Antibiotic prescribing patterns in the presence of Carbapenem-Resistant Enterobacteriaceae at the Witwatersrand Donald Gordon Medical centre in Johannesburg, Gauteng

A mini-dissertation submitted by

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DECLARATION

I AK Sithole, declare that the mini-dissertation hereby submitted to the Sefako Makgatho Health Sciences University, for the degree of Master of Science (Medical) in Pharmacy, in the Faculty of Health Sciences, School of Health Care Sciences has not previously been submitted by me for a degree at this or any other university; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

__________________________________________  _________________________
AK Sithole (Miss)                              Date
ACKNOWLEDGEMENTS

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- The Wits Donald Gordon medical centre, the Chief Executive Office, the Research Department, the Pharmacy Manager and all the staff members and
- To my Family
DEDICATION

I dedicate my dissertation to my family especially my mom. Who raised me to be the woman that I am today. For investing in my education, for teaching me and raising me to be a responsible young, independent woman that I am today. She couldn’t have done it all on her own, to my grandmother and my entire family “my village” thank you for showing up, filling the gap and lifting her up where she couldn’t. I will forever be grateful. Thank you!!
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<tr>
<td>ACI</td>
<td>Age Adjusted Charlson Comorbidity Index</td>
<td></td>
</tr>
<tr>
<td>CCI</td>
<td>Charlson Comorbidity Index</td>
<td></td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
<td></td>
</tr>
<tr>
<td>CRE</td>
<td>Carbapenem-resistant <em>Enterobacteriaceae</em></td>
<td></td>
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<tr>
<td>ESBL</td>
<td>Extended-spectrum β-lactamase</td>
<td></td>
</tr>
<tr>
<td>HAI</td>
<td>Hospital Acquired Infection</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk of death ratio</td>
<td></td>
</tr>
<tr>
<td>SASCM</td>
<td>The South African Society of Clinical Microbiology</td>
<td></td>
</tr>
<tr>
<td>SMUREC</td>
<td>Sefako Makgatho Health Science Research Ethics Committee</td>
<td></td>
</tr>
<tr>
<td>WDGMC</td>
<td>Wits Donald Gordon Medical Centre</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
<td></td>
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ABSTRACT

Introduction: According to The South African Society of Clinical Microbiology (SASCM), the threat of multi-drug resistance is global. This threat was exacerbated by the meteoric rise of carbapenemase producing Enterobacteriaceae (CPE). The common Enterobacteriaceae (the family of bacteria that includes E. coli, Serratia spp., Klebsiella spp. and Enterobacter spp.) are found in normal human intestines. When members of this family have become resistant to carbapenem antibiotics, which are considered the “drug of last resort” for infections by these organisms, by producing carbapenemase, they are called carbapenem resistant Enterobacteriaceae (CRE) or carbapenemase producing Enterobacteriaceae (CPE).

The high levels of extended-spectrum β-lactamase (ESBL) production recorded among invasive Enterobacteriaceae in South Africa, especially documented for Klebsiella pneumoniae, in both the public (55-74%) and the private sector (55-60%) shown is disconcerting. Carbapenems are the cornerstone of therapy for patients with serious infections caused by ESBL-producing organisms. The high ESBL prevalence amongst bacteraemic pathogens places a tremendous strain on the use of these agents as directed and empirical therapy. In the private sector, the use of imipenem, meropenem and ertapenem, more than doubled in terms of monthly units sold, between January 2009 and June 2011. Not only is this consumption, through selective pressure, creating the ideal environment for the development of carbapenem resistance among the Enterobacteriaceae, but at the same time, carbapenem use has been shown to be a risk factor for subsequent infections with ESBL-producing organisms.

Objectives: This study assessed the prescribing practices prior and post positive CRE microbiology cultures in all the wards in the hospital, according to antibiograms obtained from the clinical laboratories.

Method: A descriptive quantitative study was conducted retrospectively at a private hospital in Johannesburg, Gauteng Province. It was done on all patients older than 18 years of age that were admitted with a positive CRE culture during their hospitalised stay from January 2013 until December 2013 were assessed. Convenient purposive sampling was used to include all files from patients that obtained positive CRE cultures. The infection prevention practitioner was consulted to obtain information regarding the number of patients with
positive CRE culture results during this period in these units and a total of 79 patient files were identified purposely for inclusion in the study.

**Results:** During the one year study period, 79 patients in the hospital had positive CRE culture results. The majority of positive CRE results were due to *Klebsiella pneumonia* isolates (79.57%). Antibiotics prescribed post positive CRE culture were prescribed according to the antibiogram in 68% of patients. Forty nine patients survived, 21 patients died, six patients were transferred and no information could be found for three patients. Antibiotic exposure may lead to increased risk of acquiring CRE. Specific antibiotics and antibiotic classes have been frequently implicated as risk factors for colonisation or infection with CRE, and these include all the carbapenems, cephalosporins, fluoroquinolones, aminoglycosides and β-lactam/β-lactamase inhibitors (Gupta, Lumbago, Patel & Alexander, 2011). This is similar to what was found in this study where 65% of the patients received other β-lactam antibiotics prior to the positive CRE producing organisms were cultured, these included antibiotics like cephalosporins, piperacillin/tazobactam and penicillins, which might play a role in contracting an CRE producing related infection. Other antimicrobial exposure includes carbapenems (41%), fluoroquinolones (6%), and aminoglycosides (7%).

During this study patients who received appropriate therapy according to the microbiology results showed a better clinical outcome than those with inappropriate therapy, indicating the importance of appropriate prescribing practises after positive microbiology results. Looking at the clinical outcomes it indicated that sensitivity and microbiology results cannot be interpreted alone without taking into consideration the co-morbid conditions that the patient might have.

**Conclusion:** Patients who received sensitive therapy according to the microbiology results and who had a low ACCI score showed a better clinical outcome than those with resistant therapy, this underlines the importance of prescribing according to the microbiology results in combination with co-morbid conditions. According to the forum of infectious diseases, the burden of antimicrobial resistance among gram-negative pathogens, particularly CRE, is increasing rapidly worldwide. Treatment options for serious CRE infections remain extremely limited. Optimization of dosing of currently available agents and combination therapy may be the most appropriate treatment strategies. However, continued research is desperately needed, in particular randomized controlled trials, to determine the most effective treatment for serious CRE infections.
1.1 INTRODUCTION

In this chapter the background and rationale for this study are discussed. This is followed by the research question, aim and objectives of the study. The importance and significance of this study are described. The chapter ends with a short overview of the outline of the dissertation.

1.2 BACKGROUND AND RATIONALE FOR THE STUDY

According to The South African Society of Clinical Microbiology (SASCM), the threat of multi-drug resistance is a global one. This threat was exacerbated by the meteoric rise of carbapenem resistant Enterobacteriaceae (CRE). South Africa is no exception, with well-documented reports of New Delhi metallo-beta-lactamase (NDM), Klebsiella pneumoniae carbapenemase (KPC) and active oxacillin-48-like (OXA-48-like) CRE (Lowman, Bamford, Govind, Swe Swe Han, Kularatne, Senekal, Brink, Moodley, Thomas, Smit & Perovic, 2014).

The high levels of extended-spectrum β-lactamase (ESBL) production recorded among invasive Enterobacteriaceae in South Africa, especially documented for Klebsiella pneumoniae, in both the public (55-74%) and the private sector (55-60%) is disconcerting. Carbapenems are the cornerstone of therapy for patients with serious infections caused by ESBL-producing organisms. The high ESBL prevalence amongst bacteraemic pathogens places a tremendous strain on the use of these agents as directed and empirical therapy. In the private sector, the use of imipenem, meropenem and ertapenem, more than doubled in terms of monthly units sold, between January 2009 and June 2011. Not only is this consumption, through selective pressure, creating the ideal environment for the development of carbapenem resistance among the Enterobacteriaceae, but at the same time, carbapenem use has been shown to be a risk factor for subsequent infections with ESBL-producing organisms (Coetzee & Brink, 2011).

New, effective antibiotics are only likely to become available in 15 - 20 years. To prevent deaths from untreatable gram-negative infections in South Africa, the rights of any doctor, whether in general or in hospital practice, to indiscriminately prescribe whatever antibiotic they wish, and in whatever fashion, must be challenged.
Furthermore, although prevention of the emergence and subsequent spread of carbapenem-resistant Enterobacteriaceae (CRE) has focused on acute and chronic care facilities and inter alia on antibiotic exposure in these institutions, CRE may soon become an issue within entire communities, highlighting a role for public health authorities in CRE prevention efforts (Brink, Coetzee, Clay, Corcoran, van Greune, Deetlefs, Nutt, Feldman, Richards, Nordmann & Poirel, 2012).

There has been an increase in the usage of carbapenems at Wits Donald Gordon medical centre (WDGMC) in the past year (2013), which lead to the implementation of antibiotic stewardship in order to optimize antimicrobial therapy administered to patients, to ensure cost-effective therapy and improve patients' outcome while containing bacterial resistance. A protocol was also put in place for infection control purposes. This study was conducted to determine antibiotic prescribing patterns in the presence of CRE.

1.2.1 Screening of patients

A protocol for “Screening of patients for infection prevention purposes on admission” is available in the hospital (Le Roux, 2016). This protocol states that, on admission it is essential that patients are screened to better enable the hospital to manage and plan the future treatment while in hospital. These patients include:

- All patients transferred in from other hospitals.
- All foreign patients admitted for elective or emergency treatment.
- All patients from care facilities e.g. old age homes, rehab centers etc.
- All patients who have had any hospital admission in the past three months for longer than 7 days.
- All patients who have an indwelling urinary catheter: urine test only, if none of the above criteria is met.
### Table 1.1: Screening SOP: All the swabs must be dry swabs (i.e. the swab without the gel medium).

<table>
<thead>
<tr>
<th>Which Sample?</th>
<th>How is the sample taken?</th>
<th>Labeling of the specimens for investigation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Swab</td>
<td>Swab from inside the nose</td>
<td>MRSA</td>
<td>Make sure to touch the nasal hairs</td>
</tr>
<tr>
<td>Rectal Swab</td>
<td>Swab from inside the anal canal.</td>
<td>CRE/ VRE/PCR</td>
<td>Approximately 2 cm</td>
</tr>
<tr>
<td>Wound Swab</td>
<td>From wound bed</td>
<td>MRSA</td>
<td>If any exudates present after cleaning with Saline</td>
</tr>
<tr>
<td>Urine sample</td>
<td>From the catheter.</td>
<td>MC&amp;S</td>
<td>Not the bag</td>
</tr>
</tbody>
</table>

**Abbreviations:** MRSA - Methicillin resistant *Staphylococcus aureus*, CRE - Carbapenem resistant *Enterobacteriaceae*, VRE - Vancomycin-resistant enterococci, PCR - Polymerase chain reaction, MC&S - Micro-Culture and sensitivity.

Limiting the spread of epidemic multidrug-resistant members of the *Enterobacteriaceae* inside hospitals requires early detection of gastrointestinal carriers by screening as an integral component of successful infection control strategies. Using a PCR-based method for CRE surveillance has several disadvantages: first, the species carrying the KPC gene and the phenotype remain unknown, i.e., susceptibilities to clinically relevant antibiotics such as the polymyxins and tetracyclines are not tested. This may lead to a delay in identifying epidemiologically important clusters or the emergence of new phenotypes or the spread of KPC to previously unaffected species (Schechner, Straus-Robinson, Schwartz, Pfeffer, Tarabeia, Moskovich, Chmelnitsky, Schwaber, Carmeli & Navon-Venezia, 2009).

Patients were screened according to protocol and 49% of the rectal swabs tested positive for CRE. Screening is used to identify unrecognized CRE colonization as clinical cultures alone will identify only a fraction of all patients with CRE. Generally, this testing has involved stool, rectal, or peri-rectal cultures and sometimes cultures of skin sites, wounds or urine (if a urinary catheter is present).

