SCHOOL OF LABORATORY MEDICINE AND MEDICAL SCIENCES

THE PHENOTYPIC AND CLINICAL CHARACTERISATION OF TIGECYCLINE CO-RESISTANT CARBAPENEM-RESISTANT ENTEROBACTERALES OBSERVED AT INKOSI ALBERT LUTHULI CENTRAL HOSPITAL, DURBAN, SOUTH AFRICA

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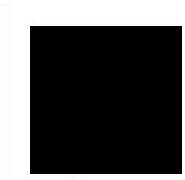
Declaration of Authorship

I, Dr Khathija Bibi Sheik Aboo, declare as follows:

1. That the work described in this dissertation has not been submitted to UKZN or any other institution for the purposes of an academic qualification, whether by me or any other party.
2. That my contribution to the project is as follows:
   2.1 I formulated the research topic, developed the protocol, sought, and obtained ethical approval, collected, and analysed the data and drafted the manuscript.
3. That the contributions of others to the project are as follows:
   3.1 Dr Khine Swe Swe Han supervised the project in its entirety.
   3.2 Prof Sabiha Yusuf Essack co-supervised the original topic formulation and the original protocol development.
   3.3 Prof Vivienne Russell offered mentorship in manuscript writing and contributed to editing the manuscript.
   3.4 Catherine Connolly performed the statistical analysis.

Student Signature: 

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Dedication

I dedicate this work to my husband, Fahim, for his selflessness.

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LIST OF ABBREVIATIONS

AMR: Antimicrobial resistance

AMS: Antimicrobial stewardship

AMU: Antimicrobial use

ARB: Antimicrobial resistant bacteria

ARDS: Acute respiratory distress syndrome

ARG: Antimicrobial resistance genes

ART: Antiretroviral therapy

AST: Antimicrobial susceptibility testing

BMD: Broth microdilution

BSI: Bloodstream infection

CFU: Colony-forming unit

CLSI: Clinical and Laboratory Standards Institute

CPE: Carbapenemase-producing Enterobacterales

CRE: Carbapenem-resistant Enterobacterales

ESBL: Extended-spectrum β-lactamase

EUCAST: European Committee on Antimicrobial Susceptibility Testing

FDA: The United States Food and Drug Administration

HCAI: Healthcare-associated infections

ICU: Intensive care unit

MIC: Minimum inhibitory concentration

MDR: Multidrug-resistant

MDRO: Multidrug-resistant organism

MODS: Multiple organ disfunction syndrome

NICU: Neonatal intensive care unit

PCR: Polymerase chain reaction

TGC Co-R CRE: Tigecycline co-resistant carbapenem-resistant Enterobacterales

UTI: Urinary tract infection

XDR: Extensively drug-resistant

CHAPTER 1

1.1 Introduction

The global advent and dissemination of antimicrobial resistance (AMR) in the order Enterobacterales portends ill for the management of infectious syndromes caused by such resistant strains (1). The prevalence of carbapenem-resistant Enterobacterales (CRE) is increasing, with rates of carbapenem resistant *Klebsiella pneumoniae* in some regions of approximately 10% (1-8). Resistance mechanisms in CRE include carbapenemases [carbapenemase-producing Enterobacterales (CPE)] and those that evince porin loss or efflux, in combination with the production of extended-spectrum β-lactamases (ESBL) or AmpC cephalosporinases [non-carbapenemase-producing CRE (non-CPE)] (1, 9, 10).

Rising rates of CPE are being recorded in South Africa, with the possibility of such strains becoming endemic (11). CPE strains in the South African setting have been shown to elaborate carbapenemases belonging to classes A (*bla*GES), B (*bla*NDM and *bla*VIM) and D (*bla*OXA-48, along with its variants), with a recent transition to predominance from *bla*NDM to *bla*OXA-48 and variants (12). Therapeutic options against infections caused by CRE are limited, with the glycylcycline tigecycline, serving as an antimicrobial of last resort (1, 3-5, 13-19). The clinical dependence on tigecycline in the treatment of infections caused by CRE has seen the rates of tigecycline co-resistance in CRE rising; a phenomenon associated with poor clinical outcomes (3-5, 15-19).

Tigecycline, a minocycline derivative with broad spectrum activity, inhibits protein synthesis by interacting with the 30S subunit of the ribosome and obstructing the entrance of amino-acyl tRNA into the ribosomal A-site (15, 20). This has the effect of halting the addition of amino acids to the growing peptide sequence (21). Tigecycline resistance often develops during use of the drug in the treatment of infections caused by CRE (4). Resistance is related primarily to enhanced efflux and associated regulatory pathway modifications, as well as to target site modification (3-5). Chromosomal genes coding for the efflux pump, AcrAB-TolC, are overexpressed, due to overexpression of the associated transcriptional activator *ramA*, in turn caused by mutations in the genes coding for the negative regulator *ramR* (5). The *rpsJ* gene, meanwhile, serves as the resistance determinant that codes for target site modification at ribosomal level (5).

