST REGISTERED FOR THIS DEGREE:	TERM: 2	YEAR: 2019	
DEPARTMENT: Clinical Microbiology and Infectious Diseases			
TITLE OF PROPOSED RESEARCH: Ceftazidime-avibactam and Aztreonam susceptibility of Carbapenem Resistant Enterobacterales and Pseudomonas aeruginosa isolates at Chris Hani Baragwanath Academic Hospital			
CANDIDATE'S SIGNATURE: 			DATE: 20/04/2022
SUPERVISOR 1 (NAME & SURNAME): Dr Lesego Mothibi			% Supervision: 100%
SUPERVISOR'S QUALIFICATIONS: MBChB, FCPATH (Micro) (SA), MMed (Micro)			
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SUPERVISOR'S QUALIFICATIONS:			
SUPERVISOR'S ADDRESS / TEL / E-MAIL:			
SYNOPSIS OF RESEARCH: <i>(Brief summary of proposed research project; between 200-300 words only; with sub-headings: an introduction and justification for study, aim/s, proposed methodology and expected outcome/s)</i> [Use reverse side of this page if more space is required]			

WITS ETHICS NOT REQUIRED: WITS ETHICS PENDING: WITS ETHICS APPROVED: (circle appropriate symbol)*	<table border="0"> <tr> <td>Yes</td> <td>No</td> </tr> <tr> <td><u>Yes</u></td> <td>No</td> </tr> <tr> <td>Yes</td> <td>No</td> </tr> </table>	Yes	No	<u>Yes</u>	No	Yes	No	IF Y SUPPLY ETHICS CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE ETHICS NUMBER HERE:
Yes	No							
<u>Yes</u>	No							
Yes	No							
*Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research								
As supervisor/s, I confirm that I have read the protocol which has been submitted for assessment.								
SIGNATURE OF SUPERVISOR/S:								
SIGNATURE PG OFFICE STAFF	REGISTERED YES..... NO.....	STAMP						

SYNOPSIS OF RESEARCH:

Multi drug resistant organisms such as carbapenem resistant Enterobacterales (CRE) and difficult to treat (DTR) *Pseudomonas aeruginosa* have become a global health threat as their increasing prevalence as nosocomial infections has led to immense treatment challenges leaving very limited options for treatment. The same narrative is true in South Africa where such infections are associated with high mortality rates.

Both *Pseudomonas aeruginosa* and CRE are represented in the ESKAPE pathogens classified by the World Health Organization (WHO) as critical priority pathogens needing novel antibiotic intervention due to their rising resistance to last resort antibiotics such as carbapenems, colistin and tigecycline.

Ceftazidime-avibactam has been shown to be effective against KPC and OXA-48 producing strains and its use in treating such infections has been approved by the US Food and Drug Administration. Aztreonam's efficacy on the other hand, is seen mostly with MBL producing strains. Moreover, synergistic activity of these two antimicrobials has been demonstrated in the treatment of some of these very resistant infections.

The clinical use and laboratory testing of CZA and Aztreonam in South African public hospitals is very limited as these are new unregistered drugs albeit available. The aim of this study will be to determine Ceftazidime-avibactam and Aztreonam susceptibility of CRE and *Pseudomonas aeruginosa* isolates at Chris Hani Baragwanath Academic Hospital (CHBAH), a public tertiary referral center found in Gauteng province in South Africa which sees the highest prevalence of such infections. Knowing the susceptibility profile of CRE and *Pseudomonas aeruginosa* population at CHBAH to Ceftazidime-avibactam and Aztreonam, will hopefully open channels in beginning to use these lifesaving drugs.