If a person is colonised with Carbapenem resistant *Enterobacteriaceae* (CRE), they do not need to be treated. However, if the bacteria have caused an infection then antibiotics will be required (Centres of Disease Control and prevention, 2014).
1.3 RESEARCH QUESTION

The following research questions were formulated:

1. What were the antibiotic prescribing patterns in patients with positive cultures for Carbapenem-resistant Enterobacteriaceae at WDGMC?

2. What was the prevalence of CRE at WDGMC?

1.4 AIM OF THE STUDY

The aim of this study was to analyse the antibiotic prescribing patterns in patients with positive cultures for CRE at Witwatersrand Donald Gordon Medical Centre (WDGMC).

1.5 OBJECTIVE OF THE STUDY

The objectives of the study were as follows:

- To describe prescribing practices prior to positive CRE microbiology cultures.
- To describe prescribing practices after CRE was cultured.
- To compare and then describe the differences/similarities of prescribing practices prior to and after positive CRE cultures.

1.6 IMPORTANCE OR SIGNIFICANCE OF THE STUDY

Carbapenem-resistant Enterobacteriaceae (CRE) is a family of gram-negative bacteria that are almost immune to the class of carbapenem antibiotics, drugs which are considered the "drug of last resort" for infections such as these. Enterobacteriaceae are common bacteria that form part of the normal flora that can be infectious agents. Bloodstream infections from Enterobacteriaceae can cause mortality in up to half of patients infected, a rate much higher than other resistant infections such as Methicillin-resistant Staphylococcus aureus (MRSA) or Clostridium difficile. Some CRE bacteria have become resistant to most available antibiotics. Infections with these microorganisms are very difficult to treat, and can be fatal (Centres of Disease Control and prevention, 2014).

South Africa has a high burden of infectious disease among its population and antibiotics have been widely used to combat these. Since antibiotics first entered common use in the 1950s, many South Africans in the country’s private and public healthcare sectors have been
treated using these drugs. The widespread use of antibiotics has led to bacterial organisms developing resistance against antimicrobial drugs. This raises concerns on the long-term sustainable effectiveness of antibiotics to treat infectious diseases and the potential impact on the country’s health and economy.

Antimicrobial resistance (AMR) is driven by many factors, primarily associated with the inappropriate management and consumption of antibiotics. For example, the incorrect prescription of antibiotics for the treatment of common colds and flu which are caused by viral and not by bacterial infections, have contributed to the increasing presence of AMR. Healthcare professionals (HCPs) do not necessarily have instant tests at hand that can tell them whether their patient has a bacterial infection or not. In the absence of instant tests to identify the specific bacterial strain causing an infection, HCPs tend to prescribe broad-spectrum antibiotics to treat as broad a range of bacteria as possible. Patients are also a contributing factor, as they demand HCPs to prescribe antibiotics as part of their treatment. The absence of an antibiotic on a prescription is regarded by many patients as “sub-optimal” treatment by the HCP. Drug companies also play their part in driving the increased use of antibiotics to increase sales. Recognising the long term threat of AMR, many HCPs have accepted the responsibility to adjust their treatment regimens to limit the use of antibiotics in an effort to reduce AMR (Krugel, 2011).

According to the CDC, antibiotics must be used judiciously in humans and animals because both uses contribute to the emergence, persistence, and spread of resistant bacteria. Resistant bacteria in food-producing animals are of particular concern. Food animals serve as a reservoir of resistant pathogens and resistance mechanisms that can directly or indirectly result in antibiotic resistant infections in humans. For example, resistant bacteria may be transmitted to humans through the foods we eat.

- Some bacteria have become resistant to more than one type of antibiotic, which makes it more difficult to treat the infections they cause.
- Preserving the effectiveness of antibiotic drugs is vital to protecting human and animal health.

This shows that antibiotic use in food-producing animals also plays a role in antimicrobial resistance.

The Witwatersrand Donald Gordon Medical Centre (WDGMC) is the first private teaching hospital in South Africa (SA). The hospital was established to create a facility in the private
sector for training of sub-specialists. This training programme is part of and complementary to the existing training programmes at The Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath and Helen Joseph hospitals. The first laboratory-confirmed case of a New Delhi Metallo-β-lactamase (NDM-1) producing isolate from South Africa was reported at Charlotte Maxeke Johannesburg Academic Hospital. The emergence of NDM-1 in Enterobacteriaceae has heightened global concern regarding antimicrobial resistance. The Multiple drug resistance (MDR) nature, propensity for horizontal transfer, and rapidity of global spread and emergence of micro-organisms harbouring this gene means that the threat of untreatable infections has arrived. The NDM resistance mechanism in Enterobacteriaceae threatens to cause serious gram-negative infections that will be untreatable. The NDM-1 enzyme hydrolyses all available penicillin, cephalosporin and carbapenem antibiotics, and is commonly accompanied by additional resistance mechanisms to multiple antibiotic classes. Initially identified as a significant healthcare risk on the Indian sub-continent, it has rapidly become a global problem, posing significant diagnostic and management challenges (Lowman, Sriruttan, Nana, Bosman, Venturas, Clay & Coetzee, 2011). It is for the reasons discussed above that this study was conducted on the antibiotic prescribing patterns in the presence of CRE at WDGMC.

Since 1 March 2010 the Molecular Biology Laboratory at the Ampath National Reference Laboratory has been screening Enterobacteriaceae for the presence of these novel genes. Recently the emergence of NDM-1 for the first time in South Africa, and KPC-2 for the first time in Africa, was documented among clinical isolates of K. pneumoniae and E. cloacae in hospitalised patients in Johannesburg and Pretoria, respectively (Brink et al., 2012).

The presence of these novel genes pose a threat to the health system, as carbapenem antibiotics are considered “drug of last resort” for infections such as these. Therefore measures need to be taken to prevent resistance. This includes monitoring antibiotic prescribing patterns in the hospitals.

In 2011, the National Department of Health (NDoH) of South Africa established the domain of patient safety, clinical governance and clinical care, which gave guidelines on how to ensure quality nursing, clinical care and ethical practice; how to reduce unforeseen harm to health care users or patients in identified cases of greater clinical risk; how to prevent or manage problems or adverse events; and support any affected patients or staff (NDoH, 2011). It is the responsibility of the health care provider to ensure that: adverse events or patient safety incidents are instantly identified and managed to minimise patient harm and
suffering, and to ensure that adverse events are routinely investigated and managed to prevent repetition and to learn from errors.

1.7 OUTLINE OF THE DISSERTATION

This dissertation consists of five chapters. Chapter 1 introduces the background and rationale of the study, together with the stated research question, aim and objectives of the study. It will also include an overview of the importance of the study. Chapter 2 focuses more on the review of literature relating to carbapenems and carbapenem resistance, risk factors for acquisition of carbapenem-resistant Enterobacteriaceae and the role of antibiotics in in carbapenem-resistant Enterobacteriaceae. Chapter 3 discusses the methodology related to this study. This includes the study design, study site, study population and the sample selection. It further elaborates on the study period, the process of data collection, the analysis of data, the reliability and validity of the study. Ethical principles of the study are also discussed. Chapter 4 presents the manuscript containing the results of the study followed by the discussion of the results. Chapter 5 concludes the dissertation with the limitations of the study, recommendations for future studies and a conclusion. Figure 1 provides a short illustration of the layout of this dissertation.
1.8 SUMMARY

Carbapenem antibiotics are the cornerstone of therapy for patients with serious infections caused by ESBL-producing organisms. The high ESBL prevalence amongst bacteraemic pathogens places a tremendous strain on the use of these agents as directed therapy, and empirically as well. According to The South African Society of Clinical Microbiology (SASCM), the threat of multi-drug resistance is a global one. This threat was exacerbated by the meteoric rise of carbapenem-resistant Enterobacteriaceae (CRE). This retrospective study aims to analyse the antibiotic prescribing patterns in patients with positive cultures for CRE producing organisms in a private academic hospital in South Africa.
CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

In this chapter, an overview of published literature on the study topic and previous research done in this particular field is provided. The chapter begins with the development of CRE resistance, infections caused by CRE, the role of antibiotics in CRE, carbapenem antibiotics, CRE prevalence, the risk factors for acquisition and the role of antibiotic stewardship.

2.2 DEVELOPMENT OF RESISTANCE

Carbapenem-resistant Enterobacteriaceae (CRE) is gram-negative bacteria that are resistant to the carbapenem class of antibiotics. In Enterobacteriaceae, carbapenem resistance arises from two main mechanisms: (i) acquisition of carbapenemase genes that encode for enzymes capable of degrading carbapenems, or (ii) a decrease in the uptake of antibiotics by a qualitative or/and quantitative deficiency of porin expression in association with overexpression of b-lactamases that possess very weak affinity for carbapenem (Nordmann, Dortet and Piorel, 2012). According to Guh, Bulens, Mu, Jacob, Reno, Scott et al (2015), resistance can vary from moderate to severe. Carbapenem-resistant Enterobacteriaceae have been defined as carbapenem-nonsusceptible, and extended-spectrum cephalosporin-resistant Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae complex, Klebsiella pneumoniae, or Klebsiella oxytoca. Some exclude ertapenem resistance from the definition.

The b-lactam family of antibiotic molecules consists of four groups: cephalosporins, monobactam, penicillins, and carbapenems. These antibiotics share common structure and mechanism of action. They enter the periplasmic space through porins, where they then inhibit transpeptidases (which are also known as penicillin-binding proteins (PBPs), enzymes that facilitate peptide cross-links during cell wall synthesis. Their binding to the PBP active site is facilitated in part by their common structure, which causes transpeptidases to irreversibly lose their catalytic activity. Inhibition of transpeptidases prevents the formation of cross-links between peptidoglycan polymers and causes a build-up of peptidoglycan precursors. Newly formed peptidoglycan is weakened from the absence of cross-linkages. The continued activity of autolysins, that function like lysozymes and cleave glycosidic and peptide bonds of peptidoglycan in periplasm, weakens the cell wall and leads to osmotic bursting of the bacterial cell. A unique quality of carbapenems is their resistance to
hydrolysis by bacterial plasmid and chromosomally mediated extended-spectrum β-lactamases (ESBL). Carbapenems, β-lactam antibiotics, targets cells by inhibiting transpeptidases (penicillin-binding proteins).

Carbapenem-resistant Enterobacteriaceae produce enzymes called carbapenemase, a form of β-lactamase. These enzymes cleave the β-lactam ring, an essential component of β-lactam antibiotics that are recognized by and bound to PBPs. Carbapenemase are divided into different classes, depending on the structure of the enzyme and the mechanism by which they hydrolyze the β-Lactam ring. The two broad categories of carbapenemase are serine-carbapenemase, which contain serine at the active site, and metallo-carbapenemase, which contain zinc at the active site. Class A carbapenemase are serine carbapenemase and are encoded on either the chromosome of the bacteria or a plasmid. A serine at position 70 at the active site of this class of enzymes is required for hydrolysis of β-lactams to occur. Class D carbapenemase, also referred to as the OXA β-lactamases, are serine β-lactamases. They are encoded on plasmids and contain a large variability in amino acid sequence. The mechanism for Class D carbapenemase forms an acyl intermediate when breaking the β-lactam ring. Class B carbapenemase are metallo-lactamases and require a zinc at the active site for hydrolysis (Nordman, Dortet & Poirel, 2012; Patel, Gopi & Bonomo, 2013).

2.3 INFECTIONS CAUSED BY CRE

An infection or colonization can be represented by CRE. Colonization means that the organism can be found on the body but it is not causing any symptoms or disease. Colonizing CRE strains can go on to cause infections if they gain access to body sites that are usually sterile like the bladder, the lungs, or the bloodstream. Infections are usually associated with symptoms which vary based on the site that is infected (e.g., cough when in the lungs, urinary tract infection symptoms when in the bladder) but can also include general symptoms like fever or chills. CRE can cause infections in almost any part of the body including bloodstream infections, ventilator- associated pneumonia, and intra-abdominal abscesses. Types of CRE are sometimes known as KPC (Klebsiella pneumoniae carbapenemase) and NDM (New Delhi Metallo-beta-lactamase). KPC and NDM are enzymes that break down carbapenems and make them ineffective. Both of these enzymes, as well as the enzyme VIM (Verona Integron-Mediated Metallo-β-lactamase) have also been reported in Pseudomonas spp. Based on information from a CDC pilot surveillance system most CRE infections involve the urinary tract, often in people who have a urinary catheter or
have urinary retention. It is also important to note that CRE kill up to half of patients who get bloodstream infections from them (Centers of Disease control and prevention, 2015).