Disturbingly, multidrug-resistant (MDR) Enterobacterales phenotypes are linked to increased expression of multidrug resistance transcriptional regulators *marA* and *rarA*, and increased expression of corresponding efflux pump genes *acrAB*, and *oqxAB* (22, 23). Another alarming finding was the discovery of the plasmid-borne mutant efflux pump gene *tet(A)* described in tigecycline-resistant CRE and correlated with phenotypic tigecycline resistance; with such transferrable tigecycline resistance constituting a public health threat due to the possibility of horizontal gene transfer (4). Tigecycline therapy in the case of CPE may lead to the development of tigecycline-resistant CPE, by inducing the expression of a plasmid-borne variant *tet(A)* gene (13).

Molecular characterisation of CPE strains of clinical origin from South Africa have revealed the presence of resistance determinants against a multiplicity of antimicrobials, including, fosfomycin, aminoglycosides, colistin and quinolones, in addition to tigecycline (24). This is reflective of our rapidly emptying store of antimicrobial choices, particularly those prescribed as part of combination therapy against infections caused by extensively drug-resistant (XDR) CRE (24). Curbing this threat demands comprehensive characterisation of CRE strains (2).

Antimicrobial stewardship (AMS), with its focus on rational antimicrobial use (AMU) is critical to halting the spread of tigecycline co-resistance in CRE (15, 16). Conserving last resort antimicrobials such as tigecycline, demands ongoing surveillance (1-3, 13, 15, 16, 24, 25). The characterisation of tigecycline co-resistant CRE (TGC Co-R CRE) will inform the design of evidence-based strategies to limit the propagation of these pathogens, with potential benefits to clinical care, infection prevention and control, as well as public health (2, 24).

1.2 Critical Literature Review

Tigecycline belongs to the glycycline antimicrobial class (26). The drug, a semisynthetic molecule, is intended for use in the medical management of polybacterial infections due to both MDR Gram-positive and Gram-negative microbes (26). A third generation tetracycline, displaying structural similarity to minocycline, tigecycline acts by inhibiting protein synthesis at the translation step, specifically by interacting with the 30S subunit of the bacterial ribosome and obstructing the ingress of amino-acyl tRNA into the ribosomal A-site (20).

Tigecycline, administered intravenously, is indicated for the treatment of adult patients evincing complicated skin and skin structure infections (apart from diabetic foot infection), complicated intra-abdominal infections and community-acquired bacterial pneumonia (26). Tigecycline demonstrates bacteriostatic antibacterial activity against the order Enterobacterales, including against CRE, but excluding the Proteae (*Proteus spp*., *Providencia spp.* and *Morganella morganii*), which display intrinsic resistance to tigecycline) (27, 28). Resistance to tigecycline in the order Enterobacterales is due largely to efflux (29). Mechanisms of resistance include AcrAB efflux pump gene overexpression and mutational inactivation of negative regulation of the OqxAB efflux pump (29).

With respect to the categorisation of tigecycline resistance in Enterobacterales, the European Committee on Antimicrobial Susceptibility Testing (EUCAST)-derived clinical breakpoints for tigecycline against *E. coli and Citrobacter koseri* are a minimum inhibitory concentration (MIC) of ≤ 0.5 mg/L for susceptibility and an MIC of > 0.5mg/L for resistance (30). The United States Food and Drug Administration (FDA) clinical interpretive breakpoints for tigecycline against Enterobacterales are an MIC of ≤ 2 µg/mL for susceptibility, an MIC of 4 µg/mL for the intermediate categorisation, and an MIC of ≥ 8 µg/mL for resistance (31).

The bacterial order Enterobacterales plays a frequent role in the aetiology of clinical infections (32). Carbapenem resistance in Enterobacterales refers to resistance to one or more of the carbapenem antimicrobials, as defined by clinical breakpoints developed by the Clinical and Laboratory Standards Institute (CLSI) (32). The clinical breakpoints denoting carbapenem resistance in Enterobacterales are an MIC of ≥ 4µg/mL for meropenem, imipenem and doripenem and an MIC of ≥ 2µg/mL for ertapenem (32).

CRE are linked to infections with poor outcomes (10). Resistance determinants in CRE include hydrolytic enzymes, efflux and impermeability, with carbapenemase production predominating (9). Carbapenemases are plasmid-encoded, facilitating global dissemination (9). Disturbingly, such plasmids frequently encode resistance determinants relating to other classes of antimicrobials; their dissemination and presence is thus associated with MDR phenotypes (32). In South Africa, *Klebsiella pneumoniae* is the commonest species of CRE; and OXA-48 and NDM are the most

frequently recorded carbapenemases (6). In-situ medical devices and carbapenem exposure are linked to acquisition (33). Prevention efforts necessitate antimicrobial stewardship, infection control, rapid diagnostics and surveillance (33, 34).

The emergence of tigecycline resistance in CRE is cause for great concern, with past AMR surveillance programmes describing rates in excess of 10% in some regions (19). The development of tigecycline resistance during use of the drug is a well-recognised occurrence in the medical management of infections due to CRE (4). Other potential risk factors for the acquistion of TGC Co-R CRE include host exposure to antimicrobials (specifically to penicillin, broad spectrum cephalosporins, aminoglycosides and fluoroquinolones), nasal catheter and venous catheterisation (15). Of note, the use of tigecycline for the treatement of urinary tract infections (UTI) has been associated with the emergence of TGC Co-R CRE, plausably due to subtherapeutic urinary concentrations of the drug (35, 36).