11 March 2019/MP

University of The Witwatersrand

Faculty of Health Sciences

Department of Microbiology and Infectious Diseases

**Ceftazidime-avibactam and Aztreonam susceptibility of Carbapenem Resistant
Enterobacterales and *Pseudomonas aeruginosa* isolates at Chris Hani Baragwanath
Academic Hospital**

Bontle Phooko

0603473v

Master of Medicine (MMed)

Supervisor: Dr Lesego Mothibi (Pathologist: Clinical microbiology)

a. Title

**Ceftazidime-avibactam and Aztreonam susceptibility of Carbapenem Resistant
Enterobacterales and *Pseudomonas aeruginosa* isolates at Chris Hani Baragwanath
Academic Hospital.**

Applicant: Bontle Phooko

Student number: 0603473v

Degree: Master of Medicine (MMed)

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Qualifications: MBChB, FCPATH (Micro) (SA), MMed (Micro)

b. Background

Carbapenem-resistant Enterobacterales (CRE), since their emergence in 1990, have grown to become a threat to global public health (1). Their worldwide spread and increasing prevalence as causes of nosocomial infection has led to immense treatment challenges leaving very limited options for clinicians (1-2). Moreover, these organisms are associated with high mortality rates ranging from 24-74% (2).

The American Centre for Disease Control and Prevention's (CDC) definition of a CRE is a member belonging to the Enterobacterales order with resistance against at least one carbapenem antibiotic or producing a carbapenemase enzyme (3). The main mechanism of resistance for CRE, although others exist, is through carbapenemases which are enzymes that cause hydrolysis of carbapenems (1-2). The Ambler classification system organises these carbapenemases according to their molecular structure into class A, e.g., *Klebsiella pneumoniae* carbapenemases (KPCs), class B, e.g., metallo- β -lactamases (MBLs) such as NDM, IMP, or VIM, and class D, e.g., OXA-48 type carbapenemase (1). The MBLs in class B require zinc for β -lactam hydrolysis unlike, Class A and D carbapenemases which require serine at their active sites. This renders currently available β -lactamase inhibitors ineffective against MBLs (1).

Pseudomonas aeruginosa, a Gram negative non fermentative organism, is an important cause of hospital-associated infections accounting for up to 14% of these (4). Due to its ability to acquire several antimicrobial resistance mechanisms, *P. aeruginosa* can present as multidrug-resistant (MDR) and extensively drug-resistant (XDR) thus making it difficult to treat (4). MDR *P. aeruginosa* is defined as *P. aeruginosa* not susceptible to at least one antibiotic in at least three antibiotic classes for which *P. aeruginosa* susceptibility is generally expected: penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems (5). Mechanisms of antimicrobial resistance seen in *P. aeruginosa* involve overexpression of

efflux pumps and acquisition of β -lactamases including carbapenemases (6). Following the introduction of the concept “difficult -to-treat” resistance (DTR) in 2018, *P. aeruginosa* resistant to all the following antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem, ciprofloxacin and levofloxacin is referred to as DTR- *P. aeruginosa* (3). Additionally, co-infection of *P. aeruginosa* and Enterobacterales has been seen in the clinical setting further complicating treatment (4). Consequently, novel therapeutics for both CRE and DTR *P. aeruginosa* infections are an urgent global need.

The Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa (GERMS-SA) reported a significant increase in the number of CRE bacteraemia across South Africa when comparing the year 2018 and 2019, 601 cases to 1052 (7). The prevalence of these organisms appears to be the highest in Gauteng with 64% of the cases followed by Kwa- Zulu Natal claiming 20% of the cases (7). The commonly isolated carbapenemase gene is OXA-48 and its variants (61%) followed by the MBL (29%) (7). This shows that there is a growing problem of CRE in South Africa which will have an impact on the health system and patient outcomes. Mortality rates of up to 40.9% from these infections have been noted within the country with antibiotic exposure being one of the leading risk factors (8).

The presence of drug resistant *P. aeruginosa* in South Africa is similarly of concern. The National Antimicrobial Resistance (AMR) surveillance in South Africa reports a 20% and 25% of *P. aeruginosa* blood stream isolates found to be resistant to - piperacillin/tazobactam (usually first line treatment) and carbapenems (usually second line treatment) respectively (9). A study done in the rural Eastern Cape province of South Africa, involving a range of clinical specimens, shows a higher prevalence of resistance within the country (6). As expected, there is a high mortality associated with such infections (6).