2.4 THE ROLE OF ANTIBIOTICS IN CARBAPENEM-RESISTANT ENTEROBACTERIACEAE

The emergence of carbapenem resistance in Enterobacteriaceae is a threat to global health. Reported outcomes of infections with carbapenem-resistant Enterobacteriaceae (CRE) are poor. Very few viable antibiotic options remain for the treatment of these resistant organisms. Antibiotics which are currently in use to treat CRE infections include aminoglycosides, polymyxins, tigecycline, fosfomycin, and temocillin. The role of combination therapy, including carbapenem containing regimens, remains to be defined. There are a number of important concerns with regard to all of these treatment options such as limited efficacy, increasing reports of resistance, and specific toxicities. Data from retrospective-studies favours combination therapy, over single-agent therapy for the treatment of CRE bloodstream infections. In summary, new antibiotics are urgently needed, as is additional prospective research (van Duin, Kaye, Neuner & Banomo, 2013).

2.5 CARBAPENEMS

Carbapenems differ from penicillin and cephalosporins by a methylene replacement for the sulfur in the 5-membered ring structure. The different types of carbapenems include imipenem, meropenem, doripenem and ertapenem. This class of drugs is very active against gram-negative, gram-positive, and anaerobes. They were developed to deal with β-lactamase-producing gram-negative organisms resistant to penicllins. A short discussion on the drugs now ensues (Katzung, 2007):

- **Imipenem**- it has a wide spectrum with good activity against many gram-positive and gram-negative aerobic and anaerobic bacteria, including *P. aeruginosa*. Imipenem is inactivated by dehydropeptidase in renal tubules, resulting in low urinary concentrations. Consequently, it is administered together with an inhibitor of renal dehydropeptidase, cilastin for clinical use.

- **Meropenem** has a similar spectrum activity to imipenem but has slightly greater activity against gram-negative aerobes and slightly less activity against gram-negative. In addition, it penetrates well into the CSF and is a suitable alternative for bacterial meningitis. Meropenem is more stable to renal inactivation thus given without cilastatin.
• **Ertapenem** has a narrow spectrum lacking activity against enterococci, *P. aeruginosa*, *A. baumannii* and other non-fermenters. It has the longest half-life (4 hours) therefore administered once daily by IV or IM routes.

### 2.6 CARBAPENEM-RESISTANT ENTEROBACTERIACEAE PREVALENCE

According to the U.S. Centres for Disease Control (CDC), CRE was first detected in a North Carolina hospital in 2001. Since that time, it has been identified in health care facilities in 41 other states. Studies showed that in 2012, 3% of patients in Chicago-area ICUs carried CRE. The same data indicated a 30% infection rate in long-term care facilities (e.g. nursing homes), though not all patients are symptomatic. During just the first half of 2012, almost 200 hospitals and long-term acute care facilities treated at least one patient infected with these bacteria (LaBore, 2013).

Carbapenem resistance among Enterobacteriaceae can be conferred by several genetic mechanisms, but epidemiologically the most important of them results in the production of β-lactamases (carbapenemase), which hydrolyse carbapenems and most other β-lactams. These genes mostly reside on large plasmids which frequently contain other resistance determinants, such as those that confer resistance to the aminoglycosides and reduced susceptibility to the fluoroquinolones.

The carbapenemase belong to different classes and include:

- **Klebsiella pneumonia** carbapenemase (KPCs) and Guiana extended-spectrum β-lactamases (GESs) (class A)
- The metallo-β-lactamases (MBLs), such as the Verona integron-encoded (VIMs) and the recently described New Delhi metallo-β-lactamases (NDM-1) (class B)
- Oxacillins-type carbapenemase such as OXA-48 and its derivatives, which also occur in Enterobacteriaceae (class D) (Brink *et al.*, 2012).
2.7 RISK FACTORS FOR ACQUISITION OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE

The following may lead to increased risk of acquiring CRE:

- **Antibiotic exposure**
  
  Exposure to antibiotics is a major risk factor for infection with CRE. Specific antibiotics and antibiotic classes have been frequently implicated as risk factors for colonisation or infection with CRE, and these include all the carbapenems, cephalosporins, fluoroquinolones, aminoglycosides and β-lactam/β-lactamase inhibitors (Gupta, Lumbago, Patel & Alexander, 2011). Studies have also shown that prior carbapenem therapy is not a prerequisite for carbapenem resistance among *E. coli* or *K. pneumoniae*. The plasmids that confer such resistance frequently carry additional resistance determinants that confer cross-resistance to most other antibiotic classes. Consequently, prior use of any antibiotic may select for a carbapenemase-producing GNB.

- **Healthcare exposure**
  
  The major risk factors for acquiring KPCs colonisation and/or infection are similar to those for extended-spectrum β-lactamases (ESBLs), the prevalence of which is extremely high in South Africa. For example, the ESBL rate for *K. pneumoniae* cultured from complicated intra-abdominal infections in private hospitals is 41.2% and that for bacteraemic isolates in the public sector 55 - 74% (Brink et al., 2012).

These risk factors include:

- Organ or stem cell transplantation
- Intensive care unit admission
- Poor functional status
- Severe illness
- Mechanical ventilation
- Prolonged hospitalisation
- Surgery.
Similar to ESBLs, it appears that long-term care facilities are reservoirs of KPCs because they act as a point of convergence of patients at high risk, amplified by cross-transmission, and this facilitates regional dissemination (Brink, et al., 2012).

Emerging evidence suggests that the presence of a high invasive-device is also a predictor of CRE. For *K. pneumoniae*, the presence of a central venous line and a urinary catheter are significantly associated with acquisition of KPC.

Transfer of patients who have been hospitalised in medical facilities or countries where CRE isolates have been established is also a major risk factor (Brink, et al., 2012).

2.8 ANTIBIOTIC STEWARDSHIP

2.8.1 International

According to the Association for professionals in Infection control and Epidemiology, Antimicrobial stewardship is a coordinated program that promotes the appropriate use of antimicrobials (including antibiotics), improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms.

Antimicrobial resistance is increasing; however, antimicrobial drug development is slowing. Now more than ever before, antimicrobial stewardship is of the utmost importance as a way to optimize the use of antimicrobials to prevent the development of resistance and improve patient outcomes (Doron & Davidson, 2011).

Improving the use of antibiotics is an important patient safety and public health issue as well as a national priority (CDC, 2013). The 2006 CDC guideline “Management of Multi-Drug Resistant Organisms in Healthcare Settings” stated that control of multi-drug resistant organisms in healthcare “must include attention to judicious antimicrobial use” (Siegel, Rhinehart, Jackson & Chiarello, 2006). In 2009, CDC launched the “Get Smart for Healthcare Campaign” to promote improved use of antibiotics in acute care hospitals and in 2013 (CDC, 2013) the CDC highlighted the need to improve antibiotic use as one of four key strategies required to address the problem of antibiotic resistance in the U.S (CDC, 2013).

2.8.2 National (South Africa)

According to the Federation of Infectious Diseases Societies of Southern Africa (FIDSSA, 2016) decades of injudicious antibiotic prescribing and a disregard for basic infection control practice have left the international community facing a return to the age of untreatable
bacterial infections. The rise of extended spectrum β-lactamase (ESBL)-producing bacteria and subsequently carbapenemase-resistant strains has left colistin as the sole antibiotic in the armamentarium for these infections, an antibiotic from the 1960s with a high toxicity profile. Colistin resistance has already been reported, rendering some patients untreatable. The recent identification and subsequent spread of New Delhi Metallo-beta-lactamase-1 (NDM-1) and \textit{Klebsiella pneumonia} carbapenemase (KPC)-producing Enterobacteriaceae signify the latest 'super-bugs' to threaten public health. The number of NDM-1 infections in Gauteng is rising rapidly with spread to other South African cities. South Africa needs a strong, coordinated, and urgent response to this threat. Clinical governance of antibiotic prescribing (antibiotic stewardship) through dedicated programmes and infection control practice must be strengthened if we are to control the situation (FIDDSA, 2016).

In South Africa, the National Department of Health came up with the Antimicrobial resistance (AMR) national strategy framework 2014 – 2024. A national response to AMR required to complement the development of a global action plan, as articulated in the WHO resolution EB134/37 “Combating antimicrobial resistance including antibiotic resistance”, adopted by the World Health Assembly in May 2014. The purpose of the Antimicrobial Resistance National Strategy framework is to provide a structure for managing AMR, to limit further increases in resistant microbial infections, and improve patient outcomes (National Department of Health Antimicrobial Resistance National Strategy Framework, 2014 – 2024).

2.9 SUMMARY

The literature on the topics relevant to this study has been reviewed in this chapter. ESBL-producing Enterobacteriaceae are emerging worldwide. This trend is of particular concern, because gram-negative bacteria account for a large proportion of healthcare–associated infections, especially in intensive care units.

ESBL-producing organisms are difficult to treat because the organisms are frequently resistant to multiple antibiotics. The remaining treatment option for these bacteria is one of the antibiotics belonging to the carbapenem-group.

To preserve the antibiotics available currently there should be a global focus on implementing antibiotic stewardship programs. Antimicrobial stewardship is a coordinated program that promotes the appropriate use of antimicrobials, reduces microbial resistance, improves patient outcomes and decreases the spread of infections caused by multidrug-resistant organisms.
Chapter 2: Literature Review

The subsequent chapter provides an overview on the methodology used for the study. The literature on the topics relevant to this study has been reviewed in this chapter. The topics explored in the literature review included:

- Carbapenems
- Carbapenem-resistant Enterobacteriaceae (CRE) prevalence
- Risk factors for acquisition of CRE
- The role of antibiotics in CRE
CHAPTER 3
METHOD

3.1 INTRODUCTION

This chapter describes the methodology used to conduct the study in detail. The chapter starts with an introduction and is followed by the purpose of the study, a description of the study site, a description of the study design, a description of the study period and the study population and sample. Then follows with data collection and data collection instruments, statistical considerations, reliability and validity of data, ethical considerations and lastly bias. The data collection instruments are individually discussed after which the data capturing and analysis procedures are outlined. Measures to ensure reliability and validity of data are provided followed by the ethical considerations for this study, which concludes the chapter.

3.2 PURPOSE OF THE STUDY

The purpose of the study was to analyse antibiotic prescribing patterns in the presence of CRE at WDGMC.

3.3 STUDY SITE

The Witwatersrand Donald Gordon medical centre is a 190 bed private hospital. It specializes in transplant surgery, oncology (paediatric and adult), gastroenterology (medical and surgical), geriatric medicine, interventional radiology ophthalmology, ear- nose and throat, urology, nephrology, cardiology, women’s health and orthopaedics. The study was conducted at all the wards at WDGMC health facility, Gauteng Province, South Africa.

3.4 STUDY DESIGN

The study was a phased descriptive quantitative study conducted retrospectively. Information for the purpose of this research was retrieved from the files of patients treated during the study period. Antibiotics prescribed prior to the CRE positive culture was documented as well as the antibiotics prescribed post positive cultures for CRE.
3.5 STUDY PERIOD

Data was collected over a period of twelve months, from January 2013 to December 2013, at the WDGMC.

3.6 STUDY POPULATION AND SAMPLE SELECTION

Study population and duration of the study.

The files of adult patients that were admitted to all the units at the hospital that had a positive CRE culture, during their hospitalised period were used for the purposes of the study. This was done according to the information provided by the infection control practitioner at the hospital. The study population included all the files of adult participants that were admitted to WDGMC that had a positive CRE culture for the 12 month study period, January 2013 to December 2013.