Management of infections caused by multidrug-resistant organisms (MDRO) such as TGC Co-R CRE is challenging. Therapeutic options for the management of uncomplicated cystitis include ciprofloxacin, levofloxacin, cotrimoxazole, nitrofurantoin, aminoglycosides or colistin (37). The novel β-lactam/β-lactamase inhibitors, ceftazidime-avibactam, meropenem-vaboractam, imipenem-cilastatin-relebactam; and the siderophore cephalosporin, cefiderocol, may be utilised in the management of pyelonephritis and complicated UTI (37).

Infections in other body sites, if due to KPC-producing CRE, may be treated with ceftazidime-avibactam, meropenem-vaborbactam and imipenem-cilastatin-relebactam (37). If such infections are due to metallo-β-lactamase-producing CRE (e.g. NDM) however; ceftazidime-avibactam plus aztreonam, or alteratively cefiderocol, is recommended for therapy (37). Finally, if such infections are due to OXA-48-like-producing CRE, ceftazidime-avibactam is recommended (37).

Prevention efforts to mitigate the onward dissemination of MDRO such as TGC Co-R CRE necessitate a multi-pronged approach. Healthcare provider-focussed educational campaigns, an AMS-inspired antimicrobial prescribing rationale, enhanced surveillance of AMU and AMR, and promotion of the development and utilisation of rapid diagnostics that facilitate the adoption of infection prevention and control measures within a clinically actionable timeframe are areas that require attention (38).

The capacitation of clinical microbiology laboratories to offer real-time microbial phylogenetic typing services as an aid to outbreak response is vital (38). The implementation of robust and monitored infection prevention and control measures is imperative, and this should include adherence to standard precautions (that embrace the importance of hand hygiene) and contact precautions, isolation measures, patient/provider cohorting, patient decolonisation, the availability of dedicated medical supplies and environmental cleaning/disinfection (38).

1.3 Problem Statement

1.3.1 Nature of the Problem

Infections due to CRE represent a worldwide management dilemma that impacts both clinical care and public health (39). Additionally, infections caused by CRE are linked to poor clinical outcomes; and CRE may display multidrug resistance with clinical management thus dogged by

limited therapeutic options (6, 10, 29, 40). The glycylcycline tigecycline serves as an antimicrobial of last resort for the medical management of infections caused by CRE (17). Unfortunately, this has seen the rates of concomitant tigecycline resistance in CRE rise, imperilling health at both the individual and collective level (15).

1.3.2 Knowledge Gaps

Research is needed to shed light on the magnitude of the problem of TGC Co-R CRE, as well as on the microbial and clinical epidemiology associated with this emergent phenotype (15, 29).

1.4 Research Question

We sought to determine the burden of TGC Co-R CRE at Inkosi Albert Luthuli Central Hospital; and to elucidate the pathogen- and host-related risk factors associated with the emergence and acquisition of TGC Co-R CRE.

1.5 Aim

The aim of the study was to characterise TGC Co-R CRE recorded at Inkosi Albert Luthuli Central Hospital, a quaternary academic referral hospital in Durban, KwaZulu-Natal, Republic of South Africa from 2017 to 2019 with respect to frequency of occurrence, microbiologic and patient profiles.

1.6 Objectives

The objectives of the study were two-fold. We sought to determine the frequency of occurrence of the TGC Co-R CRE phenotype recorded at Inkosi Albert Luthuli Central Hospital from 1 January 2017 to 31 December 2019. Additionally, we sought to describe the associated microbiologic (clinical sample type; microscopic and cultural characteristics; and antimicrobial resistance categorisation) and patient (patient age and gender; infection onset; comorbidities; ward setting; antimicrobial exposure; and clinical outcome) profiles.

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CHAPTER 2

2.1 Abstract

Background

Rising rates of carbapenem-resistant Enterobacterales (CRE) and limited therapeutic options have resulted in clinical dependence on tigecycline with subsequent emergence of tigecycline co-resistant CRE (TGC Co-R CRE). Characterisation of TGC Co-R CRE is imperative to limiting its propagation.

Objective

We sought to determine the frequency, clinical implication, and microbiologic characteristics of TGC Co-R CRE at Inkosi Albert Luthuli Central Hospital from 2017 to 2019.

Methodology

We undertook a retrospective descriptive study. Data sources comprised the laboratory and hospital information systems. The frequency of TGC Co-R CRE was calculated. Specimen type, species, antimicrobial resistance, infection onset, antimicrobial exposure, age, sex, comorbidities, ward, and clinical outcome were characterised.

Results

The frequency of TGC Co-R CRE was 2/53 (3.8%) in 2017, 0/90 (0.0%) in 2018 and 4/123 (3.3%) in 2019. The decrease was not significant ($p = 0.148$). Six isolates were recorded. Acquisition was uniformly healthcare associated. Most cases (5/6; 83,3%) were female and two-thirds (4/6; 66.7%) were paediatric. Most cases were ICU patients (5/6; 83,3%). Most cases (5/6; 83.3%) were carbapenem-exposed. None were tigecycline-exposed. Comorbidities included HIV (2/6; 33.3%), SLE (1/6; 16.7%), burns (1/6; 16.7%) and surgery (2/6; 33.3%). Half the patients (3/6; 50.0%) demised. Specimens comprised peritoneal dialysis fluid (1/6; 16.7%), blood culture (1/6; 16.7%), endotracheal aspirate (2/6; 33.3%), catheter urine (1/6; 16.7%) and wound swab (1/6; 16.7%). Species comprised *Klebsiella pneumoniae* (3/6; 50.0%), *Enterobacter cloacae* (2/6; 33.3%) and *Serratia* species (1/6; 16.7%). All isolates were multidrug-resistant (MDR).