Ceftazidime-avibactam (CZA) contains a combination of ceftazidime, a broad-spectrum cephalosporin with antipseudomonal cover, as well as avibactam which is a novel, non- β -lactam, β -lactamase inhibitor (1) (3). Avibactam belongs to the class of diazabicyclones acting as a reversible covalent inhibitor (1). CZA has been approved both in Europe and in the USA for treatment of Enterobacterales and *P. aeruginosa* infections and its use for CREs and DTR-*P. aeruginosa* is supported by the guidelines (3) (4). CZA has been shown to be effective against class A, C, and D β -lactamase-producing isolates, however, it is hydrolysed by MBLs (3). The US Food and Drug Administration has approved its use for treatment of complicated urinary tract infections as well as complicated intra-abdominal infections in combination with metronidazole for patients as young as 3 months and older (10). Additionally, CZA has been used for bacteraemia, hospital acquired pneumonia and ventilator associated pneumonia (10). CZA has also been shown to be cost effective when taking into consideration overall mortality, morbidity and length of hospital stay (10).

Aztreonam (ATM) is a monobactam shown to be efficacious against MBL producing organisms as it is not hydrolysed by this enzyme (11). ATM is easily inactivated by extended spectrum β lactamases (ESBLs), KPCs, and AmpC β lactamases. As MBLs-carrying *Enterobacterales* may frequently harbour additional ATM-inactivating β -lactamases, the activity of ATM against these isolates is often reduced (12). Its activity against MBLs is mostly enhanced when used in combination with other drugs namely CZA, that would inhibit ESBLs, KPC and AmpC β lactamases. (11,12). Clinical evidence of this was seen in Spain where 6 out of the 10 patients with *K. pneumoniae* CRE infections were cured after treatment with combined aztreonam and CZA (13). Combination treatment with aztreonam and CZA

has demonstrated reasonable efficacy with a lower mortality rate in MBL producing CRE and in some DRT-*P. aeruginosa* infections albeit less pronounced in the latter (11). Moreover, use of this combination treatment has been shown to be associated with a good safety profile (11).

P. aeruginosa and CRE are represented in the ESKAPE pathogens classified by the World Health Organisation (WHO) as critical priority pathogens needing novel antibiotic intervention due to their resistance patterns (14). Resistance to last resort antibiotics such as carbapenems, colistin and tigecycline has been observed even in South Africa, particularly in Gauteng province (15). There is therefore an urgent need for use of alternative antibiotics against these highly resistant Gram-negative bacteria. The clinical use and laboratory testing of CZA and ATM in South African public hospitals is very limited as these are new unregistered drugs albeit available (16). These drugs have been shown to be effective against CRE and MDR *P. aeruginosa*.

The Clinical Laboratory Standard Institute (CLSI) approved susceptibility testing methods for CZA and ATM for both Enterobacterales and *P. aeruginosa* is disk diffusion and minimum inhibitory concentration (MIC) testing (17). A disk content of 30/20 µg for CZA and that of 30µg for ATM is recommended for both groups of organisms. For CZA when testing Enterobacterales susceptibility, a zone diameter between 20-22 mm requires confirmatory MIC testing to avoid false susceptible or false resistant results (17).

Knowing the susceptibility profile of CRE and *P. aeruginosa* population at CHBAH, a tertiary referral centre found in Gauteng province in South Africa, will be a steppingstone in beginning to use these lifesaving drugs. Due to the lack of susceptibility data of ceftazidime-avibactam (CZA) and ATM against CRE and *P. aeruginosa* isolates at CHBAH, the aim of

the study is to demonstrate the in vitro susceptibility data of CZA and aztreonam against these organisms.

c. Study objective.

I Primary objective

- a) To determine the proportion of CRE isolates that are Ceftazidime-avibactam and Aztreonam susceptible at CHBAH.
- b) To determine the proportion of MDR-*P. aeruginosa* isolates that are Ceftazidime-avibactam and Aztreonam susceptible at CHBAH.