3.6.1 The inclusion criteria

Records of participants older than 18 years admitted to WDGMC with a positive culture for CRE.

3.6.2 The exclusion criteria

- Records of participants younger than 18 years
- CRE culture negative adult participants

3.6.3 Sample selection

All the files of adult patients that were admitted to the WDGMC units that had a CRE microbiology result during the study period were included in this study. Convenient purposive sampling was used to include all files from patients that obtained positive CRE cultures. The infection prevention practitioner was consulted to obtain information regarding the number of patients with positive CRE culture results during this period in these units and a total of 79 patient files were identified purposely for inclusion in the study.
3.7 DATA COLLECTION AND DATA COLLECTION INSTRUMENTS

The retrospective data was requested from the infection prevention practitioner, filing department and pharmacy. Data was captured on a collection sheet (Appendix 1), and transferred into a Microsoft Excel™ spread sheet for further analysis. Laboratory data was obtained from the following laboratories: Lancet and Wits laboratory.

The Data Collection Instrument (DCI) (see Appendix 1) was repeatedly used during the study period. It was developed and compiled by the researcher in a data collection sheet form. It was designed according to the recommendations by Gregory and Radovinsky (2012). The designed data collection tool was a form, which recorded patient demographics, the source of a positive culture, the date it was cultured, the antibiotics prescribed prior/post positive CRE culture and the clinical outcome. The aim of the data collection sheet was to record the antibiotic prescribing prior/post CRE positive culture in all the wards in the hospital. The DCI assisted the researcher by providing a framework to summarise all information required for this study.

3.7.1 Data entry and Analysis

A Microsoft Excel™ spreadsheet was used to analyse data categorically. The statistical analysis was of a descriptive nature. The prescribed antimicrobials were categorised and summarised descriptively. The clinical outcome of the treatment was categorised per patient:

- Death
- Infection resolved
- Infection not resolved (patient still admitted)
- Transferred (unable to follow up)

For each of these categories the prescribing patterns (prior and post a positive CRE culture) were summarised by frequency counts and percentage calculations. The Fisher exact test was used for comparison of percentage as appropriate.

All statistical procedures were performed SAS, Release 9.3 or IBM SPSS statistics, running under Microsoft windows for a personal computer. Statistical significance testing will be at the 0.05 (5%) level.
Table 3.2: Data collection instrument used in the research study

<table>
<thead>
<tr>
<th>Data Collection instruments</th>
<th>Discussion of the Data Collection instruments</th>
</tr>
</thead>
</table>
| Data collection sheet (Appendix 1)                  | **AIM:** This form was completed by the pharmacist, collecting the following information: patient demographic information, antibiotic therapy prior to CRE positive results, microbiology results and antibiotic therapy after positive results. All microbiology results will be included.  
**RATIONALE:** To assist the researcher to have participants’ information on one sheet, to keep track of antibiograms and antibiotic consumption.  
**USE:** This was used to document the participant’s demographical information, co-morbid conditions, and all microbiology results. |
| Informed consent: Wits Donald Gordon medical centre (Appendix 2) | **AIM:** To obtain informed consent from Wits Donald Gordon to conduct the study  
**RATIONALE:** Provided all the relevant information relating to the study, so as to ensure that the Hospital Manager is consequently able to make an informed decision regarding the study.  
**USE:** It will be signed by the hospital manager before the study can be conducted at the site. |

3.8 RELIABILITY, VALIDITY AND BIAS OF DATA

Reliability is the extent to which results are consistent over time and an accurate representation of the total population under study is referred to as reliability and if the results of a study can be reproduced under a similar methodology, then the research instrument is considered to be reliable (Joppe, 2000).

Validity determines whether the research truly measures that which it was intended to measure or how truthful the research results are. Researchers generally determine validity by asking a series of questions, and will often look for the answers in the research of others (Joppe, 2000). According to Phelan and Wren (2006) this was achieved by:
• Ensuring the goals and objectives are clearly defined and operationalized.

• Matching the assessment measure to the goals and objectives. Additionally, have the test reviewed by faculty at other schools to obtain feedback from an outside party who is less invested in the instrument.

• If possible, compare your measure with other measures, or data that may be available.

Reliability and validity was achieved by standardizing the measurement procedure so that the procedures are always the same. The same data collection sheet was used to assess all cases; this ensured complete reliability of the tool when comparing cases as the data collection sheet reported exact information obtained from each patient file. All captured data was proof-read and cross-checked by the researcher to ensure accuracy.

During research, bias is any condition or influence that misrepresents the data obtained (Leedy & Ormrod, 2001). Bias was minimised by the researcher recording all information relevant to the study from patient files as it was captured by relevant healthcare professionals. The study population over a twelve month period aim to be a transparent representation of the amount of CRE positive patients in facility. The population was selected through CRE positive patient statistics obtained from the infection prevention practitioner statistical report.

3.9 ETHICAL CONSIDERATION

Ethical approval was obtained from the Sefako Makgatho Health Science University Research, Ethics and Publications Committee (SMUREC), approval number SMUREC/H/51/2015: PG (Appendix 3&4). Permission was obtained from the Hospital Chief Executive Officer; a letter of intent is included (Appendix 2). Data was handled confidentiality.

Participant’s identities were kept confidential by assigning each patient a study number. An arrangement was made, for the study documentation to be kept in a locked office until the end of the study. Participants' privacy was maintained throughout the study and all information was handled confidentially. This implies that only the researcher had access to the participant’s information during the study. Research data was stored appropriately in locked patient cupboard, to ensure the participants privacy. The study was an epidemiological observation. Participant personal information was only used to match the
laboratory report obtained from the laboratory dataset. Once this was done, the patient’s personal data was anonymised.

3.9.1 Informed consent

No consent was obtained from the participant as this was a retrospective study utilising patient files. No individual patient, prescriber or provider could be identified. There was no interaction with individual patients, prescribers or providers, nor were participants directly used in the project.

3.9.2 Anonymity

The patients were not identified in any sub-study. Patients were randomly allocated a study number to each record. No patient can therefore be identified or contacted, thus ensuring that anonymity and confidentiality was maintained.

3.9.3 Confidentiality

All copies/extracts of datasets on laptops or individual researchers’ computers were stored under new file names.

No individual patient can or was identified, thus ensuring confidentiality of the information and maintenance thereof.

All data was stored on password protected computers. All computers were furthermore protected by a firewall and anti-virus software.

All data will be kept (including a dedicated backup system) by the University for a period of five years, whereupon the data will be destroyed.

On completion of this study locally held files were deleted from personal computers. Computers were also formatted to ensure that no copies of the data remained.

3.10 SUMMARY

This chapter described in detail the methodology used in this study. This was a phased descriptive quantitative study that was conducted retrospectively.

Data were captured on MS Excel™ spreadsheets and analysed in consultation with a statistician using SAS®. Ethical approval was obtained from the Sefako Makgatho Health
Chapter 3: Method

Science University Research, Ethics and Publications Committee (SMUREC), approval number SMUREC/H/51/2015: PG (Appendix 2). Permission was obtained from the Hospital Chief Executive Officer and was adhered to throughout the study.

The results of the data collected over the 12-month study period will be presented in Chapter 4 in the format of a manuscript.
CHAPTER 4
MANUSCRIPT

4.1 INTRODUCTION

The results of the study with the discussion thereof will be presented in the form of a manuscript. The manuscript will be presented to the South African Journal of Infectious Diseases for publishing. See Appendix 5 for Author guidelines.

4.2 MANUSCRIPT FOR PUBLICATION

Antibiotic prescribing patterns in the presence of Carbapenem-resistant Enterobacteriaceae at the Witwatersrand Donald Gordon Medical Centre in Johannesburg, Gauteng.

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Key words: antimicrobial, Carbapenem-resistant Enterobacteriaceae, prescribing patterns, appropriate antimicrobial use, antimicrobial stewardship

Footnotes:

- The authors declare that they do not have any commercial interest or association that may pose a conflict of interest
ABSTRACT

Introduction: According to The South African Society of Clinical Microbiology (SASCM), the threat of multi-drug resistance is global. This threat was exacerbated by the meteoric rise of carbapenem-resistant Enterobacteriaceae (CRE). The common Enterobacteriaceae (the family of bacteria that includes *E. coli*, *Serratia spp.*, *Klebsiella spp.* and *Enterobacter spp.*) are found in normal human intestines. When members of this family have become resistant to carbapenem antibiotics, which are considered the “drug of last resort” for infections by these organisms, by producing carbapenemase, they are called carbapenem-resistant Enterobacteriaceae (CRE) or carbapenemase producing Enterobacteriaceae (CPE).

Objectives: This study assessed the prescribing practices prior and post positive CRE cultures in all the wards in the hospital, according to antibiograms obtained from the clinical laboratories.

Method: A descriptive quantitative study was conducted retrospectively at a private hospital in Johannesburg, Gauteng Province. It was done on all patients older than 18 years of age that
were admitted with a positive CRE culture during their hospitalised stay from January 2013 until December 2013 were assessed.

**Results:** During the one year study period, 79 patients in the hospital had positive CRE culture results. The majority of positive CRE results were due to *Klebsiella pneumonia* isolates (79.57%). Antibiotics prescribed post positive CRE culture were prescribed according to the antiibiogram in 68% of patients. Forty nine patients survived, 21 patients died, six patients were transferred and no information could be found for three patients.

**Conclusion:** Patients who received sensitive therapy according to the microbiology results and who had a low Age-Adjusted Charlson Comorbidity Index score showed a better clinical outcome than those whom were continued on resistant therapy. This underlines the importance of appropriate prescribing practices in combination with co-morbid conditions.
INTRODUCTION

Antimicrobial resistance (AMR) threatens not only the fundamentals of modern medicine, but also the sustainability of an effective global health response to infectious disease. A global action plan was initiated (O’Neill, 2016). In support of this action plan, a high-level meeting, the fourth of its kind, was held by the general assembly of the United Nations, primarily to summon and maintain strong national, regional, and international political commitment in addressing AMR both comprehensively and multi-sectorally, and to improve awareness (Un.org, 2016).

This threat was exacerbated by the meteoric rise of carbapenem resistant Enterobacteriaceae (CPE). The common Enterobacteriaceae (the family of bacteria that includes E. coli, Serratia spp., Klebsiella spp. and Enterobacter spp.) are found in normal human intestines. When members of this family have become resistant to carbapenem antibiotics, which are considered the “drug of last resort” for infections by these organisms, by producing carbapenemase, they are called carbapenem-resistant Enterobacteriaceae (CRE). South Africa has well-documented reports of New Delhi metallo-β-lactamase (NDM), Klebsiella pneumoniae carbapenemase (KPC) and active oxacillin-48-like (OXA-48-like) CRE (Lowman, Bamford, Govind, Swe Swe Han, Kularatne, Senekal, Brink, Moodley, Thomas, Smit & Perovic, 2014).

Carbapenem-resistant Enterobacteriaceae (CRE) is a family of gram-negative bacteria that are almost immune to the class of carbapenem antibiotics. Enterobacteriaceae are common bacteria that form part of the normal flora which can also be infectious agents. Bloodstream infections from Enterobacteriaceae can cause mortality in half of patients infected, a rate higher than other resistant infections such as Methicillin-resistant Staphylococcus aureus (MRSA) or Clostridium difficile. Some CRE bacteria have become resistant to most available antibiotics. Infections with these microorganisms are very difficult to treat, and can be fatal (Centers of Disease Control and prevention, 2014).