Conclusion

The advent of TGC Co-R CRE is a phenomenon warranting further research into prevalence, resistance mechanisms and acquisitional risk factors. The results of this study are of hypothesis-generating value for subsequent research.

2.2 Introduction

The evolution and global dissemination of antimicrobial resistance (AMR) in Enterobacterales portends ill for the management of infectious syndromes (1). The prevalence of carbapenem-resistant Enterobacterales (CRE) is increasing, with rates of carbapenem resistant *Klebsiella pneumoniae* of approximately 10% in some regions (1-8). Resistance mechanisms in CRE include the elaboration of carbapenemases [carbapenemase-producing Enterobacterales (CPE)]; and the combination of porin loss, or efflux, with the production of either extended-spectrum β-lactamases (ESBL) or AmpC cephalosporinases (non-CPE) (1, 9, 10). Rising rates of CPE have been recorded in South Africa, with the possibility of such strains becoming endemic (11).

Therapeutic options against infections caused by CRE are limited, with the glycylcycline tigecycline serving as an antimicrobial of last resort (1, 3-5, 13-19). The dependence on tigecycline in the treatment of infections caused by CRE has seen the rates of tigecycline co-resistance in CRE rising; with associated poor clinical outcomes (3-5, 15-19). The risk of clonal propagation of such multidrug-resistant (MDR) strains in the hospital environment represents an additional public health concern (41).

Oftentimes, the evolution of tigecycline resistance occurs during treatment with the drug, of infections caused by CRE (4). Disturbingly, exposure to tigecycline may predispose to the emergence of MDR Enterobacterales phenotypes (22, 23). Resistance is related primarily to enhanced efflux and associated regulatory pathway modifications, as well as to target site modification (3-5). However, an alarming discovery was that of plasmid-borne tigecycline resistance determinants, including a mutant efflux pump gene and degradative enzymes; with such transferrable resistance constituting a public health threat due to the possibility of horizontal gene transfer (4, 13, 42, 43).

Tigecycline co-resistant CRE (TGC Co-R CRE) from South Africa display a high degree of multidrug-resistance (24). This is reflective of our rapidly emptying store of antimicrobials, particularly those prescribed as part of combination therapy against infections caused by extensively drug resistant (XDR) CRE (24). Curbing this threat demands the comprehensive characterisation of CRE (2).

Antimicrobial stewardship (AMS), with its focus on rational antimicrobial use (AMU), is critical to halting the spread of TGC Co-R CRE (15, 16). Conserving last resort antimicrobials, such as tigecycline, necessitates ongoing surveillance (1-3, 13, 15, 16, 24, 25). The characterisation of TGC Co-R CRE will inform the design of evidence-based strategies to limit the propagation of these pathogens, with potential benefits to clinical care, infection prevention and control, as well as public health (2, 24).

The aim of this study was to thus to determine the frequency of occurrence, microbiologic and patient profiles reported to be associated with TGC Co-R CRE in patients admitted to Inkosi Albert Luthuli Central Hospital, in Durban, South Africa, between 1 January 2017 and 31 December 2019.

2.3 Methodology

Ethical approval was obtained from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal. A retrospective descriptive study, covering the years 2017 to 2019 was then undertaken. The study focused on Inkosi Albert Luthuli Central Hospital, a quaternary academic hospital in Durban, KwaZulu-Natal, Republic of South Africa. The institution is a provincial referral hospital that admits the full spectrum of patient profiles – rural- and urban-dwelling, presenting with medical conditions of community- and healthcare-onset.

The clinical microbiology laboratory information system at the hospital was accessed. Laboratory requisition form details, microscopy, culture, and antimicrobial susceptibility test (AST) results pertaining to clinical isolates of CRE were collected. CRE for which phenotypic tigecycline susceptibility results were available, were targeted for analysis. Data were consolidated and de-duplicated. Enterobacterales genera evincing intrinsic resistance to tigecycline (*Proteus* spp.,

Providencia spp., and *Morganella* spp.) were excluded. The hospital information system was accessed. Data, de-linked from patient identifiers, was collected for infections caused by TGC Co-R CRE with respect to antimicrobial therapy, risk factors for the emergence of CRE and TGC Co-R CRE, and clinical outcome.

A data analysis was performed to determine the frequency of tigecycline co-resistance in CRE for the period 2017 to 2019 and to characterise the associated microbiologic and patient profiles. The frequency of tigecycline co-resistance in CRE was calculated for each year of analysis. Microbiologic characterisation focused on the type of specimen (whether sourced from sterile body sites or normally colonised body sites), the species diversity and the phenotypic resistance categorisation. A microbial isolate was classified as MDR if the isolate was recorded as being non-susceptible to a minimum of one drug in a minimum of three antibacterial categories (44). Patient characterisation (aided by a mini case series format) centred on whether the infection was community acquired (< 72 hours from the date of admission) or healthcare associated (≥ 72 hours from the date of admission or recent previous health care contact or referral from another health care facility) (6), antimicrobial therapy administered (exposure to carbapenems and/or to tigecycline), age, sex, comorbidities, ward setting [intensive care unit (ICU) and non-ICU)] and clinical outcome (all-cause mortality).