II Secondary objective

- a) To determine susceptibility of CRE and MDR-*P. aeruginosa* isolates that are resistant to Ceftazidime-avibactam and Aztreonam to the combination of Ceftazidime-avibactam and Aztreonam at CHBAH respectively.

d. Methods

Study design and site.

This will be a prospective laboratory-based study. The study will be conducted at the National Health Laboratory Services (NHLS), Department of Microbiology, CHBAH. CHBAH is a 3200-bed hospital in Soweto, Johannesburg, South Africa serving as a referral centre for the surrounding smaller hospitals. (18).

Sample and sample size.

Samples used will be known CRE and *P. aeruginosa* isolates cultured from different body sites (blood, cerebrospinal fluid, pus, tissue, fluid, urine, and respiratory samples) submitted

as part of the routine laboratory hospital procedure for microscopy, culture, and susceptibility at CHBAH NHLS microbiology laboratory. Swabs including surveillance swabs will be excluded. Samples will be anonymised.

The current CRE rate at CHBAH ranges between 17- 44% and the DTR-*P. aeruginosa* rate is between 0- 33% annually.

The average number of Enterobacterales and *P. aeruginosa* isolates seen at CHBAH is 300 and 30 a month respectively. Sample size is calculated using the formula: $n = (Z/e)^2 \times P (1-P)$

Where:

- Z = value from standard normal distribution corresponding to desired confidence level

(Z=1.96 for 95% CI)

- P is expected true proportion: The average global CRE resistance rate is estimated to be 67% and 17% for CZA and ATM respectively (19).

For MDR *P. aeruginosa*, the average global resistance rate is 74.5% and 30.7% CZA and ATM respectively (19).

- e is desired precision (half desired CI width) will be 5% or 0.05

- Population size: the number of Enterobacterales and *P. aeruginosa* isolates seen a month at CHBAH.

Therefore, the sample size needed for CRE CZA susceptibility is 340 and for CRE ATM susceptibility is 217. However, to minimise costs, a sample of 100 CRE isolates, which amounts to 33,3% of Enterobacterales isolates per month, will be used.

The sample size needed for *P. aeruginosa* CZA susceptibility is 292 and for ATM susceptibility is 327.

A sample size of 30 *P. aeruginosa*, depending on the availability, will be used to limit cost.

CRE and MDR *Pseudomonas aeruginosa* detection.

The known CRE and MDR *P. aeruginosa* isolates that will be used, will be those that have been cultured and identified according to the CHBAH Microbiology Laboratory Standard Operating Procedures for the respective sample types. The specimens will be inoculated on 5% blood agar, chocolate agar and MacConkey agar plates and incubated overnight at 35°C–37°C. Bacterial species identification will be achieved using the API® 20E kit (bioMérieux SA, France) or the MicroScan WalkAway system. Routine antimicrobial susceptibility testing will be performed using the disk diffusion method or the MicroScan WalkAway system as prescribed by Clinical and Laboratory Standards Institute (CLSI). Enterobacterales resistant to any of the carbapenems (ertapenem, imipenem or meropenem) will be identified as CRE. Those that are intrinsically resistant to imipenem without acquired resistance to carbapenems such as those in the *Proteeae* family will be excluded. Further CRE molecular characterisation will be detected using the RESIST-4 O.K.N.V® (Coris Bioconcept, Belgium) which is an invitro rapid diagnostic test for the detection of OXA-48, KPC, New Delhi Metallo-beta-lactamase (NDM), and Verona integron-encoded Metallo-beta-lactamase (VIM) carbapenemases in bacterial culture. The antibiotics that are routinely tested at NHLS CHBAH for *P. aeruginosa* are piperacillin-tazobactam, ceftazidime, cefepime, meropenem, imipenem, ciprofloxacin and aminoglycosides (tobramycin, amikacin, gentamicin). The isolates that will be used in this study, will be those that are MDR meaning there is no susceptibility of at least one antibiotic in at least 3 antibiotic classes.