The Witwatersrand Donald Gordon Medical Centre (WDGMC) is a 190 bed hospital, the first private teaching hospital in South Africa. The hospital was established to create a facility in the private sector for training of sub-specialists. This training is part of and complementary to the existing training programmes at The Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath and Helen Joseph hospitals.
The first laboratory-confirmed case of a New Delhi Metallo-β-lactamase (NDM-1) producing isolate from South Africa was reported at Charlotte Maxeke Johannesburg Academic Hospital (Lowman, Sriruttan, Nana, Bosman, Venturas, Clay & Coetzee, 2011). The emergence of NDM-1 in Enterobacteriaceae has heightened concern regarding antimicrobial resistance. The Multiple drug resistance (MDR) nature, propensity for transfer, rapidity of global spread and emergence of micro-organisms harbouring this gene means that the threat of untreatable infections has arrived. The NDM resistance mechanism in Enterobacteriaceae threatens to cause serious gram-negative infections that will be untreatable. The NDM-1 enzyme hydrolys all available penicillin, cephalosporin and carbapenem antibiotics, and is commonly accompanied by additional resistance mechanisms to multiple antibiotic classes. Initially identified as a significant healthcare risk on the Indian sub-continent, it has rapidly become a global problem, posing significant diagnostic and management challenges (Lowman, et al, 2011). As these organisms were prevalent in WDGMC, and for the reasons discussed above, this study was conducted on the antibiotic prescribing patterns in the presence of CRE at WDGMC.

**METHODS**

*Study design and population*

A descriptive quantitative study was conducted retrospectively. The Witwatersrand Donald Gordon medical centre is a 190 bed private hospital. It offers transplant surgery, oncology, gastroenterology, geriatric medicine, interventional radiology ophthalmology, ear- nose and throat, urology, nephrology, cardiology, women’s health and orthopaedics. The study was conducted in all the wards at WDGMC health facility, Gauteng Province, South Africa.

*Data collection, analysis and study period*

Purposive sampling was used. All records of patients (>18 years) with a positive CRE culture from January 2013 until December 2013 within the hospital were included in the study.

The Data Collection Instrument (DCI) was repeatedly used during the study period. It was developed and compiled by the researcher in a data collection sheet form. It was designed according to the recommendations by Gregory and Radovinsky, 2012. The designed data collection tool was a form, which recorded, patient demographics, the source of a positive culture the date it was cultured, the antibiotics prescribed prior/post positive CRE culture and
the clinical outcome. The aim of the data collection sheet was to record the antibiotic prescribing prior/post CRE positive culture in all the wards in the hospital. The DCI assisted the researcher by providing a framework to summarise all information required for this study.

The data from the patient files were collected managed and analysed using the IBM Statistical Package for Social Sciences Statistics® (SPSS) programme. Descriptive statistics were used to analyse data for prescribing patterns in the presence of CRE. Antibiotics that were prescribed prior and post CRE results were determined. Thereafter, the appropriateness of the prescribed antibiotics was determined against sensitivity patterns.

**Risk factors for infection due to CRE**

Each patient with a positive CRE culture was included as a case. If a CRE was isolated on multiple occasions, all episodes of infection were recorded. Hospital acquired infections was defined by the Centres of Disease Control (CDC) as an infection that occurred >48 hours after admission to the hospital, infection up to three days after discharge and/or infection up to 30 days after an operation.

The presence of mechanical ventilation was also assessed. Finally, all antimicrobial therapy that was administered prior- and post positive CRE cultures were documented. The presence of the following comorbid conditions was documented: malignancy, diabetes mellitus, renal insufficiency, HIV infection and neutropenia (Lautenbach, Patel, Bilker, Edelstein & Fishman, 2001)

Several instruments have been developed to assess quantity and grade the degree of comorbid burdens using ordinal scales. One of the most widely applied is the Charlson Comorbidity Index (CCI), which has been extensively used to evaluate the impact of comorbidity in a variety of medical conditions. The CCI was developed in 1987 and is a prognostic taxonomy that was initially developed to account for the influence of patients’ adverse medical conditions in longitudinal studies and has been validated in many clinical settings (Charlson, Pompei, Ales & MacKenzie, 1987). This index is calculated by the summation of weight scores for 19 medical conditions and high scores were found to be associated with poorer prognosis (Charlson, Charlson, Peterson, Marinopoulos, Briggs & Hollenberg, 2008).

Age has been determined to be associated with overall survival, thus was the CCI modified by Charlson et al. in 1994 (Charlson, Szatrowski, Peterson &Gold, 1994). This modification
called Age-Adjusted Charlson Comorbidity index (AACI) included the age of the patient as a correction variable of the final score of the Charlson index. Peterson, Paget, Lachs, Reid, and Charlson, (2012) reported that each decade of age ≥50 years is equivalent to a 1-point increase in comorbidity (ie, 50–59 years=1 point; 60–69 years=2 points) (Peterson, Paget, Lachs, Reid, and Charlson, 2012). The AACI was used to assess the patient estimated relative risk of death as only two patients in this study was younger than 50 years of age.

*Role of CRE-resistance in outcomes*

To evaluate the effect of infections due to CRE producing organisms on clinical outcome, the following outcomes were assessed: clinical outcome, mortality and the antimicrobial exposure before and after the positive CRE culture.

**Ethical considerations**

Ethical approval was obtained from the Sefako Makgatho Health Science University Research and Ethics Committee, reference number: SMUREC/H/51/2015:PG and from the research operations committee of the private hospital.

Participant consent was not obtained for this study. This study was considered an epidemiological observation study. Participant personal information was only used to match the laboratory report obtained from the laboratory dataset. Once this was done; the patient’s personal data was anonymised and stored in a locked cupboard.

**Results**

*Study population and socio-demographic characteristics*

During the study 79 patients had positive CRE producing isolates. Included in the study, 62 patients received antibiotics prior to the positive CRE cultures (78%) and the remaining 17 patients (22%) only received antibiotics after the positive CRE cultures.

Table 1 provides an overview of the baseline characteristics of the patients. Most patients were female (54%). The median age was 57 years (IQR=28- 64), and 77% of infections were acquired in the hospital.

The samples with positive results were as follows: blood (18.28%), urine (12.90%), rectal swab (48.39%), abdominal swab (5.38%), fluid (6.45%), sputum (2.15%), bronchial aspirate
(2.15%), tracheal aspirate (1.08%), bile (1.08%), biopsy (1.08%) and groin swab (1.08%). The majority of positive CRE producing isolates were due to *K. pneumoniae* spp (79.57%).

### Table 1: Baseline patient demographics and study characteristic information.

<table>
<thead>
<tr>
<th>Data characteristics</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic data</strong></td>
<td></td>
</tr>
<tr>
<td>Total number of patients</td>
<td>79 (100%)</td>
</tr>
<tr>
<td>Male</td>
<td>36 (46%)</td>
</tr>
<tr>
<td>Female</td>
<td>43 (54%)</td>
</tr>
<tr>
<td>Age</td>
<td>N= 79</td>
</tr>
<tr>
<td>Mean (+/-SD)</td>
<td>53.4 (+/-15.74)</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>57 (38-64)</td>
</tr>
<tr>
<td>Min/Max</td>
<td>20/98</td>
</tr>
<tr>
<td><strong>Wards</strong></td>
<td>Patients where CRE was cultured</td>
</tr>
<tr>
<td>ICU</td>
<td>31</td>
</tr>
<tr>
<td>High care</td>
<td>13</td>
</tr>
<tr>
<td>J (Surgical)</td>
<td>12</td>
</tr>
<tr>
<td>E (Medical)</td>
<td>8</td>
</tr>
<tr>
<td>C (General)</td>
<td>7</td>
</tr>
<tr>
<td>F (Oncology)</td>
<td>6</td>
</tr>
<tr>
<td>B (Hepato/Renal)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Source of culture</strong></td>
<td>N=93</td>
</tr>
<tr>
<td>Blood</td>
<td>17 (18.28%)</td>
</tr>
<tr>
<td>Urine</td>
<td>12 (12.90%)</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>45 (48.39%)</td>
</tr>
<tr>
<td>Abdominal swab</td>
<td>5 (5.38%)</td>
</tr>
<tr>
<td>Fluid</td>
<td>6 (6.45%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>2 (2.15%)</td>
</tr>
<tr>
<td>Bronchial aspirate</td>
<td>2 (2.15%)</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Count (Percentage)</th>
</tr>
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<tbody>
<tr>
<td>Tracheal aspirate</td>
<td>1 (1.08%)</td>
</tr>
<tr>
<td>Bile</td>
<td>1 (1.08%)</td>
</tr>
<tr>
<td>Biopsy</td>
<td>1 (1.08%)</td>
</tr>
<tr>
<td>Groin swab</td>
<td>1 (1.08%)</td>
</tr>
<tr>
<td>Charlson score: Median (IQR)</td>
<td>3.04 (1.45-6.38)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of Microorganism (+/- class)</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>74 (79.57%)</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>2 (2.15%)</td>
</tr>
<tr>
<td>Klebsiella ozaenae</td>
<td>2 (2.15%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6 (6.45%)</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>2(2.15%)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>5 (5.38%)</td>
</tr>
<tr>
<td>Raoultella ornithinolytica</td>
<td>1 (1.08%)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1 (1.08%)</td>
</tr>
<tr>
<td>Hospital acquired infection</td>
<td>77%</td>
</tr>
</tbody>
</table>

**Comorbidity index**

The Charlson Comorbidity Index (CCI) provides away of quantifying this impact in terms of survival and is also used as a prognostic comorbidity index. Indices, such as CCI, are useful for estimating the prognosis of real-world patients with comorbidities. CCI was developed empirically 26 years ago as a prognostic index of comorbid conditions for patients admitted to a general medical service with a variety of medical conditions which, alone or in combination, might alter the risk of short-term mortality for patients enrolled in longitudinal studies. The comorbidities were weighted by Charlson et al. in 1994 using a point system. One point was assigned to: past history of myocardial infarction (MI), heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic lung disease, connective tissue disease, peptic ulcer disease, mild liver disease and diabetes. The comorbidities weighted with 2 points were: diabetes with target organ damage, hemiplegia and moderate to severe renal disease, malignant neoplasm, leukaemia and lymphoma. Moderate to severe liver disease was weighted with 3 points and metastatic solid tumour and AIDS (stage C) were weighted with 6 points. Therefore, patients without comorbidities had CCI0, those with only one comorbidity weighed as 1 had CCI1, patients with 2 comorbidities...
where both were weighted 1 or one comorbidity was weighted 2 had CCI2, and the patients in which the sum of the weighted points of comorbidities was 3 or above had CCI≥3.

Age has been determined to be associated with overall survival, thus was the CCI modified by Charlson et al. in 1994 (Charlson, Szartrowski, Peterson & Gold, 1994). This Age-Adjusted Charlson Comorbidity index (AACI) included the age of the patient as a correction variable of the final score of the Charlson index (Peterson, Paget, Lachs, Reid & Charlson, 2012), reported that each decade of age ≥50 years is equivalent to 1-point increase in comorbidity (i.e. 50-59 years=1 point, 60-69 years=2 points).

The ACCI score for patients in this study was calculated and relative risk of death ratio (RR) compared to the patients’ clinical outcome. These were all recorded in Table 2.

Table 2: ACCI and Clinical outcome

<table>
<thead>
<tr>
<th>ACCI score</th>
<th>Number of Patients N=79</th>
<th>Relative risk of death</th>
<th>Clinical outcome Resolved/Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 1</td>
<td>21</td>
<td>0 – 1.45</td>
<td>14 (67%) / 4 (19%) (two patients transferred/ one patient no information was provided)</td>
</tr>
<tr>
<td>2 – 3</td>
<td>24</td>
<td>2.10 – 3.04</td>
<td>18 (75%) / 5 (21%) (one patient no information was provided)</td>
</tr>
<tr>
<td>4 – 5</td>
<td>25</td>
<td>4.40 – 6.38</td>
<td>13 (52%) / 8 (32%) (three patients transferred/ one patient no information was provided)</td>
</tr>
<tr>
<td>&gt;6</td>
<td>09</td>
<td>9.23 – 19.37</td>
<td>4 (44%) / 4 (44%) (one patient transferred)</td>
</tr>
</tbody>
</table>

The ACCI score corresponds with the clinical outcome of the patients. Patients with a high ACCI score (>6) had the highest mortality (44%). Patients with a low ACCI score (between 0 – 1 and 2-3) had a higher percentage of survival (71% and 75% respectively) compared to patients with a higher score. There was a total number of 45 patients with a low ACCI score (0-3) with a positive CRE culture. Nineteen of these patients were colonised without septic
markers and 12 of them were treated with antibiotics. Out of the colonised patients, 11 cases were resolved, five passed on, one patient was transferred to another institution and for two further information could not be found from the files.