2.4 Results

The frequency of occurrence of tigecycline co-resistance in CRE recorded at Inkosi Albert Luthuli Central Hospital was 2/53 (3.8%) in 2017, 0/90 (0.0%) in 2018 and 4/123 (3.3%) in 2019. There was no significant decrease in frequency over the three-year study period ($p = 0.148$). There were six isolates of TGC Co-R CRE ($n = 6$) recorded during the study period. Acquisition in all cases (6/6; 100.0%) of TGC Co-R CRE was deemed healthcare associated (6). Most patients (5/6; 83,3%) were female. Regarding age, two-thirds of the cases (4/6; 66.7%) were paediatric patients, with half (3/6; 50.0%) being under 1 year of age. The remaining one-third of the cases (2/6; 33.3%) comprised adult patients. Most patients (5/6; 83,3%) were in ICU. Of the ICU patients, a single patient (1/6; 16.7%) was in the neonatal ICU (NICU), two patients (2/6; 33.3%) were in paediatric ICU and a further two patients (2/6; 33.3%) were in surgical ICU. The single ward patient (1/6; 16.7%) was in the paediatric burns ward. Most patients (5/6; 83.3%) had been exposed to carbapenem therapy. None of the patients (0/6; 0.0%) had been exposed to tigecycline therapy. Of the comorbidities noted, two patients (2/6; 33.3%) were HIV-infected, a single patient (1/6; 16.7%) had SLE, a single patient (1/6; 16.7%) had burn injuries, and two patients (2/6; 33.3%) had undergone abdominal surgery. The latter two patients had multiple comorbidities, with the one patient also having rheumatoid arthritis and gestational diabetes, whilst the other patient was born preterm and developed necrotising enterocolitis. With respect to clinical outcome, half the patients (3/6; 50.0%) demised and half (3/6; 50.0%) were discharged.

Of the specimen types received, two (2/6; 33.3%) emanated from sterile sites and four (4/6; 66.7%) originated from non-sterile sites. Sterile site specimens comprised a single specimen of peritoneal dialysis fluid (1/6; 16.7%) and a single blood culture (1/6; 16.7%). Non-sterile site specimens comprised two endotracheal aspirates (2/6; 33.3%), a single catheter urine specimen (1/6; 16.7%) (45) and a single wound swab (1/6; 16.7%). Half the isolates (3/6; 50.0%) were identified as *Klebsiella pneumoniae subspecies pneumoniae*, a further two isolates (2/6; 33.3%) were identified as *Enterobacter cloacae* complex (46) and a single isolate (1/6; 16.7%) was identified as *Serratia*

species. The results of phenotypic AST showed that all isolates (6/6; 100.0%) were MDR (44), in addition to displaying carbapenem and tigecycline co-resistance.

Further clinicopathologic correlation is presented below as individual case summaries.

Case 1

The first case was that of a female infant, admitted into the paediatric ICU. The working diagnosis was severe acute respiratory distress syndrome (ARDS) with new-onset sepsis. The patient was 97 days old at the time of microbiologic sampling. The infant was infected with HIV. Carbapenem exposure (imipenem), but not tigecycline exposure was documented. Additional documented antimicrobial exposures included piperacillin-tazobactam, amikacin, ciprofloxacin, cefotaxime, trimethoprim-sulfamethoxazole, colistin, voriconazole, ganciclovir and valganciclovir. The clinical condition of the infant continued to deteriorate, and septic shock refractory to inotropic support supervened. The infant demised.

The clinical rationale given for microbiologic sampling was the presence of sepsis. Microscopy of the Gram-stained endotracheal aspirate revealed moderate numbers (2+; 6-15 cells per oil immersion field) of leukocytes, scanty numbers (1+; 1-5 cells per oil immersion field) of epithelial cells, scanty numbers (1+; 1-5 microorganisms per oil immersion field) of Gram-positive cocci and scanty numbers (1+; 1-5 microorganisms per oil immersion field) of Gram-negative cocci. Culture yielded a monomicrobial growth of MDR *Klebsiella pneumoniae subspecies pneumoniae*. Acquisition was categorized as healthcare associated (6). AST by broth microdilution (BMD) indicated that the isolate was susceptible to colistin, with a minimum inhibitory concentration (MIC) of 1 µg/mL. The AST result was released with a comment however, that heteroresistance to colistin may still be possible. Of additional significance, antibiogram-concordant MDR *Klebsiella pneumoniae subspecies pneumoniae* was repeatedly isolated from endotracheal aspirate and urine specimens whilst on a microbiologically appropriate colistin-containing antimicrobial regimen.