Ceftazidime-avibactam and Aztreonam antimicrobial susceptibility testing.

The disk diffusion method will be used to test susceptibility of both CZA and Aztreonam against CRE and MDR *P. aeruginosa* isolates. Disk concentrations 30/20 µg for CZA and that of 30µg for ATM will be used. Interpretation of susceptibility will be according to the CLSI, with *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 being used as quality control strains. In vitro susceptibility for CZA will be interpreted as a disk zone diameter of ≥ 21 mm as according to the (CSLI) (17). For Enterobacterales, the CLSI recommends confirmatory minimum inhibitory concentration (MIC) testing for isolates that have a zone diameter of between 20 - 22mm for CZA to avoid reporting false-susceptible or false-resistant results (18). CZA with an MIC of $\leq 8/4 \mu\text{g/mL}$ will be reported as susceptible as per CLSI guidelines (17). For Aztreonam, a disk zone of ≥ 21 mm and ≥ 22 mm will be read as susceptible for CRE and *P. aeruginosa* isolates respectively (17).

Ceftazidime-avibactam and Aztreonam combination susceptibility testing.

Isolates that are resistant to both CZA and Aztreonam will be further tested using both drugs in combination. The strip cross method described by Khan et.al will be used. For this method, Muller Hinton agar plates will be inoculated with the isolate, the Aztreonam gradient strip will be placed directly on the agar followed by the CZA gradient strip crossing perpendicular to the Aztreonam strip at the intermediate breakpoint of 8µg/ml for CRE and 16µg/ml for the *P. aeruginosa* isolates (20) . These will be incubated at 37 ° Celsius over 24 hours. A strain that will be considered synergy positive will be one that was resistant to both CZA and Aztreonam, but the Aztreonam MIC is susceptible or intermediate in the presence of the CZA strip (20) .

e. Data analysis

To determine the proportion of CRE and MDR- *P. aeruginosa* isolates that are susceptible to CZA and Aztreonam; several sub measures will be calculated:

- The number of CRE that are susceptible to CZA.
- The number of MDR- *P. aeruginosa* that are susceptible to CZA.
- The number of CRE that are susceptible to Aztreonam.
- The number of MDR- *P. aeruginosa* that are susceptible to Aztreonam
- The number of CRE and MDR- *P. aeruginosa* that are resistant to both CZA and Aztreonam that are synergy positive to the combination of CZA and Aztreonam.

To determine the susceptibility of these organisms to the drugs of concern, the number of isolates included in the study will be represented on a table or graph as a number and percentage of those that are susceptible to CZA and Aztreonam respectively. Similarly, the isolates that are resistant to both CZA and Aztreonam will be depicted as those that are synergy positive and those that are synergy negative after testing combination use of CZA and Aztreonam.

A biostatistician will be consulted as necessary.

f. Ethics

Ethics approval will be obtained before initiating the study. Approval will also be sought from the NHLS.

g. Timing

	Jan 2022	Feb 2022	Mar 2022	Apr 2022	May 2022	June 2022	Jul 2022	Aug 2022	Sep 2022	Oct 2022	Dec 2022	Jan 2023
Literature Review												
Preparing Protocol												
Ethics Application												
Protocol Assessment												
Processing & Collecting Data												
Data Analysis												
Writing up paper												

h. Funding

Funding will not restrict the completion of this study.

<u>Resources needed</u>	<u>Estimated cost</u>	
Publishing	R10000	NHLS K Funding
Stationary	R500	NHLS K Funding
Processing equipment • Media • CZA disks • Aztreonam disks • CZA gradient strips • Aztreonam gradient strip • Swabs • Normal saline • Disposable loops	R24697,8	NHLS K Funding

i. Anticipated problems

An anticipated challenge in the study will be acquiring a sufficient number of MDR-*P.*

aeruginosa isolates as the prevalence of these is currently low and may differ from month to month. Due to financial constraints the sample number of CRE isolate will be less than the calculated number. Another problem may be the fact that the RESIST-4 O.K.N. V® (Coris Bioconcept, Belgium), only detects 4 carbapenemases. Other less common CPEs may be missed.