Prescribing patterns

The prior antimicrobial exposures documented include antibiotics the patient received for suspected infections prior to samples being sent, which reported the positive CRE. Most patients that were started on antimicrobial treatment without coverage for CRE were changed to a second antibiotic with coverage for CRE.

In this study, 191 different antimicrobials were prescribed for 79 patients prior to the positive CRE culture, thus each patient received on average 2.4 antibiotics. The antimicrobial that were prescribed prior positive CRE culture were as follows; β-lactam (52%), tigecyclines (10%), linezolid (9%), sulfa/trimethoprim (4%), aminoglycosides (7%), fluoroquinolone (6%), glycopeptide (5%), macrolides (5%) clindamycin (1%) and colistin (1%).

Antibiotic prescribing practices of antibiotics post CRE positive culture

All antibiotics administered after the day of positive CRE culture were recorded. These antibiotics were considered correct when stated sensitive, or incorrect when reported resistant, according to the antibiogram. The data of the prescribing patterns post CRE positive culture is summarised in Table 3.
Table 3: Prescribing practices of antibiotics prescribed post positive CRE culture

<table>
<thead>
<tr>
<th>Culture and Microorganism</th>
<th>Prescribed antibiotics (Number of cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td><strong>Klebsiella pneumoniae</strong></td>
<td></td>
</tr>
<tr>
<td>• Blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amikacin (2)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (2)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin (1)</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (2)</td>
</tr>
<tr>
<td>(Oxa-48)</td>
<td>Meropenem (1)</td>
</tr>
<tr>
<td></td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td></td>
<td>Sulfa/Trimethoprim (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td>• Urine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meropenem (2)</td>
</tr>
<tr>
<td></td>
<td>Fosfomycin (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td>(Oxa-48)</td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td></td>
<td>Sulfa/Trimethoprim (1)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (1)</td>
</tr>
<tr>
<td></td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td>• Bronchial aspirate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin (1)</td>
</tr>
<tr>
<td></td>
<td>Sulfa/Trimethoprim (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td>(Oxa-48)</td>
<td>Ertapenem (1)</td>
</tr>
<tr>
<td></td>
<td>Tazobactam/Piperacillin (1)</td>
</tr>
<tr>
<td>• Fluid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin (1)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td>(Oxa-48)</td>
<td>Levofloxacin (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Antibiotics</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Abdominal swab</td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (1)</td>
</tr>
<tr>
<td></td>
<td>(Oxa-48)</td>
</tr>
<tr>
<td></td>
<td>Ertapenem (1)</td>
</tr>
<tr>
<td></td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td></td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (2)</td>
</tr>
<tr>
<td>Biopsy</td>
<td>Amikacin (1)</td>
</tr>
<tr>
<td></td>
<td>Colistin (1)</td>
</tr>
<tr>
<td>Groin swab</td>
<td>Tigecycline (1)</td>
</tr>
<tr>
<td>Rectal swab</td>
<td>Ertapenem (1)</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin clavulanate (2)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin (1)</td>
</tr>
<tr>
<td></td>
<td>Tazobactam/Piperacillin (1)</td>
</tr>
<tr>
<td></td>
<td>Amikacin (2)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (1)</td>
</tr>
<tr>
<td></td>
<td>Meropenem (2)</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin (1)</td>
</tr>
<tr>
<td></td>
<td>Tigecycline (2)</td>
</tr>
<tr>
<td></td>
<td>Sulfa/Trimethoprim (1)</td>
</tr>
<tr>
<td></td>
<td>Imipenem/Cilastin (1)</td>
</tr>
<tr>
<td></td>
<td>Cefuroxime (1)</td>
</tr>
<tr>
<td>(Oxa-48)</td>
<td>Sulfa/Trimethoprim (1)</td>
</tr>
<tr>
<td></td>
<td>Ertapenem (2)</td>
</tr>
<tr>
<td><strong>Enterobacter aerogenes</strong></td>
<td><strong>Enterobacter aerogenes</strong></td>
</tr>
<tr>
<td>(Oxa-48)</td>
<td>Rectal swab</td>
</tr>
<tr>
<td></td>
<td>Ertapenem (1)</td>
</tr>
<tr>
<td><strong>Enterobacter cloacae</strong></td>
<td><strong>Enterobacter cloacae</strong></td>
</tr>
<tr>
<td></td>
<td>Rectal swab</td>
</tr>
<tr>
<td></td>
<td>Ertapenem (1)</td>
</tr>
<tr>
<td><strong>Klebsiella oxytoxa</strong></td>
<td><strong>Klebsiella oxytoxa</strong></td>
</tr>
<tr>
<td></td>
<td>Abdominal and Rectal swab</td>
</tr>
<tr>
<td></td>
<td>Tazobactam/Piperacillin (1)</td>
</tr>
</tbody>
</table>
Sensitivity and clinical outcome

A total number of 75 antibiotics were prescribed post positive CRE culture. The sensitivity and resistance results of the antibiotics prescribed were recorded in Table 4 below.

Table 4: Antibiotic sensitivity and resistance

<table>
<thead>
<tr>
<th>Name / class of antibiotics</th>
<th>Total number prescribed</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbapenems</td>
<td>28 (37.33%)</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>13 (17.33%)</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Amikacin</td>
<td>12 (16%)</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Fluoroquinolones (ciprofloxacin and levofloxacin)</td>
<td>10 (13.33%)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Sulfathiazide / trimethoprim</td>
<td>5 (6.66%)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Amoxicillin / clavulanate</td>
<td>3 (4%)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Cephalosporins (ceftazidime and cefuroxime)</td>
<td>2 (2.66%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>1 (1.33%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Colistin</td>
<td>1 (1.33%)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

The majority (65%) of antibiotics prescribed post positive CRE culture were appropriate prescribed according to the antibiogram. Forty-nine (62%) patients survived, 21 (27%) patients died, six patients were transferred and no information could be found for three patients.

As per hospital protocol, patients are screened on admission as part of infection control strategies (Le Roux 2016). All patients were screened as per protocol with twenty-four patients rendering a positive CRE culture which showed colonisation. If a person is colonised with CRE, they don’t need to be treated. However, if the bacteria caused an infection then antibiotics will be required (CDC 2014). The clinical outcomes for patients were summarised in Table 5.
Table 5: Clinical Outcomes for patients as compared to appropriate vs inappropriate therapy received.

<table>
<thead>
<tr>
<th>Clinical Outcome of Patients (N = 79)</th>
<th>Antibiotics prescribed for patients (N=40)</th>
<th>No antibiotics prescribed Patients screened (+ CRE culture) (N=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive Therapy (27; N=40)</td>
<td>Resistant Therapy (13; N=40)</td>
</tr>
<tr>
<td>Survived (N = 49)</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Deceased (N = 21)</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Transferred (N = 6)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No information (N = 3)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

DISCUSSION

The study enlisted patient files from 79 patients. More female patients (54%) had a positive CRE organism in this study compared to males (46%). The median age was found to be 57.

ICU/High care had the highest number of 44 (57%) patients with positive CRE culture.

The Centres for Disease Control and Prevention estimates that more than 9000 healthcare-associated infections are caused by the two common types of CRE, carbapenem-resistant *Klebsiella* species and *Escherichia* species, each year in the United States (CDC, 2013). In this study also, out of 79 patients with CRE-producing organisms, 93 positive isolates were reported and the organisms *K. Pneumoniae spp* (79.57%) and *E. coli* (6.45%) were the most common types of CRE found followed by *E. cloacae* (5.38%), *K. oxytoca* (2.15%), *E. Aerogenes* (2.15%), *R. ornithinolytica* (1.08%) and *S. marcescens* (1.08%).

OXA-48-type carbapenem-hydrolysing class D β-lactamases are increasingly reported in enterobacterial species. To date, six OXA-48-like variants have been identified, with OXA-48 being the most widespread.

Almost fifty five percent (54.85%) isolates identified with positive CRE during this surveillance, belonged to the oxacillinase-type carbapenemase (OXA-48). The laboratory did not report on the other genes at the time. The OXA-48 type genes are always plasmid-borne.
and have been identified in association with insertion sequences involved in their acquisition and expression. OXA-48-type carbapenemase have been identified mainly from North African countries, the Middle East, Turkey and India, are constituting the most important reservoirs; however, occurrence of OXA-48 producers in European countries is now documented, with some reported hospital outbreaks. Since many OXA-48-like producers do not exhibit resistance to broad-spectrum cephalosporins, or only decreased susceptibility to carbapenems, their recognition and detection can be challenging. Adequate screening and detection methods are therefore required to prevent and control their dissemination (Poirel, Potron and Nordmann, 2012).

Since 1 March 2010 the Molecular Biology Laboratory at the Ampath National Reference Laboratory has been screening Enterobacteriaceae for the presence of these novel genes, the emergence of NDM-1 for the first time in South Africa, and KPC-2 for the first time in Africa, was documented among clinical isolates of *K. pneumoniae* and *Enterobacter cloacae* in hospitalised patients in Johannesburg and Pretoria, respectively. The emergence of the broad-spectrum antibiotic-inactivating enzyme, OXA-48, and its derivatives among Enterobacteriaceae from hospitalised patients in Johannesburg, Cape Town and Port Elizabeth has been confirmed. Furthermore, VIMs have been detected in *K. pneumoniae* in Johannesburg and GESs in Enterobacteriaceae in hospitals in Cape Town (*K. pneumoniae*), Bloemfontein (*K. oxytoca*), Witbank (*E. cloacae*) and Port Elizabeth (*Serratia marcescens*) (Brink, *et al.*, 2012).

The samples with positive results were as follows: blood (18.28%), urine (12.90%), rectal swab (48.39%), abdominal swab (5.38%), fluid (6.45%), sputum (2.15%), bronchial aspirate (2.15%), tracheal aspirate (1.08%), bile (1.08%), biopsy (1.08%) and groin swab (1.08%).

In this study 19% positive CRE culture were found in blood samples. Blood stream infections (BSIs) due to carbapenem-resistant Enterobacteriaceae (CRE) are associated with high hospital mortality rates and present a challenge to clinicians. The optimal treatment remains undefined. A Pooled data of 215 patients with BSIs due to CRE followed in Greek hospitals between 2004 and 2011 revealed that the overall success rate of combination therapy was significantly higher than that of monotherapy (*p* = 0.01; OR 2.41, 95% CI 1.2–4.7). Carbapenem-containing combination therapies with two or more active drugs (colistin the most common) showed a significantly higher success rate when compared to non-carbapenem-containing regimens (93.3%; *p* = 0.04; OR 5.15, 95% CI 1.1–24.5), (Hirsch &
Tam, 2010). Colistin was reported to be the least effective agent in the monotherapy group in the same study (Akova, Daikos, Tzouvelekis & Carmeli, 2012).

**Prescribing practises prior positive CRE cultures**

Antibiotic exposure may lead to increased risk of acquiring CRE. Specific antibiotics and antibiotic classes have been frequently implicated as risk factors for colonisation or infection with CRE, and these include all the carbapenems, cephalosporins, fluoroquinolones, aminoglycosides and β-lactam/β-lactamase inhibitors (Gupta, Lumbago, Patel & Alexander, 2011). This is similar to what was found in this study where 65% of the patients received other β-lactam antibiotics prior to positive CRE producing organisms cultured; these included antibiotics like cephalosporins, piperacillin/tazobactam and penicillins, which might play a role in contracting a CRE related infection. Other antimicrobial exposure includes carbapenems (41%), fluoroquinolones (6%), and aminoglycosides (7%).

**Prescribing practises post positive CRE culture results and clinical outcome**

Sensitivity or resistance data of the antibiotics prescribed post positive CRE could only be found for 40 patients, 24 patients’ results were screenings and no information could be found for 15 patients. The majority (65%) of all antibiotics prescribed post positive CRE culture were sensitive according to the antibiogram.

Patients who received therapy which was sensitive according to the microbiology results showed a better clinical outcome than those with resistant therapy, indicating the importance of consulting antibiograms when prescribing antibiotics. Looking at the clinical outcomes it indicated that sensitivity and microbiology results cannot be interpreted alone without taking into consideration the co-morbid conditions that the patient might have.

The aforementioned results, provides the motivation to establish an antimicrobial stewardship programme in order to preserve the antibiotics available currently. Antimicrobial stewardship is a coordinated program that promotes the optimal use of antimicrobials, reduces microbial resistance, improves patient outcomes and decreases the spread of infections caused by multidrug-resistant organisms (Doron & Davidson, 2011).
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Recommended treatment options for CRE

According to an article on the Treatment Options for carbapenem-resistant Enterobacteriaceae the most optimal treatment for CRE infections is largely unknown. Current treatment options include the use of older agents, such as polymyxins, fosfomycin, and aminoglycosides, which have been rarely, used due to efficacy and/or toxicity concerns. Optimization of dosing regimens and combination therapy are additional treatment strategies being explored (Morrill, Pogue, Kaye & LaPlante, 2015). This article is in support of the study findings where no resistance was found for both fosfomycin and polymyxin, and the resistance to aminoglycoside was minimal.

Dual-carbapenem combination treatment may be an effective option for infections caused by pandrug-resistant CRE; however, data are limited to selected case reports (Wiskirchen, Crandon & Nicolau, 2013). Dual-carbapenem combination treatment is a promising option, which may be effective in combination with a third drug. 20% of patients in this study were on dual-carbapenem combination treatment. Around 50% cases were resolved, there was 44% mortality and for 6% of cases a follow up could not be made.

Combination therapy for CRE infections may decrease mortality compared with monotherapy. It is also an important consideration when a CRE is suspected. Monotherapy with antibiotics has limitations in severe infection; combination therapy can be an option to optimize therapy. However, with combination therapy, there is a potential for an increased risk for the development of Clostridium difficile infection, colonization or infection with other resistant bacteria, and adverse effects such as nephrotoxicity (Paul, Carmeli, Durante-Mangoni, Mouton, Tacconelli, Theuretzbacher, Mussini & Leibovici, 2014). Benefits of combination therapy include reduction of initial inappropriate antimicrobial therapy, potential synergistic effects, and suppression of emerging resistance (Petrosillo, Giannella, Lewis, Viale, 2013). In this study 49 cases of CRE were resolved. In 22 (44%) of those cases the patients were found to be on combination therapy of two or more of the following: carbapenem, aminoglycoside, polymyxin, tigecycline and fosfomycin antibiotics. In the presence of the recommended combination therapy fluoroquinolones, cephalosporins and sulfa/trimethoprim were also found to form part of the prescribed antibiotics.
Limitations of the study

No indication was given if the infections were colonisations, which are not to be treated, or an active infection which is an important part of antimicrobial stewardship. The clinical picture of the patient could not be evaluated as it was a retrospective file review. It is recommended that future studies of this nature be limited in scope to ensure effective focus and in depth evaluations of specific guidelines related to antibiotic prescriptions.

SUMMARY

According to the forum of infectious diseases, the burden of antimicrobial resistance among gram-negative pathogens, particularly CRE, is increasing rapidly worldwide. Treatment options for serious CRE infections remain extremely limited. Optimization of dosing of currently available agents and combination therapy may be the most appropriate treatment strategies. However, continued research is desperately needed, in particular randomized controlled trials, to determine the most appropriate treatment for serious CRE infections.
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Le Roux, A., 2016, Admission screening of patients for infection prevention purposes.


CHAPTER 5
LIMITATIONS, RECOMMENDATIONS AND CONCLUSIONS

5.1 INTRODUCTION

In this chapter, a summary of the results, conclusions, limitations and recommendations will be presented. Based on the results of the study, limitations encountered during the study. The chapter will end with recommendations for practice offered.

5.2 SUMMARY OF RESULTS

During the study 79 CRE producing isolates were cultured in patients. More female patients (54%) had a positive CRE organism in this study compared to males (46%). The median age was found to be 57 which indicate that older patients were prone to colonization with CRE producing isolates. A high number of patients with CRE positive culture were found in ICU/high care and the surgical ward.

Out of 79 patients with CRE-producing organisms, 93 positive isolates were reported and the organisms were as follows: K. pneumoniae (79.57%), K. oxytoca (2.15%), K. ozaenae (2.15%), E. coli (6.45%), E. aerogenes (2.15%), E. cloacae (5.38%), R. ornithinolytica (1.08%) and S. marcescens (1.08%). Almost fifty percent (54.85%) isolates identified with positive CRE belonged to the oxacillinase-type carbapenemase (OXA-48).

The samples with positive results were as follows: blood (18.28%), urine (12.90%), rectal swab (48.39%), abdominal swab (5.38%), fluid (6.45%), sputum (2.15%), bronchial aspirate (2.15%), tracheal aspirate (1.08%), bile (1.08%), biopsy (1.08%) and groin swab (1.08%).

Fifteen patients out of the 79 post positive CRE culture treatment information could not be found. Seventy five recorded different antimicrobials were prescribed for 79 patients post positive CRE culture. More than 44% of patients received other β-lactam antibiotic post positive CRE culture, these included antibiotics like cephalosporins, piperacillin/tazobactam, ertapenem, meropenem and penicillin. The remainder of the patients received aminoglycosides (16%), tigecycline (15%) fluoroquinolones (13%), sulfa/trimethoprim (7%), polymixin E (1%) and fosfomycin (1%).

The majority (65%) of all antibiotics prescribed post positive CRE culture were sensitive according to the antibiogram and 35% of the antibiotics prescribed post positive CRE were
found to be resistant. The ACCI was used to assess the patient estimated relative risk of death ratio according to their co-morbid conditions and age. The clinical outcome of the sample population corresponds with the prediction calculated by the AACI. Patients with a high ACCI score (>6) had the highest mortality (44%). Patients with a low ACCI score (between 0 – 1 and 2-3) had a higher percentage of survival (71% and 75% respectively) compared to patients with a higher score.

5.3 CONCLUSION

The overall aim of the study was to analyse the antibiotic prescribing patterns in patients with positive cultures for CRE. The objectives of the study were to describe prescribing practices prior to positive CRE microbiology cultures and post positive CRE culture.

Carbapenem antibiotics are the cornerstone of therapy for patients with serious infections caused by ESBL-producing organisms. The high ESBL prevalence amongst bacteraemic pathogens places a tremendous strain on the use of these agents as directed therapy, and empirically as well. According to The South African Society of Clinical Microbiology (SASCM), the threat of multi-drug resistance is a global one. This threat was exacerbated by the meteoric rise of carbapenem-resistant Enterobacteriaceae (CPE). To preserve the antibiotics available currently, there should be a global focus on implementing antibiotic stewardship programs. Antimicrobial stewardship is a coordinated program that promotes the appropriate use of antimicrobials, reduces microbial resistance, improves patient outcomes and decreases the spread of infections caused by multidrug-resistant organisms.

5.4 LIMITATIONS AND RECOMMENDATIONS

The study followed a retrospective, epidemiological observational approach. This limited the study in a sense that some patient files were incomplete. In addition, due to its design, focus and scope, findings were based on the data captured by the infection prevention practitioner of the hospital where the study was conducted. Mostly patient records were used to collect the data retrospectively, so other data that could be valuable in deciding if treatment were appropriate or not, like infection markers and clinical profiles of patients were not available.

There was no indication if the infections were colonisation or active infection which is an important part of antimicrobial stewardship, to only treat infections and not colonisations. Hence, it is recommended that future studies of this nature be limited in scope to ensure effective focus and depth in evaluating specific guidelines related to antibiotic prescriptions.
It is also important that future researchers look into a mix of quantitative and qualitative study designs which will allow authentic views of the prescribers when prescribing for patients with antibiotic resistance and also conduct a prospective study involving other risk factors of acquisition e.g. prolonged hospitalisation, catheterisation and ventilation, where infection markers and MICs are taken into consideration.

Recommendations to the institution include writing and implementing an antibiotic policy in the wards taking into consideration ventilator status and co-morbid conditions to limit antimicrobial exposure. There should be a strong urge to establish an antimicrobial stewardship team to monitor these efforts.
REFERENCES


Centres for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States, Atlanta, GA.


References


LaBore, K., 2013. CRE Infection in Nursing Homes.


OpenOffice.Org, computer software, viewed 28 December 2016, from [http://www.apic.org/Professional-Practice/Practice-Resources/Antimicrobial-Stewardship](http://www.apic.org/Professional-Practice/Practice-Resources/Antimicrobial-Stewardship)


References


Appendices

APPENDICES

Appendix 1: Data collection sheet

Study ID: _________________________

The analysis of antibiotic prescribing patterns of Carbapenem-Resistant Enterobacteriaceae at the Witwatersrand Donald Gordon Medical Centre in Johannesburg, Gauteng.

Patient initials: _________________________ Ward: __________

Patient Hospital number: ________________ Gender: Male/Female

Date of birth: ______/____/_______ Race/Ethnicity: ______________

Clinical Data Points (study variables):

Initial admitting diagnosis: ________________________________

Date of diagnosis of initial CRE positive culture: ______/____/______

Weight at diagnosis (kg): ________________ ICD 10 code: ________

Duration of stay in the ward: ________________

Source of first positive culture: ________________

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<th>Date CRE cultured</th>
<th>Source of culture</th>
<th>Antibiotics prescribed post CRE</th>
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<th>Clinical outcome:</th>
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Appendix 2: Letter of intent

UNIVERSITY OF LIMPOPO

Medunsa Campus

The CEO: Dr Tyger

The Wits Donald Gordon Medical Centre

Letter of intent to conduct an operational research

I Sithole A.K request to conduct a research at WDGMC. I am registered for MSc (Med) in clinical Pharmacy at the University of Limpopo. Please find the attached proposal for the study entitled: “The analysis of CRE at WDGMC”

The proposal will be submitted to the School of Health Care Sciences and the Medunsa Research and Ethics Committee at the University of Limpopo (Medunsa Campus).

The aim of the study is:

The main objectives of the study are as follows:

- The objectives of the study are as follows: To describe prescribing practises prior to positive CRE microbiology cultures (blood or urine).
- To describe prescribing practises after CRE was cultured.
- To describe prescribing practices of antimicrobials according to patient diagnosis.
- To compare and then describe the differences/ similarities of prescribing practices prior to and after positive CRE cultures.

Regards

_________________

Sithole A.K
Appendices

Appendix 3: Letter of addressed recommendations made before the ethics clearance certificate

Miss A Sithole
Department of Pharmacy
P. O Box 218
Medunsa
0204

Sefako Makgatho Health Sciences University
Research & Postgraduate Studies Directorate
Sefako Makgatho University Research Ethics Committee
(SMUREC)

Dear Sir/Madam

RE: ANTIBIOTIC PRESCRIBING PATTERNS IN THE PRESENCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEA AT THE WITWATERSRAND DONALD GORDON MEDICAL CENTER IN JOHANNESBURG, GAUTENG.

My protocol was considered at the SMUREC meeting that was held on 05 March 2015. The committee approved the protocol on provision that I address the recommendations made before the clearance certificate is issued.

Informed consent letter to hospital on SMU letterhead and wording to be accordingly changed

Ethical consideration: aim of the study to be included in permission letter (Appendix 2)

The recommendations stated above have been corrected and the revised protocol that addresses all the concerns is attached

Yours Sincerely

Asia Sithole
Appendices

Appendix 4: Ethical approval certificate

Sefako Makgatho Health Sciences University
Research & Postgraduate Studies Directorate
Sefako Makgatho University Research Ethics Committee
(SMUREC)

Motloulegi Street, Ga-Rankuwa 0208
Tel: (012) 521 5617/3698 | fax: (012) 521 3749
Email: lbrato.phir@smu.ac.za
P.O. Box 183 Medunsa 0204

Miss A Sithole
Department of Pharmacy
P.O Box 218
MEDUNSA, 0204

Dear Miss Sithole

RE: ANTIBIOTIC PRESCRIBING PATTERNS IN THE PRESENCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE AT THE WITWATERSRAND DONALD GORDON MEDICAL CENTER IN JOHANNESBURG, GAUTENG.

Your protocol was considered at the SMUREC meeting held on 05 March 2015.

The committee PROVISIONALLY APPROVED and RECOMMENDED that the researcher must still address the following recommendations before the CLEARANCE CERTIFICATE is issued:

(i) Informed consent letter to Hospital on SMU letterhead and wording to be accordingly changed.
(ii) Ethical Considerations: Aim of the study was omitted from permission letter (Appendix 2). Please correct this.

SMUREC awaits your response to above recommendations and submission of a revised protocol that addresses all these concerns.

Yours Sincerely,

[Signature]

PROF GA OGUNSANJO
CHAIRPERSON SMUREC

05 March 2015

Cc.: Ms E Bronkhorst

Members of the Interim Council:
Prof O Shibane (Chairperson), Ms SA Mchunu, Mr P Black, Dr N Simelane, Prof AM Beceno, Dr E van Staden
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**Appendix 5: South African Journal of Infectious Diseases Author Guidelines**

Manuscripts submitted to the SAJID must be in the form of *Research Articles, Brief Reports, Clinical Case Studies, Correspondence, Reviews, State-of-the-Art Articles, Commentaries and Opinion Papers, Editorials or Supplement Articles*. The Journal welcomes the publication of *Guidelines, Conference Proceedings, Newsletters or Press Releases, and Book Reviews*. Articles, Brief reports and Reviews are peer reviewed; other categories are reviewed by the Editors. Commentaries and Editorials are generally invited contributions, indicating the authors' identity, while manuscripts in the form of Reviews, and State-of-the-Art Articles may also be requested by the Editors.

All manuscripts must have conflict of interest and funding statements. When authors submit a manuscript, whether an article or a letter, they are responsible for disclosing all financial and personal relationships that might bias their work. To prevent ambiguity, authors must state explicitly whether potential conflicts do or do not exist. Authors should do so in the manuscript on a conflict-of-interest notification page that follows the title page.

**Manuscripts describing research in human subjects or animals must indicate ethics clearance from appropriate research review committees.** When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

*Articles* describe original investigations at an acceptable degree of completion, constituting an advance in the field. Articles must not exceed 3500 words of text, without counting the abstract, references or legends, and illustrations and tables must be limited to the minimum necessary for clear and concise presentation. The abstract must either be structured, using *Background, Methods, Results,* and *Conclusions* as headings and comprising no more than 250 words, or unstructured with a 200 word limit. Articles are limited to a maximum of 7 insets (tables and figures combined) and 50 references.

*Brief Reports* present complete studies that are narrower in scope than those described in Articles or that present new developments. Manuscripts that are descriptive or primarily methodological in nature, or that describe in vitro chemotherapeutic studies should, in general, be submitted as Brief Reports. Brief Reports include an abstract (no more than 100 words) and are limited to a total of no more than 2000 words of text, a total of 2 inserts (tables or figures), and 15 references.

*Correspondence (letters)* must be submitted in reference to a previous publication in SAJID (within the previous 12 months), or relate to a topical matter in line with the interests of FIDSSA, PHASA or their affiliated societies. Please prepare the letter in manuscript format, including a title page. The letter must not exceed 750 words of text, 1 insert (table or figure) and 10 references.

*Commentaries and Editorials* are generally invited by the Editor and are overviews of articles in SAJID, or of other research in epidemiology or infectious diseases, or matters relating to public health and other issues of special interest to FIDSSA, PHASA or their associated societies. Unsolicited commentaries are also considered.

*Reviews* and *State-of-the-Art Articles* that are research oriented or fall within the fields of interests of FIDSSA, PHASA or any of their affiliated societies will be considered for publication by SAJID. Prospective authors of such manuscripts are advised to communicate with the Editor in advance to ensure that a specific contribution is deemed appropriate and timely. Manuscripts of Reviews and State-of-the-Art Articles will be peer-reviewed.

**Reviewers**

The Journal would encourage authors to supply the names of at least 2 potential reviewers for their manuscript, as well as to indicate any reviewers they would feel may have a potential conflict of interest with regard to their submission.
Review procedure. The Editor-in-Chief and Emeritus Editor screen all unsolicited manuscript submissions and some of these are rejected without further review. All other manuscripts are sent to a minimum of two outside experts for review. After receipt of the reviewers' reports, the Editor-in-Chief and the Emeritus Editor with administrative assistance of the Journal Secretary discuss the merits of the manuscripts and the Editor-in-Chief makes the final decision to accept, reject, or request revision of the manuscript. A request for revision does not guarantee ultimate acceptance of the revised manuscript.

Related manuscripts. If there appears to be significant overlap between a manuscript submitted to SAJID and another submitted manuscript by the same authors to SAJID or another journal, the editors will take the matter up with the corresponding author, and based on the response, take appropriate action (ask for modification, or reject with detailed explanation). Further action may include informing the appropriate authority in the authors' resident institution and if overlapping is discovered after publication in SAJID, publishing an appropriate announcement to that effect in the journal.

**DOCUMENT REQUIREMENTS**

**Checklist**

The following are required for your manuscript to be processed:

- Covering letter
- Word count limits
- Conflict of interest statement
- Funding statement
- List of potential reviewers

**Covering Letter**

All manuscripts submitted to SAJID must be accompanied by a letter declaring that the manuscript has not been submitted or accepted for publication elsewhere. This letter must confirm and declare that all authors have seen and approved the content and have contributed significantly to the work. Authors should suggest potential unbiased reviewers who are qualified to review their manuscript. A covering letter must also accompany a revised submission and must address issues raised in the review process.

**Manuscript Preparation**

The SAJID complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journal Journals (Ann Intern Med 2000; 133:229-231 [editorial]; http://www.icmje.org, full text). Text, tables, references, and legends must be double-spaced. Italics should be used for genus and species names and for genes but not for in vivo, in vitro, in situ, et al., or other Latin-derived expressions. For layout of manuscript and appropriate style see a recent issue of SAJID.

**Title page.** On the title page, please supply a running head of not more than 40 characters and spaces, a title of not more than 160 characters and spaces, the names and affiliations of all the authors, and word counts of the abstract and text. Each author's first name, subsequent initials and surname must be used.

**Footnote page.** Footnotes must include:

- Statement that authors either have or have not a commercial or other association that might pose a conflict of interest (e.g. pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding)
- Statement naming sources of financial support (including grant numbers)
- Name, date (month and year), and location (city, and country if not South Africa) of a meeting at which all or part of the information has been presented (include an abstract number, if available)
- Name, address, telephone and fax numbers, and e-mail address of the person to whom correspondence should be addressed.
- Current affiliations and addresses for authors whose affiliations have changed since completion of the study

**Abstract.** The abstract for an Article may be structured with the headings Background, Methods, Results, and Conclusions (250-word limit) or unstructured (200-word limit). Abstracts of Brief Reports
should be no more than 100 words. Whether structured or unstructured, the abstract must state the purpose of the research, the methods used, the results, and the conclusions. Do not cite references in the abstract. Include up to 10 key words, separate from the abstract. Please remember that the abstract is particularly useful for literature retrieval purposes.

Text. The text of Articles must be no longer than 3500 words, and that of Brief Reports no longer than 2000 words. The Methods section must include a statement that informed consent was obtained from patients or their parents or guardians, and human experimentation guidelines of the National Department of Health (http://www.doh.gov.za) or the South African Medical Research Council (MRC; http://www.sahealthinfo.org/ethics/index.htm) and/or those of the authors' institution(s) were followed in the conduct of clinical research or that animal experimentation guidelines (see MRC website above) were followed in animal studies.

References. Articles are generally limited to 50 references, Brief Reports to 15 references. Only works that have been published or accepted for publication can be included in the reference list. Unpublished observations by the authors (authors' unpublished data) personal communications (SP Stanley, personal communication), and manuscripts submitted for publication (J Odendaal, S Coovadia and J Radebe, submitted) should be mentioned parenthetically in the text Please number references in order of appearance; those cited only or first in tables or figures are numbered according to the order in which the table or figure is cited in the text. Example: If table 3 is cited in the text after reference 20, a new reference cited in table 3 will be reference 21.

References must follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org, full text). Provide all authors' (or editors') names when there are fewer than 7; for 7 or more, list the first 3 and add “et al.” Titles of journals not listed in Index Medicus should be spelt out in full. Reference to a doctoral thesis or Master's dissertation should include the author, title, institution, location, year and publication information, if published. For online resources, include a URL and date accessed. Accuracy of references is the responsibility of the authors.

Examples of the proper format are as follows:


Acknowledgment(s). The page preceding the references may include a statement thanking those who assisted substantially with work relevant to the study.

Statistical analysis. The statistical analyses used should be identified both in the text and in all tables and figures where the results of statistical comparison are shown.

Units of measure. All Data should be expressed in metric units; use of SI units is encouraged. Use ºC for temperature.

Tables and figures. Articles are limited to a maximum of seven inserts (tables and figures combined), Brief Reports to a maximum of two inserts. Data should not be repeated in both a table and a figure. Abbreviations and acronyms used in tables and figures must be explained in the table footnotes and figure legends, even if already defined in the text.

Tables should be numbered in the order of mention in the text. Tables should be typed double-spaced throughout, with no vertical or internal rules. Footnotes and accompanying explanatory material should
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be kept to a minimum. Footnotes should be placed below the table and designated by superscript lowercase letters (listed in order of location when the table is read horizontally). Each column must have an appropriate heading describing the data in the column below, and units of measure must be clearly indicated. For further instructions on the preparation of tables in Word, consult the Special Instructions for Tables.

Figures should be also numbered in the order of mention in the text and should appear at the end of the manuscript and references. Your figures should be prepared in accordance with the Guidelines for Submission of Artwork. Letters, numbers, and symbols should be clear and of sufficient size to be legible when the figures are reduced. Photomicrographs should have internal scale markers. Figures reproduced from other publications must be accompanied by permission from the copyright holder. If the manuscript is accepted, the author will be required to send one complete set of glossy, hard-copy figures.

Figure legends should be double-spaced and appear on a separate page preceding the figures. Any abbreviations or symbols used but not defined in the figure itself must be defined in the legend.

Nomenclature. SAJID recommends the latest widely accepted nomenclature, as set out in documents prepared by recognised international agencies e.g. the International Journal of Systematic and Evolutionary Microbiology, Bergey's Manual of Determinative Bacteriology (9th ed., revised, Williams & Wilkins, 1993), Virus Taxonomy - The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses (Springer-Verlag, 1995). The latter document also supplies standard abbreviations for virus species.

Clinical trials registration. All clinical trials must be registered in a registry that is electronically accessible to the public, free of charge. Registration should occur before patient enrolment and the registry's URL and the trial's registration number must be supplied at the end of the manuscript's abstract. For information on acceptable registries, consult the ICMJE Web site, http://www.icmje.org. The National Library of Medicine's registry which is free and open to all investigators, generally meets the requirements of journals for the publication of clinical trials.

Manuscript Submission

Procedure

All manuscripts must be submitted online at www.saje.co.za. Register as an author, login in, and click on the "Submit Article" button. Please submit your files as follows: Word files, PDF files, and Excel files. Figures should be uploaded separately. Please include a cover note indicating the manuscript title, authors, and contact information.

All manuscripts must be submitted online at www.saje.co.za. Register as an author, login in, and click on the "Submit Article” button. Please submit your files as follows: Word files, PDF files, and Excel files. Figures should be uploaded separately. Please include a cover note indicating the manuscript title, authors, and contact information.

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Manuscript Submission

Procedure

All manuscripts must be submitted online at www.saje.co.za. Register as an author, login in, and click on the "Submit Article” button. Please submit your files as follows: Word files, PDF files, and Excel files. Figures should be uploaded separately. Please include a cover note indicating the manuscript title, authors, and contact information.