Case 2

The second case was that of a female patient, admitted to the adult surgical ICU, after clinical deterioration on the general ward, where she was being treated for a lupus nephritis flare. The patient was 19 years old at the time of microbiologic sampling. The patient was afflicted with SLE. Carbapenem (imipenem) exposure, but not tigecycline exposure was documented. Additional known antimicrobial exposures included ciprofloxacin, cloxacillin, piperacillin-tazobactam and vancomycin. The clinical rationale given for microbiologic sampling was the presence of sepsis. The clinical scenario was that of left-sided infective endocarditis, cerebral septic embolization, multiple areas of cerebral infarction and decreased level of consciousness. The patient was anaemic. Myopericarditis was also suspected. In addition, pulmonary embolism was noted as a possible reason for the difficulty experienced with respect to mechanical ventilation. The presence of bilateral pleural effusions and ascites were recorded. Inotrope-requiring haemodynamic instability supervened. The prognosis was not favourable, and the patient succumbed to septic shock with multiple organ dysfunction syndrome (MODS) on day 11 of the ICU stay.

Microscopy of the wet preparation of the catheter urine received as part of the septic screen revealed moderate numbers (2+; 11-40 cells per high-power field) of leukocytes, numerous (3+; >40 cells per high-power field) erythrocytes and scanty numbers (1+; 2 cells per high-power field)

of epithelial cells. Additionally, bacteria and yeast were observed, whilst casts, crystals and parasites were not observed. Culture yielded a monomicrobial growth of *Serratia* species at a colony count of >100 colony-forming units (CFU)/mL. Acquisition was categorized as healthcare associated (6). Antimicrobial substances were present in the urine specimen. Catheter care, with repeat sampling and contact precautions were advised. Of additional significance, methicillin-susceptible *Staphylococcus aureus*, was recovered from blood culture.

Case 3

The third case was that of a female infant, admitted into the paediatric ICU with severe hypoxic pneumonia with air leak syndrome and ARDS, having been referred from a regional hospital for mechanical ventilation. The patient was 205 days old at the time of microbiologic sampling. The infant was infected with HIV and was receiving antiretroviral therapy (ART). The diagnosis was that of adenoviral pneumonia with MODS with secondary infection by *Elizabethkingia meningoseptica*. High-frequency oscillatory ventilation and inotropic support was required. The infant evinced seizures during the ICU admission. Ultimately though, the MODS resolved, the infant was weaned from mechanical ventilation, extubated, and discharged to the referring hospital.

The clinical rationale given for microbiologic sampling was that of admission work-up. Carbapenem exposure, but not tigecycline exposure was documented. Additional documented antimicrobial exposures included amoxicillin-clavulanate, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, and vancomycin. Microscopy of the Gram-stained endotracheal aspirate revealed scanty numbers (1+; 1-5 cells per oil immersion field) of leukocytes and scanty numbers (1+; 1-5 cells per oil immersion field) of epithelial cells, with an absence of bacteria. Culture yielded a monomicrobial growth of *Enterobacter cloacae subspecies dissolvens.* Acquisition was categorized as healthcare associated (6). The result was released with a comment questioning the significance of the cultured organism, advising clinical correlation and repeat sampling in the presence of clinical indication. Of additional significance, adenovirus polymerase chain reaction (PCR) testing was positive on endotracheal aspirate and *E. meningoseptica* was isolated from endotracheal aspirate and urine.

Case 4

The fourth case was that of male child, admitted to the paediatric burns ward, having been referred from a regional hospital. The patient was 9 years old at the time of microbiologic sampling. The child had sustained burn injuries to the right hand. No carbapenem or tigecycline exposure was documented. Antimicrobial exposure to amoxicillin-clavulanate was documented. The clinical rationale given for microbiologic sampling was that of post-operative assessment following Kirschner wire-associated skin graft fixation involving the affected hand (47). Microscopy of the Gram-stained wound swab revealed scanty numbers (1+; 1-5 cells per oil immersion field) of leukocytes and scanty numbers (1+; 1-5 microorganisms per oil immersion field) of Gram-negative bacilli. Culture yielded a monomicrobial growth of *Enterobacter cloacae subspecies cloacae*. Acquisition was categorized as healthcare associated (6). The result was released with a comment questioning the significance of the cultured organism, advising clinical correlation, repeat sampling in the presence of clinical indication and contact precautions. The clinical course was uneventful, and the child was discharged from the ward.

Case 5

The fifth case was that of a female patient, referred from a regional hospital and admitted into the surgical ICU. The patient was 32 years old at the time of microbiologic sampling. The patient was afflicted with rheumatoid arthritis and gestational diabetes. She had undergone a caesarean section for uterine rupture and foetal distress and multiple urogynaecologic surgeries, including subtotal hysterectomy, cystectomy, vaginal vault surgery and bilateral ureterostomy. The patient was managed with an open abdomen. Of additional significance, the patient had a history of receiving corticosteroid therapy for rheumatoid arthritis. ICU stay was characterised by the development of sepsis with suspected sources being abdominopelvic infection or catheter-related bloodstream infection. Acute kidney injury necessitated the receipt of haemodialysis during admission. Septic shock with MODS supervened and the patient demised.

The clinical rationale given for microbiologic sampling was the presence of sepsis. Carbapenem (meropenem) exposure, but not tigecycline exposure was documented. Additional documented antimicrobial exposures included amoxicillin-clavulanate, ciprofloxacin, metronidazole, vancomycin, colistin and amphotericin B. Microscopy of the Gram-stained peritoneal dialysis fluid revealed numerous (3+; >15 cells per oil immersion field) leukocytes, scanty numbers (1+; 1-5 microorganisms per oil immersion field) of Gram-positive cocci and numerous (3+; >15 microorganisms per oil immersion field) Gram-negative bacilli. Culture yielded a polymicrobial growth of MDR *Pseudomonas aeruginosa*, MDR *Acinetobacter baumannii*, MDR *Klebsiella pneumoniae subspecies pneumoniae* and *Enterococcus faecalis*. Acquisition was categorized as healthcare associated (6). Contact precautions were advised against the multidrug-resistant organisms (MDRO).

Case 6

The sixth case was that of a female infant, admitted into the NICU. The patient was 51 days old at the time of microbiological sampling. The infant had experienced preterm birth, was afflicted with perforated necrotising enterocolitis, and had undergone a right hemicolectomy. The post-operative course had been complicated by a superficial surgical site infection and sepsis. Carbapenem exposure (meropenem), but not tigecycline exposure was documented. Additional documented antimicrobial exposures included piperacillin-tazobactam, amikacin, metronidazole, and colistin. The clinical rationale given for microbiological sampling was that of admission work-up. Microbiologic sampling included blood cultures. Microscopy of the Gram-stained positive blood culture broth revealed Gram-negative bacilli. Culture yielded a monomicrobial growth of *Klebsiella pneumoniae subspecies pneumoniae*. Acquisition was categorized as healthcare associated (6). AST by BMD indicated that the isolate was susceptible to colistin, with an MIC of 1 µg/mL. The carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection was treated using combination therapy comprising meropenem and colistin and the infant was subsequently discharged from NICU.

2.5 Discussion

The frequency of occurrence of TGC Co-R CRE recorded in our study was in accord with the reported global rate of less than 5% (18). All recorded cases of TGC Co-R CRE in our study were healthcare-associated infections (HCAI). This was also in keeping with the reported clinical epidemiology of CRE (6, 48).

Most patients in our study were female. Male gender has, however, been described as a risk factor for CRE acquisition and infection (49-51). Further research is thus recommended to determine if female gender is a risk factor for infection with TGC Co-R CRE. Most of the patients in our study belonged to the paediatric age group. This contrasts with published findings linking age over sixty years both to infections with CRE as well as CRE-associated mortality (6, 51). Further research should, therefore, be undertaken to determine if paediatric patients constitute a risk group for infection with TGC Co-R CRE.

The ICU preponderance of CRE in our study has also been borne out in the literature (16), as has the association of CRE with burn injuries (52). ICU support necessitates the utilisation of medical devices that increase the susceptibility of the critically ill to acquiring MDRO-related HCAI (53). Indeed, the literature shows that medical device usage is an acquisitional risk factor for CRE (33). Half of the patients in our study did not survive. This correlates with high mortality rates associated with CRE that have been reported in the literature (6, 8).

The presence of comorbidities, for example diabetes mellitus, as well as prematurity, recent surgery, intra-abdominal infection, and invasive procedures involving the abdomen are associated with the acquisition of CRE (54-57). In accordance with these findings, comorbid conditions noted in our case series included gestational diabetes, preterm birth, perforated necrotising enterocolitis and abdominal surgery.

Immunosuppression, including HIV, diabetes mellitus and the receipt of immunosuppressive therapy is associated with infections by MDRO (58). Indeed, immunosuppression has been associated with CRE infection (52). Immunosuppressive comorbidities of note relating to our cases included HIV, gestational diabetes (as mentioned in the preceding paragraph) and exposure to corticosteroid therapy. Additionally, one of our reported cases evinced lupus nephritis, a condition managed with immunosuppressant therapy (59). These immunosuppressing comorbidities warrant further research to confirm their association with TGC Co-R CRE.

Most of the cases in our study were exposed to carbapenem therapy, which is a known acquisitional risk factor for CRE (33). The single case not exposed to carbapenem therapy received amoxicillin-clavulanate therapy, a factor of import, considering that antecedent exposure to antimicrobials with activity against anaerobes, such as possessed by amoxicillin-clavulanate, may serve as a predisposing factor for CRE colonisation (60). Interestingly, none of the cases in our study were exposed to tigecycline therapy, which has been established to be a predisposing factor for the emergence of and infection with TGC Co-R CRE (61-63). Possible explanations for the occurrence of TGC Co-R CRE not associated with prior tigecycline exposure include nosocomial horizontal transmission or horizontal genetic transfer (64, 65). Additional antimicrobial exposures associated with TGC Co-R CRE acquisition include penicillin, to which all our cases (6/6; 100%) had exposure; aminoglycosides, to which half our cases (3/6; 50%) had exposure and fluoroquinolones, to which two-thirds (4/6; 66.7%) of our cases had exposure (15).

AMS and infection prevention and control efforts advocating for adherence to standard and contact precautions need to be superimposed upon a background of enhanced surveillance of both AMU and AMR. Such strategies are essential to lowering the resistance-engendering selection pressure of AMU and retarding the dissemination of both antimicrobial resistant bacteria (ARB) as well as antimicrobial resistance genes (ARG) (66).

The commonest specimen type was endotracheal aspirate, in keeping with the literature describing the lower respiratory tract as the commonest site of CRE infection (51). The microbial species diversity of the isolated TGC Co-R CRE, comprising *Klebsiella pneumoniae*, *Enterobacter cloacae* complex and *Serratia* species were in accordance with published data on CRE in general and TGC Co-R CRE specifically (4, 6, 14, 15, 67). All recorded isolates of TGC Co-R CRE were MDR, in keeping with South African trends (24), and underscoring the vulnerability of our antimicrobial armamentarium against such MDRO.

2.6 Limitations

The number of cases of TGC Co-R CRE recorded in our study are insufficient to draw firm conclusions regarding prevalence and risk factors for acquisition, but nevertheless are of hypothesis-generating value for subsequent research. Additionally, time and financial constraints have precluded the genotypic characterisation of TGC Co-R CRE recorded at our centre and have thereby restricted our study to phenotypic and clinical characterisation.

2.7 Conclusion

Tigecycline co-resistance in CRE is an emerging phenomenon of great concern that threatens the viability of tigecycline as a last resort agent against CRE. Our study has served to uncover the emergence of TGC Co-R CRE at our centre. Our findings serve to alert professionals in clinical microbiology, infectious diseases, biomedical science, epidemiology, and infection prevention and control, to the need for enhanced surveillance of this MDRO.

Larger and longer prospective studies, including molecular epidemiology are needed to fully characterise the burden of TGC Co-R CRE in terms of prevalence, transmission dynamics, resistance mechanisms, and risk factors for acquisition. An important finding from our study was the absence of tigecycline exposure in any of our cases of TGC Co-R CRE. This revelation warrants further genotypic characterisation and phylogenetic analysis to elucidate the mechanisms of such non-exposure associated tigecycline co-resistance in CRE.

2.8 Recommendations

It is imperative that the utility of last resort antimicrobials such as tigecycline be conserved for the treatment of infections, such as complicated skin and skin structure infections and complicated intra-abdominal infections caused by MDRO such as CRE (26). Rational AMS, robust infection prevention and control and enhanced surveillance should be championed to stem the rising tide of AMR, as evidenced by the emergence of TGC Co-R CRE. Investment is needed into the research and development of innovative rapid diagnostics and novel antimicrobials to effect timely targeted therapy and replenish our dwindling store of therapeutic options against MDRO such as TGC Co-R CRE.

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APPENDICES

APPENDIX 1

Biomedical Research Ethics Committee Approval Letter

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

20 January 2021

Dr Khathija Bibi Sheik Aboo (200266850)
School of Laboratory Medicine & Medical Science
Medical School

Dear Dr Aboo,

Protocol reference number: BREC/00001579/2020
Project title: Molecular epidemiology of colistin and/or tigecycline co-resistance in carbapenem-resistant Enterobacteriaceae in the public healthcare sector in KwaZulu-Natal, South Africa.
Degree: Master of Medicine
***NEW TITLE:* The Characterisation of Tigecycline Co-Resistant Carbapenem-Resistant Enterobacterales observed at Inkosi Albert Luthuli Central Hospital, Durban, South Africa**

We wish to advise you that your application for amendments received on 09 November 2020 to amend the title (to the above new title), aim, objectives, study design and methodology for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

The committee will advised of the above at its next meeting to be held on 09 February 2021.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

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

APPENDIX 2

Supplementary Table of AST Results of Microbial Isolates and Clinical Outcome of Cases

			Antibacterial																	
Case	Isolate	Outcome	AMK	AMC	AMP/AMX	FEP	CTX/CRO	FOX	CAZ	CXM	CIP	CST	ETP	GEN	IPM	MEM	NIT	TZP	TGC	SXT
1	KLEPP	DEM	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R
2	SERSP	DEM	R	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R
3	ENTCD	DIS	I	R	R	R	R	R	R	R	R		R	R	I	I	R	R	R	R
4	ENTCC	DIS	S	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R
5	KLEPP	DEM	I	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R
6	KLEPP	DIS	S	R	R	R	R	R	R	R	R	S	I	R	R	I	R	R	R	R

AMK: Amikacin; AMC: Amoxicillin-clavulanate; AMP/AMX: Ampicillin/Amoxicillin; FEP: Cefepime; CTX/CRO: Cefotaxime/Ceftriaxone; FOX: Cefoxitin; CAZ: Ceftazidime; CXM: Cefuroxime; CIP: Ciprofloxacin; CST: Colistin; ETP: Ertapenem; GEN: Gentamicin; IPM: Imipenem; MEM: Meropenem; NIT: Nitrofurantoin; TZP: Piperacillin-tazobactam; TGC: Tigecycline; SXT: Trimethoprim/Sulfamethoxazole; KLEPP: *Klebsiella pneumoniae subspecies pneumoniae*; SERSP: *Serratia* species; ENTCD: *Enterobacter cloacae subspecies dissolvens*; ENTCC: *Enterobacter cloacae subspecies cloacae*; DEM: Demised; DIS: Discharged; S: Susceptible; I: Intermediate; R: Resistant

APPENDIX 3

Originality Report

Turnitin Originality Report

MMed Latest Draft by Khathija Bibi Sheik Aboo

From Khathija (Khathija)

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