Lastly, sourcing media may become problematic as we are currently facing a media shortage in Gauteng.

2.10. References

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<https://doi.org/10.1128%2FAAC.00846-21>

Abbreviations list:

CRE	Carbapenem-resistant Enterobacterales
CDC	The American Centre for Disease Control and Prevention's
KPCs	<i>Klebsiella pneumoniae</i> carbapenemases
MBLs	Metallo- β -lactamases
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
MDR	Multidrug-resistant
XDR	Extensively drug-resistant
DTR	Difficult -to-treat resistance
GERMS-SA	Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa
CZA	Ceftazidime-avibactam
ESBLs	Extended spectrum β lactamases
ATM	Aztreonam
ESKAPE	<i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> , <i>Pseudomonas aeruginosa</i> and <i>Enterobacter species</i>
WHO	World Health Organisation
CLSI	Clinical laboratory Standard Institute

2. Supplementary data

File number_CRE	Specimen type_CRE	Organism_CRE	Carbapenamase Gene_CRE	ATM zone_CRE	CZA zone_CRE	CZA-ATM synergy result_CRE	Ward_CRE
A1	CATHETER TIP	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		abu
A2	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	abu
A3	CATHETER TIP	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	abu
A4	BLOOD CULTURE	<i>E.COLI</i>	OXA-48	Resistant	Susceptible		gen surg
A5	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		gen surg
A6	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		gen surg
A7	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		gen surg
A8	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		gen surg
A9	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		gen surg
A10	PUS	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		gen surg
A11	TRACHEAL ASPIRATE	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		gen surg
A12	URINE	<i>KLEBS SPP</i>	OXA-48	Susceptible	Susceptible		gen surg
A13	URINE	<i>E.COLI</i>	OXA-48	Resistant	Susceptible		gen surg
A14	PUS	<i>KLEPN</i>	OXA-48 & NDM	Resistant	Resistant	Positive	gen surg
A15	FLUID	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	gen surg
A16	FLUID	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	gen surg
A17	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	gen surg
A18	TRACHEAL ASPIRATE	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		maternity
A19	URINE	<i>SER LIQ</i>	NEG	Susceptible	Resistant		icu
A20	PUS	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		icu
A21	PUS	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		icu
A22	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		icu
A23	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		icu
A24	TRACHEAL ASPIRATE	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		icu
A25	TRACHEAL ASPIRATE	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		icu
A26	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	icu
A27	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		maternity
A28	SPUTUM	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		maternity
A29	SPUTUM	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		maternity
A30	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	NDM	Resistant	Resistant	Positive	medical
A31	BLOOD CULTURE	<i>M.MORGANII</i>	NDM	Susceptible	Resistant		medical
A32	URINE	<i>E.COLI</i>	NDM	Resistant	Resistant	Positive	medical
A33	URINE	<i>E. CLOACAE</i>	NDM	Susceptible	Resistant		medical
A34	TRACHEAL ASPIRATE	<i>ENCL</i>	NEG	Resistant	Susceptible		medical
A35	URINE	<i>E.COLI</i>	NEG	Resistant	Susceptible		medical
A36	PUS	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		medical
A37	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A38	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A39	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A40	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A41	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A42	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A43	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A44	SPUTUM	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A45	SPUTUM	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A46	TRACHEAL ASPIRATE	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		medical
A47	URINE	<i>KLEBS SPP</i>	OXA-48	Resistant	Susceptible		medical
A48	CATHETER TIP	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical

File number_CRE	Specimen type_CRE	Organism_CRE	Carbapenamase Gene_CRE	ATM zone_CRE	CZA zone_CRE	CZA-ATM synergy result_CRE	Ward_CRE
A49	URINE	<i>E.COLI</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical
A50	URINE	<i>KLEBS SPP</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical
A51	URINE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical
A52	URINE	<i>KLEPN</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical
A53	SPUTUM	<i>K.PNEUMONIAE</i>	VIM	Resistant	Resistant	Positive	medical
A54	TISSUE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A55	TISSUE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		medical
A56	FLUID	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical
A57	BLOOD CULTURE	<i>E.COLI</i>	NEG	Resistant	Susceptible		medical paed
A58	CATHETER TIP	<i>ENTCL</i>	OXA-48 & NDM	Resistant	Resistant	Positive	medical paed
A59	BLOOD CULTURE	<i>K.OXYTOCA</i>	OXA-48	Resistant	Susceptible		neonates
A60	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A61	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A62	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A63	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A64	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A65	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A66	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A67	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A68	CSF	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		neonates
A69	CATHETER TIP	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		neonates
A70	BLOOD CULTURE	<i>E.COLI</i>	NDM	Susceptible	Resistant		neurosurgery
A71	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	NDM	Resistant	Resistant	Positive	neurosurgery
A72	PUS	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	neurosurgery
A73	PUS	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		orthopaedic
A74	BLOOD CULTURE	<i>C.FREUNDII</i>	OXA-48	Resistant	Susceptible		orthopaedic
A75	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		orthopaedic
A76	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		orthopaedic
A77	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		orthopaedic
A78	FLUID	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		orthopaedic
A79	SPUTUM	<i>SER MA</i>	NEG	Resistant	Resistant	Positive	other
A80	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A81	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A82	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A83	BLOOD CULTURE	<i>E.CLOACAE</i>	OXA-48	Resistant	Susceptible		other
A84	FLUID	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A85	SPUTUM	<i>K.PNEUMONIAE</i>	OXA-48	Susceptible	Susceptible		other
A86	PUS	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A87	PUS	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A88	TISSUE	<i>KLEPN</i>	OXA-48	Resistant	Susceptible		other
A89	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A90	CATHETER TIP	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other
A91	URINE	<i>E.COLI</i>	OXA-48	Resistant	Susceptible		other
A92	FLUID	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	other
A93	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	VIM	Resistant	Resistant	Positive	medical
A94	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Susceptible		paeds onc
A95	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	paeds onc
A96	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	NEG	Resistant	Resistant	Positive	pbu
A97	PUS	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		pbu
A98	TISSUE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Negative	pbu
A99	TISSUE	<i>K.PNEUMONIAE</i>	OXA-48 & NDM	Resistant	Resistant	Positive	pbu
A100	SPUTUM	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		urology
A101	BLOOD CULTURE	<i>K.PNEUMONIAE</i>	OXA-48	Resistant	Susceptible		other

File number_ pseudo	Specimen type pseudo	Organism_pseudo	Carbapenemase Gene_pseudo	Susceptibility_pseudo	ATM zone_ pseudo	CZA zone_pseudo	Ward_pseudo	CZA-ATM synergy result_pseudo
B1	BLOOD CULTURE	PSEUDO	NEG	XDR	Resistant	Resistant	abu	Negative
B2	BLOOD CULTURE	PSEUDO	VIM	XDR	Susceptible	Resistant	abu	
B3	TRACHEAL ASPIRATE	PSEUDO	VIM	XDR	Resistant	Resistant	abu	Positive
B4	BLOOD CULTURE	PSEUDO	VIM	XDR	Resistant	Resistant	abu	Positive
B5	FLUID	PSEUDO	VIM	XDR	Susceptible	Resistant	gen surg	
B6	FLUID	PSEUDO	VIM	XDR	Susceptible	Resistant	gen surg	
B7	PUS	PSEUDO	VIM	XDR	Resistant	Resistant	gen surg	Positive
B8	FLUID	PSEUDO	VIM	XDR	Susceptible	Resistant	gen surg	
B9	FLUID	PSEUDO	VIM	XDR	Susceptible	Resistant	gen surg	
B10	FLUID	PSEUDO	NEG	MDR	Resistant	Susceptible	gen surg	
B11	BLOOD CULTURE	PSEUDO	NDM	XDR	Susceptible	Resistant	gen surg	
B12	TRACHEAL ASPIRATE	PSEUDO	NEG	MDR	Susceptible	Susceptible	gen surg	
B13	BLOOD CULTURE	PSEUDO	NEG	XDR	Resistant	Susceptible	gen surg	
B14	CATHETER TIP	PSEUDO	VIM	XDR	Resistant	Resistant	gen surg	Positive
B15	CATHETER TIP	PSEUDO	VIM	XDR	Susceptible	Resistant	gen surg	
B16	BLOOD CULTURE	PSEUDO	NEG	MDR	Resistant	Susceptible	icu	
B17	TRACHEAL ASPIRATE	PSEUDO	NEG	MDR	Resistant	Susceptible	icu	
B18	BLOOD CULTURE	PSEUDO	NEG	XDR	Susceptible	Resistant	icu	
B19	BLOOD CULTURE	PSEUDO	VIM	XDR	Susceptible	Resistant	icu	
B20	SPUTUM	PSEUDO	NEG	MDR	Susceptible	Susceptible	medical	
B21	TRACHEAL ASPIRATE	PSEUDO	NEG	MDR	Resistant	Susceptible	medical	
B22	BLOOD CULTURE	PSEUDO	VIM	XDR	Resistant	Resistant	medical paed	Negative
B23	SPUTUM	PSEUDO	VIM	XDR	Resistant	Resistant	medical paed	Positive
B24	SPUTUM	PSEUDO	VIM	XDR	Susceptible	Resistant	other	
B25	PUS	PSEUDO	NEG	XDR	Susceptible	Susceptible	other	
B26	CATHETER TIP	PSEUDO	NEG	XDR	Resistant	Susceptible	other	
B27	BLOOD CULTURE	PSEUDO	NEG	XDR	Resistant	Resistant	other	Negative
B28	PUS	PSEUDO	NEG	MDR	Resistant	Susceptible	other	
B29	PUS	PSEUDO	VIM	XDR	Resistant	Resistant	paeds onc	Positive
B30	BLOOD CULTURE	PSEUDO	VIM	XDR	Resistant	Resistant	paeds onc	Positive
B31	URINE	PSEUDO	NEG	XDR	Susceptible	Susceptible	urology	
B32	CATHETER TIP	PSEUDO	NEG	XDR	Resistant	Resistant	other	Negative

3. Ethics Clearance



R49 Dr B Phooko

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) CLEARANCE CERTIFICATE NO. M220556

NAME: Dr B Phooko
(Principal Investigator)

DEPARTMENT: School of Pathology
Dept of Clinical Microbiology & Infectious Diseases
Medical School
University

PROJECT TITLE: Ceftazidime-avibactam and Aztreonam susceptibility of
CRE's and Pseudomonas isolates at Chris Hani
Baragwanath Academic Hospital

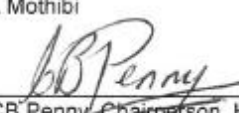
DATE CONSIDERED: Ad hoc

DECISION: Approved unconditionally

CONDITIONS:

NOTE: If contact information regarding student study participants is required,
please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>

SUPERVISOR: Dr L Mothibi

APPROVED BY: 
Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2022/06/09

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

DECLARATION OF INVESTIGATORS

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

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report.** When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **2022/05/01** and therefore reports and re-certification will be due in the month of **2022/05/01** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

Date

4. Approval of Tittle



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

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

30 January 2024
Person No: 0603473V
PAG

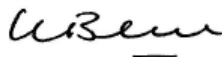
Miss BB Phooko
No 5 Cheshire Road
Hurlyvale
Edenvale
1609
South Africa

Dear Miss Bontle Phooko

Master of Medicine in Microbiological Pathology: Approval of Title

We have pleasure in advising that your proposal entitled *Ceftazidime-avibactam and Aztreonam susceptibility of Carbapenem Resistant Enterobacterales and Pseudomonas aeruginosa isolates at Chris Hani Baragwanath Academic Hospital* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

A handwritten signature in black ink, appearing to read 'S. Benn'.

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences