

Prevalence of carbapenem resistance in adult patients admitted to a private hospital in Daspoort, Tshwane

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“You anoint my head with oil; my cup runs over. Surely goodness and mercy shall follow me all the days of my life” (Psalm 23:5-6).

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LIST OF ACRONYMS, KEY TERMS AND ABBREVIATIONS

ACC	Ambler class C
Aerobic	Requires oxygen to grow and maintain life (Mosby's dictionary of medicine, nursing & health professions, 2013:49)
AGAR	Australian Group on Antimicrobial Resistance
AGISAR	WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AmpC	Class C β -lactamases (cephalosporinases) that mediates resistance to cephalosporins and penicillins (Jacoby, 2009:161)
AMR	Antimicrobial resistance
Anaerobic	Requires oxygen to grow and cannot be cultured if oxygen is present (Porter & Kaplan, 2011:1180)
ANSORP	Asia Network for Surveillance of Resistant Pathogens
Antimicrobials	A large group of drugs with a variety of mechanisms of actions against bacteria, fungi, parasites and viruses (Mosby's dictionary of medicine, nursing & health professions, 2013:113)
APACHE II	Acute Physiology Assessment and Chronic Health Evaluation
APFID	Asia Pacific Foundation for Infectious Diseases
APUA	Alliance for the Prudent Use of Antibiotics
ARC	Antibiotic Resistance Coalition
ARMed	Antibiotic Resistance Surveillance and Control in the Mediterranean Region
ASM	American Society for Microbiology
ASP	Antibiotic Stewardship Program

ATC	The anatomical therapeutic chemical classification system divides drugs according to the site or organ where it acts and their therapeutic and chemical characteristics (WHO, 2013b:15)
Bacterial resistance	The capability of bacteria to continue to grow in the presence of specific antibiotics to which they were susceptible to previously (Mosby's dictionary of medicine, nursing & health professions, 2013:175)
β-lactamases	Enzymes that hydrolyse the beta-lactam ring of β-lactam antibiotics and render the antibiotic ineffective (Bush & Jacoby, 2010:969)
β-lactam antibiotics	A group of antibiotics with a beta-lactam ring in the chemical structure for e.g. penicillin, cephalosporin and carbapenem (Mosby's dictionary of medicine, nursing & health professions, 2013:198)
BLNAR	β-lactamase-negative ampicillin-resistant
BP	Blood pressure
CAESAR	Central Asian and Eastern European Surveillance of Antimicrobial Resistance
CANWARD	Canadian Ward Surveillance Study
CAP	Community-acquired pneumonia
Carbapenems	β-lactam antibiotics with a broader spectrum of activity compared to other β-lactams due to the five-member ring and fused β-lactam ring. Examples include imipenem-cilastatin, ertapenem, meropenem and doripenem (El-Gamal <i>et al.</i> , 2016:187)
Carbapenemases	β-lactamases that hydrolyse most β-lactam antibiotics like penicillins, cephalosporins and carbapenems (Queenan & Bush, 2007:440)
Catalase	An enzyme found in biologic cells that catalyses the breakdown of hydrogen peroxide (Concise Medical Dictionary, 2015)
CAUTI	Catheter-associated urinary tract infections

CDC	Centers for Disease Control
CDDEP	Center for Disease Dynamics, Economics & Policy
Cephalosporinases	β -lactamases that act mainly on cephalosporins (Dorland, 2007)
Cephalosporins	β -lactam antibiotic similar to penicillin that is resistant to the action of penicillinase because of a beta-lactam dihydrothiazine ring instead of a beta-lactam thiazolidine (Mosby's dictionary of medicine, nursing & health professions, 2013:108)
CGIAR	Consultative Group for International Agricultural Research
CHINET	China Antimicrobial Resistance Surveillance Study
CIPARS	Canadian Integrated Program on Antimicrobial Resistance Surveillance
CLABSI	Central line-associated bloodstream infections
Classes of antibiotics	Penicillins, macrolides, tetracyclines, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems
CLSI	Clinical and Laboratory Standards Institute
CMY	Cephameycins
CNISP	Canadian Nosocomial Infection Surveillance Program
CO-ADD	Community for Open Antimicrobial Drug Discovery
Coagulase	An enzyme formed by certain bacteria of the genus <i>Staphylococcus</i> that results in the coagulation of plasma (Concise Medical Dictionary, 2015)
COMPACT	COMParative Activity of Carbapenem Testing
COPD	Chronic obstructive pulmonary disease
CPE	Carbapenemase-producing Enterobacteriaceae
CPO	Carbapenemase-producing organisms
CRAB	Carbapenem-resistant <i>Acinetobacter baumannii</i>

CRE	Carbapenem-resistant Enterobacteriaceae
CRKP	Carbapenem-resistant <i>Klebsiella pneumoniae</i>
CRNE	Carbapenem-resistant non-Enterobacteriaceae
CSAB	Carbapenem-susceptible <i>Acinetobacter baumannii</i>
CSE	Carbapenem-sensitive Enterobacteriaceae
cSSTIs	Complicated skin and soft tissue infections
CTX-M	Cefotaxime hydrolysing capabilities
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Program
DART	Deutsche Antibiotika-Resistenzstrategie
DDD	The anticipated average mean daily drug dose prescribed for its main indication in adults (WHO, 2013b:22)
DDD/100BDU	Defined daily doses per 100 bed-days used
DHA	Dhahran hospital
DHP-I	Dehydropeptidase-I
DNDi	Drugs for Neglected Diseases <i>initiative</i>
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
Efflux	Mechanism involved in the expulsion of substrates like antibiotics that are potentially harmful to the cell to the external environment (Ryan <i>et al.</i> , 2001:1409)
EFSA	European Food Safety Authority
EIP	Emerging Infections Program

Enterobacteriaceae	A family of aerobic and anaerobic gram-negative bacteria, which include genera of the families <i>Citrobacter</i> , <i>Edwardsiella</i> , <i>Enterobacter</i> , <i>Escherichia coli</i> , <i>Klebsiella</i> , <i>Proteus</i> , <i>Providencia</i> , <i>Shigella</i> , <i>Salmonella</i> , <i>Serratia</i> , <i>Hafnia</i> , <i>Morganella</i> and <i>Yersinia</i> (Mosby's dictionary of medicine, nursing & health professions, 2013:623)
Enzyme	A protein that catalyses certain biological reactions in living cells (Concise Medical Dictionary, 2015)
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
ESBL	Extended spectrum β -lactamase
ESKAPE	Acronym for the group of bacteria: <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> , <i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Acinetobacter baumannii</i> complex, <i>Pseudomonas aeruginosa</i> , <i>Enterobacter cloacae</i> complex and <i>Escherichia coli</i> (NICD-NHLS, 2016d:107; Santajit & Indrawattana, 2016)
ESPUAR	English Surveillance Programme for Antimicrobial Utilization and Resistance
ESR	New Zealand Institute of Environmental Science and Research
ESVAC	European Surveillance of Veterinary Antibiotic Consumption
EU	European Union
EWEC	Every Woman Every Child
Facultative anaerobic	A microorganism that develops in the presence of oxygen but develops quicker in an environment with no oxygen (Mosby's dictionary of medicine, nursing & health professions, 2013:672)
FAO	Food and Agriculture Organization of the United Nations
FDA	US Food and Drug Administration
Fermenting	Enzymatic breakdown of organic substances like carbohydrates into simpler substances under anaerobic circumstances (Dorland, 2007)

FIDSSA	Federation of Infectious Diseases Societies of Southern Africa
FIND	Foundation for Innovative New Diagnostics
FINRES-VET	Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents
FOX	Cefoxitin
GARD	Global Antibiotic Research and Development
GARP	Global Antibiotic Resistance Program
GASP	The Gonococcal Antimicrobial Surveillance Program
GCS	Glasgow Coma Scale
GERM-VET	German National Veterinary Antibiotic Resistance Monitoring
GES	Guyana extended-spectrum-lactamase
GHSA	Global Health Security Agenda
GISA	Glycopeptide-intermediate <i>Staphylococcus aureus</i>
GISP	Gonococcal Isolate Surveillance Program
GLASS	Global Antimicrobial Resistance Surveillance System
Gram-negative	Bacteria that do not retain the dark blue colour of Gram's stain (Dorland, 2007)
Gram-positive	Bacteria that retain the crystal violet dye as a dark blue colour after fixation and alcohol decolourisation (Porter & Kaplan, 2011:1180)
HAI	Health Action International
HAIC	Healthcare-Associated Infections Community Interface
ICU	Intensive care unit
IFPMA	International Federation of Pharmaceutical Manufacturers & Associations

IMP	Imipenemase
ITAVARM	Italian Veterinary Antimicrobial Resistance Monitoring
JANIS	Japan Nosocomial Infections Surveillance
JVARM	Japanese Veterinary Antimicrobial Resistance Monitoring System
KARMS	Korean Antimicrobial Resistance Surveillance Program
KONSAR	Korean Nationwide Surveillance of Antimicrobial Resistance
KPC	<i>Klebsiella pneumoniae</i> carbapenemase
LRTI	Lower respiratory tract infection
MARAN	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands
MDR	Multidrug-resistant
MDRP	Multidrug-resistant <i>Pseudomonas aeruginosa</i>
MIC	Minimum inhibitory concentration
MOHNARIN -	Ministry of Health National Antimicrobial Resistant Investigation Net
MOX	Moxalactam
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSF	Médecins Sans Frontières
MUSA	Medicine Usage in South Africa
NAMRU-2 PP	United States Naval Medical Research Unit 2 Phnom Penh
NARMS	National Antimicrobial Resistance Monitoring System
NARS	Network for Antimicrobial Resistance Surveillance
NARST	National Antimicrobial Resistance Surveillance Center, Thailand
NDM	New Delhi Metallo- β -lactamase

NDoH	National Department of Health
NETHMAP	Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands
NHLS	National Health Laboratory Service
NHSN	National Healthcare Safety Network
NICD	National Institute for Communicable Diseases
Non-fermenting	Lacks the capacity to ferment any sugars (Engelkirk & Duben-Engelkirk, 2008:295)
NORM	Norwegian Surveillance System for Antimicrobial Drug Resistance
Nosocomial infection	An infection acquired after at least 72 hours following admission to a hospital or healthcare facility (McGraw-Hill Concise Dictionary of Modern Medicine, 2002)
NSAR	National Surveillance of Antimicrobial Resistance Program
NTSS	National Tuberculosis Surveillance System
Obligate aerobic	Requires oxygen to grow in a culture and produce energy (Porter & Kaplan, 2011:1180)
Obligate anaerobic	Does not require oxygen to grow and cannot grow if air is present (Mosby's dictionary of medicine, nursing & health professions, 2013:1254)
OECD	Organisation for Economic Co-operation and Development
OIE	World Organization for Animal Health
ONERBA	l'Observatoire National de l'Epidémiologie de la Résistance Bactérienne aux Antibiotiques
OXA	Oxacillin hydrolysing capabilities
OXA-48	Oxacillinase-48 type carbapenemase
PAHO	Pan American Health Organization

Pathogen	A microorganism that can produce disease (McGraw-Hill Concise Dictionary of Modern Medicine, 2002)
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
PDR	Pan-drug-resistant
PISP	Penicillin-intermediate <i>Streptococcus pneumoniae</i>
Polymicrobial	Combination of a number of species of microorganisms (Mosby's dictionary of medicine, nursing & health professions, 2013:1420)
Porin	Channels across the outer membrane of gram-negative bacteria that permit passive transport of hydrophilic substances (Galdiero <i>et al.</i> , 2012:843)
PRSP	Penicillin-resistant <i>Streptococcus pneumoniae</i>
RACP	Royal Australasian College of Physicians
REACH	Retrospective Study to Assess the Clinical Management of Patients With Moderate-to-Severe Complicated Skin and cSSTI or CAP in the Hospital Setting (Leprince <i>et al.</i> , 2015:177)
ReAct	Action on Antibiotic resistance
ReLAVRA	Latin American Antimicrobial Resistance Surveillance Network
SAPC	South African Pharmacy Council
SAS	Statistical Analysis System® program
SASCM	South African Society for Clinical Microbiology
SHV	Sulfhydryl variable
SME	<i>Serratia marcescens</i> enzyme
SPM	São Paulo metallo- β -lactamase
Spore	A bacterial form that is resistant to hostile conditions (Mosby's dictionary of medicine, nursing & health professions, 2013:1679)

spp.	Species
SSI	Surgical site infections
SVARM	Swedish veterinary antibiotic resistance monitoring programme
SWEDRES	Swedish utilisation and resistance in human medicine
TATFAR	Trans Atlantic Task Force on AMR
TEM	Temoneira
TSAR	Taiwan Surveillance of Antimicrobial Resistance
TSN	The Surveillance Network
TWN	Third World Network
UN	United Nations
US	United States
VAP	Ventilator-associated pneumonia
VIM	Verona integron-encoded metallo- β -lactamase
VINARES	Viet Nam Resistance Project
Virulence	Ability of microorganism to produce disease (Concise Medical Dictionary, 2015)
VRE	Vancomycin-resistant enterococci
WAAAR	World Alliance against Antibiotic Resistance
WB	World Bank
WePARS	Western Pacific Regional Antimicrobial Resistance Surveillance
WHO	World Health Organization

ABSTRACT AND KEYWORDS

Prevalence of carbapenem resistance in adult patients admitted to a private hospital in Daspoort, Tshwane

The aim of this study was to investigate the prevalence of organisms that are resistant to the carbapenem class, in patients admitted to a private hospital. A quantitative descriptive (non-experimental) cross-sectional design was followed in order to retrospectively investigate the prevalence of resistance of hospitalised patients treated with a carbapenem between 1 January and 31 December 2014.

Mortality of patients infected with carbapenem-resistant Enterobacteriaceae (CRE) has been found to be three to six times higher compared to patients infected with carbapenem-susceptible organisms. Infections due to resistant organisms also contribute to an increased length of hospital stay and higher hospitalisation costs.

The global reported prevalence of carbapenem resistance was initially restricted to case reports and outbreaks in the intensive care unit (ICU) setting. However, a marked increase (up to 27%) has been reported in Europe and the United States (US) in recent years. Current available data in South Africa are mostly based on referred isolates of suspected carbapenemase-producing Enterobacteriaceae (CPE), which means that the prevalence of carbapenem resistance in the population at risk cannot be calculated. The World Health Organization (WHO) and the South African National Department of Health (NDoH) have identified that data regarding carbapenem resistance in Africa and specifically South Africa is incomplete and not representative of the potential burden that currently exists.

The monitoring of carbapenem resistance is an important priority because this antibiotic class has been seen as the last option in the treatment for resistant gram-negative Enterobacteriaceae for the past two decades. Organisms that exert resistance to carbapenems are often also resistant to other antibiotics like gentamycin, the quinolone and cephalosporin classes, other β -lactam antibiotics or β -lactam combinations. This results in few or no treatment options for resistant organisms. Gram-negative organisms pose a specific challenge for the development of new antibiotic treatment due to its efflux-mediated resistance mechanism, which impairs the efficiency of antibiotics. This challenge is amplified by economic and regulatory constraints that have brought the development of antibiotics to a halt in recent years.

The prevalence of carbapenem resistance found in this study was 12.7% (n=9). The majority of resistance (78% of isolates) were identified through carbapenemase production based on a rectal polymerase chain reaction (PCR) swab. Two organisms, *Pseudomonas putida* and *Enterobacter cloacae* (extended spectrum β -lactamase (ESBL) positive), expressed resistance

towards a carbapenem. *Pseudomonas putida* exhibited resistance to imipenem, meropenem and doripenem. This specific organism has inherent resistance to ertapenem. The *Enterobacter cloacae* (ESBL positive) isolate was resistant towards ertapenem only. Both these organisms, initially thought to have low virulence, have emerged as difficult-to-treat infections in nosocomial infections. Literature shows that 9.6% of CRE isolates cultured in South Africa during 2014 were *Enterobacter cloacae*. *Pseudomonas putida* has to date not formally been reported as a resistant organism in South Africa.

In conclusion, the results from the current study confirm that carbapenem resistance is likely to be prevalent at all private sector hospitals in South Africa. The heterogeneity in the reported carbapenem resistance prevalence and the underrepresentation of the true burden across South Africa means that it can only be speculated on whether a prevalence of 12.7% as seen is in this study will be found in similar private hospital settings. Healthcare professionals in this hospital should use these results to improve antibiotic prescribing practices and preserve the carbapenem class as a treatment option for difficult-to-treat infections.

KEYWORDS: carbapenem importance, resistance, prevalence, carbapenem-resistant organisms, carbapenem resistance mechanism, risk factors for carbapenem resistance.

UITTREKSEL EN TREFWOORDE

Voorkoms van karbapenemweerstandigheid in volwasse pasiënte in 'n privaat hospitaal te Daspoort, Tshwane

Die doel van die studie was om die voorkoms van weerstandigheid van organismes teenoor die karbapenemklas te ondersoek in pasiënte wat in 'n privaat hospitaal opgeneem was. 'n Kwantitatiewe beskrywende (nie-eksperimentele) deursnee-ontwerp was gevolg om die voorkoms van weerstandigheid op 'n retrospektiewe wyse te ondersoek in gehospitaliseerde pasiënte wat met 'n karbapenem behandel was tussen 1 Januarie en 31 Desember 2014.

Daar is bevind dat die mortaliteit van pasiënte wat met karbapenemweerstandige Enterobacteriaceae (CRE) geïnfekteer was, drie tot ses keer hoër was as in pasiënte wat met karbapenem-sensitiewe organismes geïnfekteer is. Infeksies wat deur weerstandige organismes veroorsaak is, dra tot 'n verlengde hospitaliseringstydperk en hoër hospitalisasie-uitgawes by.

Die voorkoms van karbapenemweerstandigheid soos internasionaal gerapporteer, was aanvanklik beperk tot gevallestudies en uitbrake in intensiewesorgeenhede. Daar is egter 'n merkbare styging (tot 27%) in Europa en die Verenigde State in die afgelope paar jaar gedokumenteer. Huidige beskikbare data is meestal gebaseer op kwekings wat ontvang is waar karbapenemase-produiserende Enterobacteriaceae (CPE) vermoed word en dit beteken dat die voorkoms van karbapenemweerstandigheid in die blootgestelde bevolking nie bereken kan word nie. Die Wêreldgesondheidsorganisasie en die Suid-Afrikaanse Nasionale Departement van Gesondheid het bevind dat data rakende karbapenemweerstandigheid in Afrika, maar meer spesifiek in Suid-Afrika onvolledig is en nie die omvang van die potensiële probleem wat tans bestaan voldoende weerspieël nie.

Die monitering van karbapenemweerstandigheid is 'n belangrike prioriteit aangesien hierdie antibiotikumklas tydens die afgelope twee dekades beskou is as die laaste keuse in die behandeling van weerstandige gram-negatiewe Enterobacteriae. Organismes wat weerstandig is teenoor karbapenems, is ook dikwels weerstandig teenoor ander antibiotika soos gentamisien, die kinoloon- en kefalosporien-klasse, ander betalaktaamantibiotika of betalaktaamkombinasies. Die gevolg is dat daar min of geen behandelingskeuses vir weerstandige organismes bestaan nie. Gram-negatiewe organismes bied 'n spesifieke uitdaging vir die ontwikkeling van 'n nuwe antibiotiese behandeling weens die uitvloeibemiddelde weerstandbiedende meganisme wat die doeltreffendheid van antibiotika verswak. Daarmee saam het die ontwikkeling van antibiotika gedurende die afgelope paar jaar tot stilstand gekom weens ekonomiese en regulatoriese beperkings.

Die voorkoms van karbapenemweerstandigheid wat in hierdie studie gevind is, was 12.7% (n=9). Weerstandigheid (78% van die gevalle) is meestal geïdentifiseer deur karbapenemaseproduksie in 'n anale depper (PCR-swab). Twee organismes *Pseudomonas putida* en *Enterobacter cloacae* (ESBL-positief) het weerstandigheid teenoor 'n karbapenem antibiotikum getoon. *Pseudomonas putida* het weerstandigheid teenoor imipenem, meropenem en doripenem getoon. Hierdie spesifieke organisme toon 'n inherente weerstandigheid teenoor ertapenem. *Enterobacter cloacae* (ESBL-positief) was slegs weerstandig teenoor ertapenem. Beide hierdie organismes is aanvanklik gesien as minder virulent, maar het egter ontwikkel tot infeksies wat veral in nosokomiale omstandighede moeilik is om te behandel. Literatuur het aangetoon dat *Enterobacter cloacae* geïdentifiseer is in 9.6% van CRE in Suid-Afrika gedurende 2014. *Pseudomonas putida* is tot op hede nog nie formeel as 'n weerstandige organisme in Suid-Afrika aangemeld nie.

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Die resultate van hierdie studie bevestig dat karbapenemweerstandigheid heel moontlik voorkom in alle privaatsektor hospitale in Suid-Afrika. Die heterogenetiese verskille in die voorkoms van die aangemelde karbapenemweerstandigheid en die ondervermelding van die ware toedrag van sake in Suid-Afrika, beteken dat die voorkoms van 12.7% gevind in hierdie studie nie noodwendig in soortgelyke privaathospitaal omgewings gevind sal word nie. Professionele verskaffers van gesondheidsorg in hierdie hospitaal behoort hierdie resultate te gebruik vir die verbetering van antibiotikum voorskryfpraktyke en die bewaring van die karbapenemklas as behandelingsopsie vir infeksies wat andersins moeilik behandelbaar is.

TREFWOORDE: Belangrikheid van karbapenem, weerstandigheid, voorkoms, karbapenemweerstandige organismes, karbapenemweerstandige meganismes, risikofaktore vir karbapenemweerstandigheid.

PREFACE

This mini-dissertation is presented in the article format as approved by the North-West University. Chapter 3 presents the findings of the empirical investigation in the form of a manuscript, which is prepared for submission to the *South African Family Practice Journal*. The manuscript is written in line with the author guidelines of the journal. The article includes a list of references written according to the required referencing style of the journal. The author guidelines are included in the annexures. Results not included in the manuscript are included separately in Chapter 3. The reference list of the mini-dissertation was written according to the Harvard style as required by the North-West University.

The mini-dissertation is divided into the following chapters:

Chapter 1 provides information on the research proposal and the methodology used for the literature review and the empirical investigation. Chapter 2 provides a review of the available literature on the topic of the global prevalence of carbapenem resistance, the risk factors for the acquisition thereof and the value of this class in the treatment of infectious diseases. Chapter 3 presents the results of the investigation in the form of a manuscript and the additional results. The manuscript contains a list of references used according to the referencing style required by the journal. Chapter 4 concludes on whether the objectives for both the literature review and empirical investigation were met. This chapter comments on the strengths and weaknesses of the investigation and makes recommendations based on all the findings of the study in the context of the literature review.

The co-authors to the manuscript presented in Chapter 3 were also the supervisor and co-supervisors for the mini-dissertation. They have given their consent that the manuscript may form part of this mini-dissertation. The authors' contributions are described in the following section.

AUTHORS' CONTRIBUTIONS (MANUSCRIPT)

Article	Author role
Prevalence of carbapenem resistance in adult patients admitted to a private hospital in Daspoort, Tshwane	M de Kock was involved in the design of the study, the interpretation of the analysed data, the drafting and writing of the manuscript. DM Rakumakoe has revised the manuscript and verified the layout of the manuscript according to the author's guidelines. JM du Plessis has provided input on the formatting and content of the manuscript according to author guidelines in preparation for submission. MS Lubbe has given guidance on the layout and content of the manuscript and provided extensive support on the interpretation and writing of the results. M Cockeran has verified the sample size and statistical techniques and the analysis and interpretation of the results. All authors have read and approved the manuscript.

The co-authors confirm their individual roles in the manuscript and give their permission that the manuscript may form part of this dissertation:

I declare that the above-mentioned contribution is representative of my actual contribution to the manuscript and I hereby give consent that this manuscript may form part of the mini-dissertation submitted in partial fulfilment for the degree Master of Pharmacy in Advanced Clinical Pharmacy for Ms M de Kock.

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CHAPTER 1: RESEARCH PROTOCOL

1.1 Introduction

Everything has been seen before and antimicrobial resistance (AMR) is no exception. However, the rapidly changing level of resistance and how it affects the human race have not been experienced before (Huttner *et al.*, 2013). Resistance to multiple antibiotic classes has left the carbapenem class of antibiotics as the only sustainable treatment option for many isolates, either as monotherapy or in combination with other active drugs (Evans *et al.*, 2013:225; Trecarichi & Tumbarello, 2017). This mini-dissertation investigated the current prevalence of carbapenem resistance at both an international and local level.

1.2 Background

Antimicrobial resistance is defined as the ability of microbes to develop a tolerance towards the effects of an antimicrobial drug that would normally suppress their growth or kill them (Mosby's dictionary of medicine, nursing & health professions, 2013:175). The consequence of AMR is that a strain of a specific organism is not eradicated or inhibited by a concentration to which most of the genetic variants of that specific microbe are susceptible (SCENIHR, 2009:13). Alexander Fleming already commented on bacterial resistance in 1945 and stated that organisms easily become resistant just by exposing them to suboptimal doses of penicillin (Fleming, 1945). Hence, the emergence of bacterial β -lactamases-mediated resistance started to threaten the use of penicillin in the 1960s (Holt & Stewart, 1964:203). One of the simplest ways to classify β -lactamases is through protein sequencing. Conserved and distinguishing amino acid motifs are used to classify β -lactamases into four molecular classes, i.e. A, B, C, and D (Bush & Jacoby, 2010:969). β -lactamases are the enzymes produced by some bacteria that have driven the necessity for the development of the most widely used class of antibiotics, i.e. β -lactam inhibitors (Bush, 2013:84).

The first β -lactam inhibitors to counter AMR were discovered by 1975 and clavulanic acid, one of the first β -lactam inhibitors discovered, is still used today. The cephalosporin class followed from here and the third-generation cephalosporins were, mainly because of the ability of chemists to tailor properties of antibiotics to meet specific clinical needs, regarded as a milestone in antimicrobial therapy (Pfeifer *et al.*, 2010:372; Wright *et al.*, 2014:8845). Unfortunately, cephalosporins exerted selective pressure on microbes, which resulted in the appearance of resistance in enterobacterial species (spp.) shortly thereafter. This resulted in the introduction of carbapenems as part of the β -lactam inhibitors (Pfeifer *et al.*, 2010:372). The development of carbapenems in the 1960s was a direct consequence to the dramatic increase in resistance against the widely used cephalosporin class (Zaffiri *et al.*, 2013:171). Examples of

currently available carbapenems include imipenem-cilastatin, meropenem, ertapenem and doripenem (El-Gamal *et al.*, 2016:186). These agents have been proven to have the broadest *in vitro* spectrum of activity amongst β -lactam antibiotics (El-Gamal *et al.*, 2016:194; Lagacé-Wiens *et al.*, 2014:16). In addition to this, its activity includes most gram-positive pathogens, except methicillin-resistant *Staphylococcus aureus* (MRSA) and most ampicillin-resistant *Enterococcus faecium*. It also includes the clinically important anaerobic bacteria (Cunha & Cunha, 2013:202; Hawkey & Livermore, 2012; Zhanel *et al.*, 2007:1032). Carbapenems currently under development, have higher selective antibacterial and bactericidal activities that include clinical activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (El-Gamal *et al.*, 2016:194). Table 1-1 provides information on the *in vitro* activity of the currently available carbapenems (Jones *et al.*, 2004; Kattan *et al.*, 2008; Sahm, 2009; Tsuji *et al.*, 1998; Zhanel *et al.*, 2007). Carbapenems have been amongst the most commonly used and the most efficient antibiotics prescribed for the treatment of serious infections due to gram-negative bacteria as well as non-fermenting gram-negative bacteria since the 1980s (Bowers & Huang, 2016; El-Gamal *et al.*, 2016:185; Papp-Wallace *et al.*, 2011:4943). Carbapenems remain, to date, a valuable class for the treatment of infections caused by gram-negative bacteria that are resistant to other β -lactams (Bassetti *et al.*, 2016:368). This can be explained by the class' stability against the majority of β -lactamases (Zhanel *et al.*, 2007:1031).

Organisms of clinical importance are methicillin-resistant *Staphylococcus aureus* as well as the Enterobacteriaceae and *Acinetobacter* families. Enterobacteriaceae include gram-negative bacilli that are naturally present in the gastro-intestinal tract of humans. However, community-acquired and healthcare-acquired infections from this family are mostly caused by *Escherichia coli*, the *Klebsiella* and *Enterobacter* spp. (Jacob *et al.*, 2013:165). *Acinetobacter* spp. are aerobic, gram-negative coccobacilli that have become one of the most problematic pathogens to treat (Evans *et al.*, 2013:223). *Acinetobacter baumannii* was initially considered to have low pathogenic potential, but is now the most frequent cause for nosocomial infection (Abbot *et al.*, 2013:395).

Penicillins, cephalosporins, aminoglycosides, quinolones and tetracyclines have essentially been eliminated as effective classes for the treatment of infections due to resistant *Acinetobacter baumannii* isolates. This has resulted in the carbapenems, due to their good activity, being the class of choice for the treatment of infections caused by *Acinetobacter baumannii* (Evans *et al.*, 2013:225; Menegucci *et al.*, 2016:380). The rapid spread of extended spectrum β -lactamases and quinolone resistance amongst Enterobacteriaceae have increased dependence on carbapenems (Livermore, 2009:i29). The class is still regarded, notwithstanding the lack of options for multidrug resistant *Acinetobacter baumannii*, as an effective treatment in combination with fosfomycin and polymyxin B (Menegucci *et al.*, 2016:382).

Table 1-1: *In vitro* activity of the carbapenem class

Obligate aerobic & facultative anaerobic gram-negative bacteria	Facultative anaerobic gram-positive bacteria	Obligate anaerobic bacteria
<i>Acinetobacter</i> spp. ^a <i>Bordetella pertussis</i> <i>Citrobacter freundii</i> <i>Enterobacter aerogenes</i> <i>Enterobacter cloacae</i> <i>Enterococcus faecalis</i> <i>Enterococcus faecium</i> ^c <i>Escherichia coli</i> <i>Haemophilus influenza</i> <i>Klebsiella pneumoniae</i> <i>Klebsiella oxytoca</i> <i>Moraxella catarrhalis</i> <i>Morganella morganii</i> <i>Neisseria gonorrhoeae</i> <i>Proteus mirabilis</i> <i>Proteus vulgaris</i> <i>Providencia rettgeri</i> <i>Pseudomonas aeruginosa</i> ^a <i>Salmonella</i> spp. <i>Serratia marcescens</i> <i>Shigella</i> spp.	<i>Staphylococcus aureus</i> ^b <i>Staphylococcus epidermidis</i> ^b <i>Streptococcus pneumoniae</i> <i>Streptococcus pyogenes</i>	<i>Bacteroides fragilis</i> <i>Clostridium difficile</i> <i>Clostridium perfringens</i> <i>Fusobacterium</i> spp. <i>Prevotella</i> spp. <i>Peptococcus</i> spp. <i>Peptostreptococcus</i> spp. <i>Propionibacterium</i> spp.

a = Ertapenem resistant b = Methicillin susceptible c = Ampicillin susceptible

Many non-fermenting gram-negative bacteria, Enterobacteriaceae and gram-positive bacteria are or are still in the process of becoming resistant to clinically available carbapenems (Papp-Wallace *et al.*, 2011:4946). Enterobacteriaceae are frequently implicated in community-acquired infections and carbapenem-resistant Enterobacteriaceae (CRE) have the potential to be transmitted from current healthcare-exposed patients to the community (Jacob *et al.*, 2013:167). Organisms express resistance to carbapenems through a number of mechanisms e.g. altered penicillin-binding proteins (PBPs) and porin function, the β -lactamases production and through efflux pumps (Nordmann *et al.*, 2012:264; Potter *et al.*, 2016:31; Zhanel *et al.*, 2007:1028). Certain bacterial species such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* exhibit a combination of these mechanisms leading to increased levels of resistance to carbapenems (Papp-Wallace *et al.*, 2011:4946). The production of β -lactamases is the most frequent cause of carbapenem resistance. Carbapenemases are a type of β -lactamases with the capability to hydrolyse carbapenems (Papp-Wallace *et al.*, 2011:4947).

Alternative treatment to the carbapenem class for the difficult-to-treat pathogen, MRSA includes ceftaroline, tigecycline, daptomycin and linezolid. Products that are awaiting launch for the

treatment of these organisms include telavancin, ceftobiprole and dalbavancin (Livermore, 2009:i29). Alternative treatment for enterococci, because of its high-level aminoglycoside resistance, is a greater problem. However, daptomycin still offers cidal potential (Livermore, 2009:i29). Few alternative treatments are currently available for CRE (Morris *et al.*, 2015). Antibiotic classes that still exhibit *in vitro* activity against CRE include polymyxins, some aminoglycosides, fosfomycin and tigecycline (Kanj & Kanafani, 2011:253; Van Duin *et al.*, 2013:116). High-dose prolonged-infusion carbapenem therapy has been used for CRE where the minimum inhibitory concentrations (MICs) are still below 4 mg/L (Kanj & Kanafani, 2011:253). It is suggested that combination therapy is superior to monotherapy for these infections (Van Duin *et al.*, 2013:116). Double-carbapenem therapy can be considered and has been shown to be effective in some instances. However, the efficacy and safety data of double-carbapenem therapy are scarce (Van Duin *et al.*, 2013:116; Yamamoto & Pop-Vicas, 2014). Treatments that are available for invasive CRE infections include polymyxins, tigecycline, and aminoglycosides (Van Duin *et al.*, 2013:116). Current resistance rates of CRE range from 9.7% to 51.3% (mean 22.6%) for colistin, 5.6% to 85.4% (mean 43.5%) for gentamicin and from 0% to 33% (mean 15.2%) for tigecycline (Trecarichi & Tumbarello, 2017). The most effective therapeutic management of CRE infections has not been established, because no clinical trials have so far been undertaken with this aim (Trecarichi & Tumbarello, 2017). Present review data found that carbapenems in association with other active drugs are likely to remain effective for CRE isolates with carbapenem MICs <8 mg/l (Trecarichi & Tumbarello, 2017).

Newer alternative non- β -lactam/ β -lactamase inhibitor combinations, ceftolozane/tazobactam and ceftazidime/avibactam, have *in vitro* activity against selected carbapenem-resistant gram-negative pathogens (Goodlet *et al.*, 2016:1814). Ceftazidime/avibactam, recently approved by the US FDA, display *in vitro* activity against CRE that produce *Klebsiella pneumoniae* carbapenemases (KPC) and class C β -lactamases (AmpC) and partial activity against class D, oxacillin hydrolysing (OXA) enzymes (Goodlet *et al.*, 2016:1814; Trecarichi & Tumbarello, 2017). The drug is however, not active against metallo- β -lactamases such as New Delhi metallo- β -lactamase (NDM), Verona integron-encoded metallo- β -lactamase (VIM) or imipenemase (IMP) (Sharma *et al.*, 2016:434; Trecarichi & Tumbarello, 2017). Clinical data on the efficacy of ceftazidime-avibactam in severe infections caused by CRE are scarce (Trecarichi & Tumbarello, 2017). The FDA has cautioned that the combination has limited clinical safety and efficacy data (Sharma *et al.*, 2016:437). The first reports of ceftazidime-avibactam-resistant *Klebsiella pneumoniae* have already emerged (Shields *et al.*, 2017). Developing alternative treatment for highly resistant bacterial infections remains challenging, with gram-negative nosocomial pathogens of particular concern due to its ability to exert resistance to a broad spectrum of structural classes (Paris, 2015; Payne *et al.*, 2007:39).

Patients with invasive infections (e.g. bloodstream infections) caused by CRE have demonstrated fatality rates of more than 40% compared to patients infected with carbapenem-susceptible Enterobacteriaceae (CSE) (Jacob *et al.*, 2013:167). Most well designed studies showed a three to six times higher mortality rate amongst CRE infected patients compared to those either infected with CSE or without a CRE infection (Temkin *et al.*, 2014:27). A study investigating the consequences of carbapenem resistance in Latin America found that the average total cost of hospitalisation of US \$11 359 for infections caused by carbapenem-resistant *Acinetobacter baumannii* (CRAB) was almost double when compared to the cost of US \$7 049 for patients with carbapenem-susceptible *Acinetobacter baumannii* (CSAB). Current CRE prevalence rates have led to added costs for third-party payers and hospitals due to an increase in hospitalisation, drug treatment and associated tests to the effect of US \$10 440 up to US \$66 031 (Bartsch *et al.*, 2017:48.e12). Longer intensive care unit (ICU) stays and higher costs from antimicrobial drugs have contributed to the higher cost in patients with CRAB (Lemos *et al.*, 2014:178). A comparison between sensitive and resistant groups showed that the median length of a hospital stay was nine vs. 23.5 days (Priyendu *et al.*, 2014:A271). Societal costs can be as high as US \$83 512, which can be attributed mainly to productivity losses and fatality (Bartsch *et al.*, 2017:48.e12).

Exposure to antibiotics, especially the carbapenem class, has demonstrated a significant relationship with carbapenem resistance (Ling *et al.*, 2015; Routsis *et al.*, 2013:1255; Voor In 't Holt *et al.*, 2014:2631). The overall mean consumption of antibiotics observed for the period October 2009 to January 2011 in private sector hospitals was 101.38 defined daily doses (DDD) per 100 patient-days (95% CI, 93.05 - 109.72) (Brink *et al.*, 2016:1022). More specifically, the consumption of penam and carbapenem units in the private sector had risen by 50% for the period, 2008 to 2011 (Essack *et al.*, 2011:565). Control of private sector antibiotic consumption is hampered, apart from those systems being put in place through quality improvement initiatives, by the lack of formal governance to control prescribing practices (National Department of Health, 2015:11).

Reports of carbapenem resistance in Enterobacteriaceae were initially infrequent and limited to case reports, tertiary care centres, intensive care units, and outbreak settings (Patel & Bonomo, 2013). A press release by the European Centre for Disease Prevention and Control (ECDC) in November 2013, however, reported on the marked increase seen in carbapenem resistance. This press release comments on the increase of *Klebsiella pneumoniae* in blood cultures found to be resistant to a carbapenem. This infection has increased from 2009 to 2012 to be more than 5% in five countries. Carbapenem-resistant *Acinetobacter* spp. is of concern in eight of eighteen countries that report resistance (ECDC, 2013). Surveillance data from China reported that the prevalence of imipenem-resistant *Acinetobacter baumannii* increased from 13.3% in

2004 to 70.5% in 2014 (Gao *et al.*, 2017:661). Global surveillance of carbapenem resistance still face several limitations in both community and healthcare settings (WHO, 2016b:7). One of the major contributors to this is the lack of high-quality data from regions like South and Southeast Asia and Sub-Saharan Africa (WHO, 2016b:7; World Bank Group, 2016:42). Bamford, Brink, *et al.* (2011:579) pointed out that although South Africa has made a good start at AMR surveillance, but it can and must be improved. The surveillance approach in South Africa does not reflect the magnitude of the problem due to surveillance by a small group of healthcare facilities only (Bamford, Brink, *et al.*, 2011:579). A review of the literature demarcating the current level of resistance to carbapenems in South Africa from early January 2000 to May 2016, found a total of 2315 reported carbapenem-resistant cases or infections over this period. The number of cases was regrettably not related to the population at risk, thus lacking comparability to other population groups in the rest of the world and South Africa (Joubert & Erlich, 2012:20; Osei-Sekyere, 2016). The National Institute for Communicable Diseases (NICD) database relies on referred isolates of suspected carbapenemase-producing Enterobacteriaceae (CPE) (NICD-NHLS, 2017a:9). This means that the frequency rate of carbapenem resistance in the population at risk cannot be calculated (Joubert & Erlich, 2012:20). The NICD has since 2014 not changed their observation that the available statistics for carbapenemase-producing Enterobacteriaceae (CPE) do not represent the current burden in South Africa (NICD-NHLS, 2014:9; NICD-NHLS, 2017a:9). This has led to the conclusion that the spread of carbapenem resistance in South Africa still remains largely undetected, especially in the private sector hospitals (CDDEP, 2015:24; Osei-Sekyere, 2016).

The impact of carbapenem resistance on our capability to effectively manage common infectious diseases and the associated devastating effects can be only limited once the true prevalence and extent of carbapenem resistance is known (WHO, 2016a, WHO, 2017a:7).

1.3 Problem statement and research questions

Hence, the question that remained unanswered was whether similar trends in carbapenem resistance existed in South Africa compared to the international situation and whether the problem was only related to current healthcare-exposed patients or not. In view of the internationally reported increase in carbapenem-resistant organisms on a broader basis and the lack of representative data on the burden of resistance, a review of the current status was performed at community level.

The following research questions were formulated for the study:

- How important is the carbapenem class for the management of infections?
- What is the extent of carbapenem resistance at an international and national level?

- What are the most prevalent resistant organisms, the mechanisms through which they exert resistance, and the risk factors for acquiring resistance?
- What types of data extraction instruments, approaches, and methods were used in similar retrospective investigations?

1.4 Research aims and specific objectives

The aim of the project was to assess the prevalence of carbapenem-resistant organisms in patients admitted to a private hospital in Daspoort, Tshwane. Specific objectives were set for the literature review and the empirical investigation to meet this aim and answer the research questions.

The literature review objectives included:

- To analyse the value of the carbapenem class in the treatment of infectious diseases.
- To determine what the prevalence of carbapenem resistance was at national and international level.
- To investigate the prevalence of specific carbapenem-resistant organisms and the mechanism through which resistance is exerted.
- To investigate risk factors for acquired carbapenem resistance.
- To evaluate the types of data extraction instruments utilised in quantitative, descriptive (non-experimental), cross-sectional retrospective investigations using the same approaches and methods as in the empirical investigation to determine carbapenem resistance.

The objectives for the empirical study were:

- To determine the prevalence of carbapenem resistance in patients where a carbapenem was prescribed during their stay in the hospital.
- To determine which organisms exhibit resistance to carbapenems in patients where a carbapenem was prescribed during their stay in the hospital.

1.5 Research methodology

The research is conducted in two phases, a literature review phase and an empirical investigation phase.

1.5.1 Literature review phase

Joubert and Erlich (2012:66) state that a literature review should involve a critical evaluation and synthesis of existing reports to justify new research. It should aim to indicate gaps in knowledge that the proposed research intends to fill and to put findings into context.

This phase in the research aimed to meet the objectives of the literature review (as stipulated in section 1.4). The search strategy was based on the topic of carbapenem resistance and the following keywords and phrases were identified for the search: carbapenem importance, resistance, prevalence, carbapenem-resistant organisms, carbapenem resistance mechanism, risk factors for carbapenem resistance, and data-extraction instruments. The search was conducted using the keywords alone or in combination.

Resources identified for this literature review included databases available through the North-West University's library system such as EBSCOhost, ScienceDirect® and Scopus. In addition, MacPLUS Federated Search and Evidence UPDATES were used to select literature based in the 6S Pyramid principle of Evidence-Based Medicine (Straus *et al.*, 2011:52). Critical reading of suitable literature involved a preliminary phase and a critical review. During the preliminary phase, the abstract and article were scanned to determine whether it met the objectives of the review. During the critical review the articles were analysed for usability, completeness and consistency with the literature review objectives (Brink, van der Walt, *et al.*, 2012:79). Relevant studies were further evaluated by assessing the validity of the study results according to evidence-based medicine principles (Straus *et al.*, 2011:68). The most up-to-date publications were required to provide an accurate overview of the context for the empirical investigation as bacterial resistance is rapidly evolving (Huttner *et al.*, 2013). The data synthesis aimed to provide a summary of the findings of primary studies while documenting consistencies and differences between studies evaluating the same topic. Conflicting findings found across studies were explained through the evaluation of influencing factors (Joubert & Erlich, 2012:72).

The emphasis was directed towards the critical evaluation of known facts about the present situation to provide the context for the proposed empirical investigation and to draw parallels between the international and the known current local situation.

1.5.2 Empirical investigation phase

The quality of the empirical investigation was dependent on the choice of design, the population, and the sample that represent the population the best. The data-collection protocol and analysis of data with the ethical implications thereof represent an important part of the empirical investigation (Brink, van der Walt, *et al.*, 2012:55).

1.5.3 Study design

Aldous *et al.* (2011:26) stated that the purpose and scope for the research determine the design of the study. Maree (2012:262) described the aim of quantitative research as a description of trends or an explanation of relationships between variables. When an existing situation needs to be described to uncover existing problems, the most suitable design is descriptive and a cross-sectional study is suitable to assess the prevalence at one point in time (Aldous *et al.*, 2011:24). Non-experimental designs are suitable when no manipulation of the independent variable takes place (Brink, van der Walt, *et al.*, 2012:112). Data is classified as retrospective when it is identified backwards (Aldous *et al.*, 2011:25).

Based on the aim of this research the proposed design of this study was a quantitative, descriptive (non-experimental), cross-sectional design using retrospective data from the hospital database of a private sector hospital. The results of the study reported in this mini-dissertation were based on the retrospective data collection from patients who were treated with a carbapenem at a certain point in time with no active intervention from the researcher. The study aimed to investigate the occurrence of carbapenem-resistant organisms in the selected population.

1.5.4 Setting and/or data source

1.5.4.1 Description of the setting and/or data source

The empirical investigation took place in a private hospital in Daspoort, situated in the Tshwane municipality of Gauteng in South Africa. The hospital in Daspoort is part of a large hospital group that operates in both South Africa and the United Kingdom. This group provides comprehensive healthcare services in South Africa and strives to deliver the best clinical outcomes. Antibiotic stewardship has been implemented in all their hospitals as part of their commitment to improve patient outcomes. The group uses the defined daily doses per 100 bed-days used (DDD/100BDU) model to monitor total acute inpatient antimicrobial consumption. This model is based on the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) index. The hospital in Daspoort was identified as the hospital with the highest antimicrobial DDD/100BDU within the group. The frequent prescription of carbapenems in this hospital was the major contributor to this antimicrobial consumption. Data has shown that a high existence of extended-spectrum beta-lactamase (ESBL) and carbapenem resistance is inevitable wherever there is a lack of infection control practices and antibiotic use policies. This results in a cycle of wide-spectrum antibiotic use and consequent resistance (Ulu *et al.*, 2015:224). These factors mentioned above, made the hospital in Daspoort a very relevant and important site to investigate the prevalence of carbapenem resistance.

The data source consisted of patient files (as identified according to the inclusion and exclusion criteria in this study) and the hospital-dispensing programme. The hospital-dispensing programme contains demographics such as age, gender, admission, and admission diagnosis. It also contains a record of the admission and discharge date and has a record of the antibiotics dispensed and dosages prescribed. The hospital-dispensing programme facilitated the process of patient identification and selection based on demographics such as admission date, age, gender and antibiotics dispensed.

The patient file contains the microbiology report regarding type of specimen taken, date and time that specimen was taken, and the culture results of the specimen. The microbiology report was used to determine whether the patient was infected with a carbapenem-resistant infection.

1.5.4.2 Reliability and validity of source

Various operators recorded data in real-time on the hospital's dispensing programme. An internal audit of the hospital's dispensing programme by the hospital's pharmacy manager showed an error report of less than 0.1%. Patient files were accessed retrospectively. The extraction of information from the patient files relied on the accuracy of the written record and on the filing of all microbiology data. Important data were not available in all files. A protocol evaluation of the data extraction form was done to assess the dependability and validity of the data sources.

1.5.5 Target population

The target population is the set of elements on which an investigator would like to base generalisations on (Brink, van der Walt, *et al.*, 2012:131). The target population for this investigation included all hospital in-patients admitted between 1 January 2014 and 31 December 2014 to the selected private hospital (as previously mentioned) that received treatment with a carbapenem during their stay.

1.5.6 Study population

The study population is also referred to as the 'accessible population'. However, such a population was not accessible to the researcher and a characteristic was added to the defined population. The researcher planned to generalise his/her findings to this particular population, rather than the entire population (Brink, van der Walt, *et al.*, 2012:131). The accessible population for this empirical investigation included hospital in-patients admitted during the defined study period to the private hospital in Daspoort and who received treatment with a carbapenem during their stay.

1.5.6.1 Sampling

Sampling aimed to select a group of individuals or units of analysis from the population defined above to gain information on carbapenem resistance in such a manner that it represents the population of interest (Brink, van der Walt, *et al.*, 2012:132).

The sampling approach for this investigation was a non-randomised consecutive method of sampling to select the sample that represented the study population. It is assumed that the patients were admitted to the hospital in a random manner between the two defined dates of 1 January 2014 and 31 December 2014.

1.5.6.1.1 Inclusion criteria

Criteria for eligibility were:

- Male and female patients 18 years of age or older. This age group was selected because more than 90% of patients admitted to the private hospital in Daspoort and treated with a carbapenem are 18 years and older as indicated by the statistics kept by the hospital.
- Admission to the private hospital in Daspoort between 1 January 2014 and 31 December 2014. This time period was selected based on the average number of patients treated per month for the period of 1 January to 31 March 2014 as well as the sample size needed as described later in this section. The sample was based on a recent time period of admission because of the evolving nature of resistance patterns among bacteria. It was estimated that ethical approval might be obtained by the end of 2014 and that the investigation could start early in 2015.
- Treated with a carbapenem during their stay in hospital.
- Subjects were required to have at least one microbiology report from a pathology laboratory in their file containing documented organism sensitivity and resistance data towards at least one of the carbapenems or documented evidence of a carbapenemase gene.

1.5.6.1.2 Exclusion criteria

Criteria for exclusion were:

- Pregnancy. This group was excluded because there are no adequate studies to support the use of carbapenems in pregnant women. Carbapenems are indicated only in situations where the potential benefit outweighs the possible risk to the fetus.

- Evidence of HIV-positive status. This group was excluded due to limited data available regarding the difference of resistance patterns between HIV-positive subjects and non-HIV subjects. A recent study found that antibiotic-resistant *Escherichia coli* isolates with limited virulence is able to cause infections in HIV patients but are not able to overcome the host defences of non-HIV patients to cause infection consequently (Padmavathy *et al.*, 2013:349). It was therefore hypothesized that a difference in resistance patterns will be seen between the two groups. The study was not powered to detect such a difference or the influence of the hosts' compromised immune system on the prevalence of resistance in the total study population.

The process to obtain the sample was as follows: Each patient admitted to the hospital was assigned a case number. These numbers were arranged in a consecutive, ascending manner. All data regarding dispensed medicine were recorded on the hospital dispensing system and linked back to a specific patient case number. The study population was identified by the pharmacy manager at the hospital in Daspoort based on the patient case number and carbapenems dispensed for the period 1 January to 31 December 2014. These aspects were put together in a list that was made available to the researcher. The case numbers were ordered in a numerically ascending manner by the researcher and the age of patients was verified on the hospital system to meet the inclusion criteria. From here, the patient files were accessed on the hospital premises in a private locked room based on the numerical case number list to verify whether microbiology data were present according to the inclusion criteria (Annexure 1). Eligible patients were then assigned a study identification code that de-identified the patient (Annexure 1 and 2). The researcher selected the sample from the code sheet of eligible subjects in a consecutive manner (Annexure 2) until the calculated sample size was reached. Only the patient study identification code was recorded on the data extraction to ensure the privacy and confidentiality of the subject (Annexure 3).

The report on infectious diseases kept at the study hospital showed that the prevalence of organisms resistant to carbapenems in the hospital was between three to five percent per month for the period 1 January 2014 to 31 March 2014. This data were based on microbiology organism statistics not linked to the class of antibiotic that the patient was treated with. The average number of patients aged 18 years and older treated monthly with carbapenems for the same period of time was 55. Out of this group it was estimated that approximately 50% of these patients had microbiology results as stated per the inclusion criteria. This estimation was based on statistics kept by the hospital infection control practitioner. An average of 27 patients per month could be sampled from. Calculations using the Epi Info™ 7 version 3.1.1 Statistical Calculator with an estimated population size 324 (27 patients per month multiplied by 12 months), expected resistance frequency of 5% and a confidence limit of 5%, calculated a

sample size of 120 for a confidence level of 95% (Centers for Disease Control and Prevention, GA). The North-West University's Statistical Consultation Services based on a Chi-square statistical significance test confirmed this sample size, a significance level of $\alpha \leq 0.05$ with the power of the test set to 0.8.

1.5.6.2 Patient identification

Identification of potential subjects was done retrospectively through the hospital's dispensing programme and the patient files kept at the hospital (Section 1.5.6.1 and Annexure 1). No manipulation of the independent variable took place. Data were extracted from the files onto the extraction form with no link afterwards to the individual patient. As described in the sampling section, it was assumed that the patients were admitted to the hospital in a random manner between the two defined dates of 1 January 2014 and 31 December 2014 (Section 1.5.6.1). Due to this reason it was assumed that each case had a fair chance of being selected when a non-random consecutive sampling method was used. Community involvement did not form part of the recruitment process because of the retrospective nature of this investigation.

Access to all records took place in a private locked room on the premises of the hospital based on the patient confidentiality protocols of the hospital and the company group that the hospital forms part of. An application for approval for research to be conducted at the mentioned facility in Daspoort was submitted to the company's Research Operational Committee.

1.5.7 Data collection

On completion of the recruitment process, data were extracted from each patient file using the data extraction form (Annexure 3). The development of the data extraction form was based on the variables to be measured and aimed to achieve the objectives (Joubert & Erlich, 2012:107).

Threats to validity and reliability of the data collection method were minimised through assessment by the supervisors of this mini-dissertation as well as an evaluation of the data extraction form. The test was done prior to commencement of the formal investigation to evaluate whether the correct information were to be extracted through the data extraction sheet. The investigator conducted an evaluation of the data extraction form and also collected the data during the formal investigation.

1.5.7.1 The possibility of random error

Random error can influence the precision of study results and is normally divided into random sampling error and random measurement error. Random error is probably always a factor due to its unforeseen nature (Joubert & Erlich, 2012:157). Errors that could have influenced the data

collection in this specific study might have been due the fact that the instrument did not measure what it was supposed to measure and that the instrument did not include all the components measured. To minimise this type of error, the extraction form was designed to extract data based on all the variables as listed in Table 1-2. The extraction form was tested prior to the formal start of the data collection to minimise the chance of this type of error.

1.5.7.2 Validity

Brink, van der Walt, *et al.* (2012:165) define instrument validity as a way to establish if an instrument accurately measures what it was designed for. Instrument validity can influence the meaning of the results. The four main measures of validity are content, face, criterion-related and construct validity.

Content validity is an indication of how well representative the instrument is of the variables that will be measured. This type of validity is used mainly for the development of questionnaires and interviews (Brink, van der Walt, *et al.*, 2012:166).

Face validity is based on the fact that the instrument appears at face value to measure what is expected and is therefore the weakest kind of instrument validity. Face and content validity should be done first to establish the accuracy of the data-collection instrument (Brink, van der Walt, *et al.*, 2012:166). Both the researcher and the supervisors of the mini-dissertation have assessed the data-collection instrument used for this study for face and content validity.

To maximise content validity the organisms listed in the form that have *in vitro* sensitivity against the carbapenems were based on information obtained from the literature as set out in Table 1-1 (Cunha & Cunha, 2013; Zhanel *et al.*, 2007). However, organisms could have been cultured in the study population that were not on this list. An extra field was inserted on the extraction form where unlisted organisms could be added to ensure content validity.

Criterion-related validity refers to predictive and concurrent validity and establishes a relationship between the measurements on the instrument and other external criteria. By comparing measurements of the instrument to a measure that is known to be valid, the researcher can establish whether the instrument measure is what is expected (Brink, van der Walt, *et al.*, 2012:167). One of the objectives of the literature review was to investigate the existence of a reliable, validated instrument in this study.

Predictive validity compares the research instrument results to a specific criterion that is expected in the test population in the future. It can only be used where the researcher is persuaded that the variable of concern has a clear predefined measure against which another

instrument can be verified (Brink, van der Walt, *et al.*, 2012:167). This was not applicable to the instrument used for this study.

On the other hand, concurrent validity compares the results of a new data-collection instrument to those of a criterion measure at the same point in time. The challenge is to find a criterion that is valid and reliable. This was investigated in the literature review of this study.

Construct validity is frequently used and validates the construct that the instrument is using. This is useful for feelings or traits and is used to explore the association of the instrument's results to measures of fundamental theoretical concept(s) of the instrument (Brink, van der Walt, *et al.*, 2012:168). This type of validity was not applicable to this study's instrument.

Inconsistent validity is the last concept of validity and refers to a measure that is valid for one group and not another (Joubert & Erlich, 2012:120). The validity of the instrument for this study was tested for consistency during the protocol evaluation of the data extraction form. The pharmacy manager identified a number of patient files admitted over a three-month period in 2013 where patients received a carbapenem during their stay and the extraction form was evaluated for the consistency of data collection from the different files.

1.5.7.3 Reliability

If the instrument is used repeatedly by the same person or by two researchers over time it should be reliable to yield consistent results. The reliability can be measured with a correlation measure that varies between 0 and 1, where a measure near 1 indicates a high correlation (Brink, van der Walt, *et al.*, 2012:169). Three commonly evaluated characteristics of reliability are stability, internal consistency, and equivalence reliability. Stability refers to its consistency over time and is applicable to interviewing, questionnaires, and observations (Brink, van der Walt, *et al.*, 2012:170). This test of stability was assessed during the protocol evaluation of the data extraction instrument as described earlier in this section.

Equivalence reliability tests whether different observers can obtain the same outcomes at the same time or determines whether similar tests given at the same moment will yield the same results. It is also referred to as 'inter-rater reliability' and is applicable to use in this study's measurement instrument (Brink, van der Walt, *et al.*, 2012:170). Although only the researcher conducted the data collection it was still useful to test whether the data collection tool was reliable to collect the data consistently if another data collector was to use the same tool.

Similar tests can also be used for the same patient file in this study to statistically determine the correlation between the two (Brink, van der Walt, *et al.*, 2012:171). The literature review did not find an alternative appropriate measurement tool.

1.5.8 Study variables

The following independent and dependent variables were measured:

1.5.8.1 Independent variables

The independent variables were:

- Prevalence of all carbapenem-resistant organisms in the total sample was recorded as a variable to establish the total prevalence of resistance among the sample.
- Resistance of specific organisms was measured to determine the contribution of each organism to the total prevalence.
- Resistance of organisms to a specific carbapenem (e.g. imipenem, ertapenem, meropenem and doripenem) was included as a variable to establish which carbapenem has the biggest contribution to total resistance prevalence.
- Age was chosen to establish the prevalence of resistant organisms across different ages.
- Gender was recorded to establish whether prevalence is similar across the two different gender groups.

1.5.8.2 Dependent variables

The dependent variables were:

- Prevalence of carbapenem-resistant organisms (%) in the sample.

1.5.9 Data analysis

Biostatistics forms an important part of the planning of a project (Joubert & Erlich, 2012:126). Data analysis requires careful planning and is aimed at answering the research questions (Brink, van der Walt, *et al.*, 2012:177).

1.5.9.1 Description of techniques

Factors to consider when choosing an appropriate analysis are the type of variable being analysed, how variables are distributed and the characteristic being tested (Joubert & Erlich, 2012:148). The type of variable influences the method of analysis and its scale of measurement will determine how the variable is set up in the statistical database (Joubert & Erlich, 2012:107).

Table 1-2: Scale of measurement classification

Study objective	Study variable		Type of data	Statistical significance test	Practical significance test
	Independent	Dependent			
Prevalence of resistance (%)	-	Prevalence (%)	Categorical	Frequency	-
Prevalence of resistance (%) across specific carbapenems	Specific carbapenem	Prevalence (%)	Categorical	Chi-square	Cramér's V-value
Prevalence of resistance (%) across age groups	Age group	Prevalence (%)	Categorical	Chi-square	Cramér's V-value
Prevalence of resistance (%) across gender groups	Gender	Prevalence (%)	Categorical	Chi-square	Cramér's V-value
Prevalence of resistance (%) across specific organisms	Specific organism	Prevalence (%)	Categorical	Chi-square	Cramér's V-value

Before the analysis was done, the data were carefully checked to identify any strange values or errors that might have occurred in the original data source document, during transcription or during data entry. Such errors can influence and bias results. Missing values or implausible codes for categorical data were checked. If any queries occurred from the data-checking procedure the researcher would go back to the raw data to verify (Joubert & Erlich, 2012:127). The variables of this investigation were classified according to the scale of measurement in Table 1-2.

1.5.9.2 Statistical analysis

Data analysis were performed using the SAS® programme, version 9.4 (Statistical Analysis System®) (SAS Institute Inc., 2002-2012). The data were described and summarised using descriptive statistics. Frequency distribution refers to the number of times a result occurs (Brink, van der Walt, *et al.*, 2012:180). This method of description was used to describe the prevalence of carbapenem resistance in this study. Frequency tables were used to display frequency counts for resistance to describe the prevalence. A contingency table is appropriate for the data where the chi-square test is used.

A statistician at the North-West University's was consulted regarding the statistical aspects of the dissertation.

1.5.9.3 Study limitations

Carlson and Morrison (2009:77) state that the main limitation of the cross-sectional study design is the lack of “a temporal relationship between exposure and outcome”. The reason for this is because exposure and outcome are simultaneously measured. This investigation was aimed at determining the prevalence of carbapenem-resistant organisms in patients and not to discover the cause of the resistance. The level of representativeness of a study sample is influenced by the sampling method (Joubert & Erlich, 2012:101). The sampling approach for this investigation was a non-randomised consecutive method of sampling and it was assumed that the patients were admitted to the hospital in a random manner between the two defined dates as mentioned previously. This aimed to increase the level of generalizability of the sample to the study population. How representative this sample is of the broader population in South Africa is unknown.

1.5.10 Ethical considerations

1.5.10.1 Permission/consent

Admission to the private hospital in Daspoort is subject to terms and conditions as part of the admission contract where patients acknowledge and give consent that the hospital and other third parties are allowed to process personal information for the purpose of service provision.

The National Health Act, 2004 (61 of 2003), section 16(1)(b) sets out that “a healthcare provider (which includes pharmacists) may examine a user's health records for the purpose of study, teaching or research with the authorisation of the user, head of the health establishment concerned and the relevant health research ethics committee”. Section 16(2) of the National Health Act, 2004 (61 of 2003), confirmed: “if the study, teaching or research reflects or obtains no information as to the identity of the user concerned, it is not necessary to obtain the authorisations contemplated in that subsection”. The researcher investigating the carbapenem resistance is a registered pharmacist with the South African Pharmacy Council (SAPC) and had a contractual obligation with the group regarding patient confidentiality.

An article investigated the ethical and legal implications of using retrospective data in Switzerland, where treating physicians wanted to reopen their own patient files for the purpose of data extraction for research (Junod & Elgar, 2010). It was found that this was not in breach of medical confidentiality (Junod & Elgar, 2010). The federal law allows the use of patient files for research when the balance of interest favours the data user and not the data provider, provided that results are published in a way that protects the patients' identities (Junod & Elgar, 2010).

The Protection of Personal Information Act, 2013 (4 of 2013) provides similar context to the National Health Act. Section 27 states the following: “if the processing is for historical, statistical or research purposes to the extent that (i) the purpose serves a public interest and the processing is necessary for the purpose concerned”.

Taking into account that the data in this investigation is retrospective and that the researcher is a health professional practising in terms of the Pharmacy Act, 2002 (53 of 1974) it would be ethical to argue that the patient was not at risk if reasonable precautions were taken to protect the identity and privacy of the patient. The results of the proposed research are critical to preserve existing antibiotics for the treatment of infection to avoid the so-called “post-antibiotic era”.

Ethics approval for this study was obtained from the Health Research Ethics Committee of the North-West University (Ethical number NWU-00004-15-A1).

1.5.10.2 Anonymity

Potential subjects were identified for inclusion as previously described. Each subject eligible for inclusion was assigned a number not linked to personal information and only this number was recorded on the data extraction form to ensure the privacy and confidentiality of the subject (Annexure 3). The only link to the patient is on the code sheet and the eligibility check form (Annexure 1) that is linked to the hospital case number only. Only the researcher had access to information as described under sections 1.5.6.1 and 1.5.6.2 to protect the identities of the study subjects.

1.5.10.3 Confidentiality

As described in sections 1.5.6.1 and 1.5.6.2, data extraction and analysis were not linked to the personal information of the patient. Hard copies of the eligibility form and code sheet were kept in a lockable cupboard with access by the researcher only. No electronic records of this link were kept. These records will be kept for five years and disposed of according to standard procedures as required by the hospital for any confidential information.

1.5.10.4 Respect for recruited participants and study communities

Case files were accessed retrospectively with no direct contact with the study participants and no active intervention by the researcher. Study participants and the community were not actively involved in this research. The information gained from this investigation will benefit the community through future services rendered at the hospital. If the current situation regarding carbapenem resistance is known, it can be better managed to prevent an escalation of

resistance, which can have potentially serious healthcare implications as described in the problem statement (Section 1.3).

1.5.10.5 Benefit-risk ratio

Major potential benefits were to be related back to the problem statement (Section 1.3). The potential impact of resistant organisms on healthcare and more specifically mortality rates has been described as part of the problem statement. The potential risk of resistance on morbidity and mortality, as well as the adverse effects of alternative treatment for carbapenem-resistant organisms, may be a reality for patients admitted to this hospital unless the current situation is assessed and managed accordingly. The benefits of conducting this investigation will outweigh the risks.

1.5.10.5.1 Direct benefits

- There is no immediate direct benefit for the study participants. However, patients are often readmitted to the same hospital for a similar or different indication. The results from the proposed study provided insight on the prevalence of resistance and direct appropriate action to prevent an increase or spread in resistance. Considering the consequences of resistance as described in section 1.2, lengthy ICU stays can be prevented, which will have an immediate impact on the cost of a stay in hospital as well as morbidity and mortality outcomes of these patients. Therefore, patients from whom data were collected will receive a direct benefit if they are readmitted in future.
- Patients with resistant organisms have to be isolated to prevent the spread of these pathogens to other patients. This might lead to inadequate nurse-patient staffing ratios and the unavailability of resources like equipment that has to undergo a lengthy sterilisation process once a patient is discharged before it can be used again. Increased resistance necessitates the infection control practitioner of the hospital to increase time spent on surveillance and the control of resistance outbreaks with limited time to complete essential functions (Hughes, 2008:41-6). If carbapenem resistance prevalence is known, measures can be put into place to prevent an increase in resistance and to aim to reduce resistance where applicable. This will result in a direct benefit for the hospital.

1.5.10.5.2 Indirect benefits

- Control of CPE spread can be done at hospital level with the use of rapid diagnostic techniques and the strict implementation of infection control measures. Antibiotic resistance that has spread to the community is very difficult to contain (Nordmann & Poirel, 2014:824). Knowledge of the current prevalence of resistance at hospital level will direct actions

regarding appropriate treatment and infection control to prevent spread to the community. The results from this study will have an indirect benefit to the community.

- The extent of the current burden in South Africa is unknown as previously mentioned, and this investigation can contribute to creating a more representative picture of the current situation in South Africa.

1.5.10.5.3 Risks

- This is a low-risk study due to the use of retrospective data. The patients included in this study have received services and interventions at the hospital based on standard treatment practices. Data were gathered retrospectively and the patients did not have direct contact with the researcher and no active intervention by the researcher.
- There may be a loss of privacy for these patients should the contents of the code sheet that contains the link between the hospital case number and the patient study identity number, not be stored securely. Data storage is described in section 1.5.11.6 and is aimed to ensure confidentiality and anonymity.

1.5.10.5.4 Precautions

Each patient was assigned a number that was not linked to personal information. Only this number was recorded on the data extraction form with no link to individual patients. Only hard copies of the eligibility form and code sheet are kept and have been stored at the office of Medicine Usage in South Africa (MUSA) in a locked cabinet since completion of the study as described in the following section.

1.5.10.5.5 Level of ethical risk

The level of risk was low for this investigation, due to the retrospective design and the lack of interaction between researcher and study subjects. The focus of the research was on the carbapenem prescribed and the organisms that exert resistance. Therefore, the focus was *not* on the individual patient, but rather the overall picture of resistance in the hospital. However, it was still of the utmost importance and the duty of the researcher to ensure that all the ethical considerations as described are applied and adhered to.

1.5.10.6 Data storage

Reasonable measures were taken to safeguard against unauthorised access to information.

1.5.10.6.1 During the research

Patient records were accessed in a private lockable room on the hospital premises. No electronic records of the link to the patient identity are kept. Hard copies of the eligibility-form, code sheet and the clinical data collection form were kept in a lockable cupboard in the office of the researcher with access by the researcher only. The code sheet was stored separately from the clinical data collection form. Computers used for any data analysis and processing during the research were password-protected and equipped with the necessary antivirus programmes and firewalls. Data analysis and report writing were based on the data extraction sheet only, which did not contain the link to the individual patient.

1.5.10.6.2 After completion of the research

Electronic data are stored on an external hard drive and kept in a safe in the locked office of MUSA. No electronic records of the link to the patient identity are kept. Hard copies of the eligibility-form, code sheet and the clinical data collection form are kept by MUSA in a locked cupboard for a five year period where after it will be disposed of appropriately. The code sheet is stored separately from the clinical data collection form. Destruction of records will take place under the direct supervision of MUSA's research assistant. The security and confidentiality of the records will be maintained during this process.

1.6 Chapter summary and description of the presentation of results

The first chapter provided a brief insight into carbapenem resistance and demonstrated the importance of conducting a literature review and empiric investigation into the topic. The second chapter consists of the literature review and the findings of the empirical investigation are presented in the third chapter. The final chapter is an interpretation of the results of the investigation with conclusions, recommendations and limitations for further studies.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature review in this chapter aims to elaborate on the initial introduction to the topic in chapter one. This is done through a detailed analysis of the value of the carbapenem class in the management of evolving infectious diseases, as well as the current prevalence and mechanisms of acquired organism resistance to carbapenems and also the risk factors contributing to this resistance.

The chapter starts with a brief description of the background to the problem, the evolution of bacterial pathogens and infections to provide context to the importance of the carbapenem class for the treatment of infectious diseases.

2.2 Background to the problem

Carbapenem resistance is an on-going global concern, especially amongst gram-negative pathogens, that has caused serious outbreaks and limited treatment options (Meletis, 2016:15). Recent publications, however, commented on the underreporting of resistance and the lack of repetitive data available on this topic (ECDC, 2014:2; NICD-NHLS, 2016a:17; WHO, 2014a). Therefore, this literature review and its objectives are of importance.

2.3 Evolution of bacterial pathogens and infection

Bacteria are defined as small unicellular microorganisms of the class Schizomycetes and are grouped according to its morphologically shapes e.g. cocci (spherical), bacilli (rod-shaped) or spirochetes (spirals) (Mosby's dictionary of medicine, nursing & health professions, 2013:175). Bacteria were observed and described for the first time around 1680 by Antony van Leeuwenhoek (Porter, 1976:265). During the 19th century, respiratory tract infections, diarrhoea and diphtheria were the leading cause of fatality and it only became possible late in the same century to correlate bacteria with the development of numerous diseases (Zaffiri *et al.*, 2012:67). The association between specific microorganisms (pathogens) and infectious disease only became important for medicine and public health at this point (Heggenhougen & Quah, 2008:282).

Hans Christian Gram classified bacteria in 1882 as either gram-positive or gram-negative based on the colour of the bacterial cell wall after stains have been administered (Heggenhougen & Quah, 2008:273; Beveridge, 1999:4725). The main clinical difference between these two classes is that gram-negative organisms are able to produce an endotoxin that can lead to tissue necrosis, shock and fatality. Further differences between these two classes are their

susceptibilities to antibiotics (Heggenhougen & Quah, 2008:273). Bacteria are in addition classified according to the organisms' growth responses to oxygen or the lack thereof. Obligate aerobes imply that these organisms always require oxygen to grow (Porter & Kaplan, 2011:1180). Facultative organisms have the ability to grow with or without oxygen whereas obligate anaerobes require the absence of oxygen to grow (Heggenhougen & Quah, 2008:273; Mosby's dictionary of medicine, nursing & health professions, 2013:1254). Bacteria that are not classified as gram-positive or gram-negative include the spirochetes, *Mycobacteria* and *Rickettsia* species. *Rickettsia* spp. and spirochetes do not take up the Gram stain. *Mycobacteria* can be identified with a special stain, the Ziehl-Neelsen (acid-fast) stain (Heggenhougen & Quah, 2008:273; Porter & Kaplan, 2011:1181). Although the presence of organisms has been recognised for centuries, the first complete list of human pathogen species was only published in 2001 (Woolhouse & Gaunt, 2007:231). Current classification of common pathogenic bacteria is listed in Table 2-1 (Porter & Kaplan, 2011:1181; Schlecht & Bruno, 2015).

Table 2-1: Classification of common pathogenic bacteria

Type	Bacteria
Obligate aerobic	
Gram-negative cocci	<i>Moraxella catarrhalis</i> , <i>Neisseria gonorrhoeae</i> , <i>N. meningitides</i>
Gram-positive bacilli	<i>Corynebacterium jeikeium</i>
Acid-fast bacilli	<i>Mycobacterium avium</i> complex, <i>M. kansasii</i> , <i>M. leprae</i> , <i>M. tuberculosis</i> , <i>Nocardia</i> spp.
Non-fermentative, non-Enterobacteriaceae	<i>Acinetobacter calcoaceticus</i> , <i>Elizabethkingia meningoseptica</i> , <i>Pseudomonas aeruginosa</i> , <i>P. alcaligenes</i> , other <i>Pseudomonas</i> spp., <i>Stenotrophomonas maltophilia</i>
Fastidious gram-negative coccobacilli and bacilli	<i>Brucella</i> , <i>Bordetella</i> , <i>Francisella</i> and <i>Legionella</i> spp.
Treponemataceae (spiral bacteria)	<i>Leptospira</i> spp.
Obligate anaerobic	
Gram-negative bacilli	<i>Bacteroides fragilis</i> , other <i>Bacteroides</i> spp., <i>Fusobacterium</i> spp., <i>Prevotella</i> spp.
Gram-negative cocci	<i>Veillonella</i> spp.
Gram-positive cocci	<i>Peptococcus niger</i> , <i>Peptostreptococcus</i> spp.
Non-spore-forming gram-positive bacilli	<i>Actinomyces</i> , <i>Bifidobacterium</i> , <i>Eubacterium</i> , and <i>Propionibacterium</i> spp.
Endospore-forming gram-positive bacilli	<i>Clostridium botulinum</i> , <i>C. perfringens</i> , <i>C. tetani</i> , other <i>Clostridium</i> spp.

Type	Bacteria
Facultative anaerobic	
Gram-positive cocci, catalase-positive	<i>Staphylococcus aureus</i> (coagulase-positive), <i>S. epidermidis</i> (coagulase-negative), other coagulase-negative staphylococci
Gram-positive cocci, catalase-negative	<i>Enterococcus faecalis</i> , <i>E. faecium</i> , <i>Streptococcus agalactiae</i> (group B streptococcus), <i>S. bovis</i> , <i>S. pneumoniae</i> , <i>S. pyogenes</i> (group A streptococcus), viridans group streptococci (<i>S. mutans</i> , <i>S. mitis</i> , <i>S. salivarius</i> , <i>S. sanguis</i>), <i>S. anginosus</i> group (<i>S. anginosus</i> , <i>S. milleri</i> , <i>S. constellatus</i>), <i>Gemella morbillorum</i>
Gram-positive bacilli	<i>Bacillus anthracis</i> , <i>Erysipelothrix rhusiopathiae</i> , <i>Gardnerella vaginalis</i> (gram-variable)
Gram-negative bacilli	Enterobacteriaceae (<i>Citrobacter</i> spp., <i>Enterobacter aerogenes</i> , <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Morganella morganii</i> , <i>Proteus</i> spp., <i>Plesiomonas shigelloides</i> , <i>Providencia rettgeri</i> , <i>Salmonella typhi</i> , other <i>Salmonella</i> spp., <i>Serratia marcescens</i> , <i>Shigella</i> spp., <i>Yersinia enterocolitica</i> , <i>Y. pestis</i>)
Fermentative, non-Enterobacteriaceae	<i>Aeromonas hydrophila</i> , <i>Chromobacterium violaceum</i> , <i>Pasteurella multocida</i>
Fastidious gram-negative coccobacilli and bacilli	<i>Actinobacillus actinomycetemcomitans</i> , <i>Bartonella bacilliformis</i> , <i>B. henselae</i> , <i>B. quintana</i> , <i>Eikenella corrodens</i> , <i>Haemophilus influenzae</i> , other <i>Haemophilus</i> spp.
Mycoplasma	<i>Mycoplasma pneumoniae</i>
Treponemataceae (spiral bacteria)	<i>Borrelia burgdorferi</i> , <i>Treponema pallidum</i>
Micro-aerophilic	
Curved bacilli	<i>Campylobacter jejuni</i> , <i>Helicobacter pylori</i> , <i>Vibrio cholerae</i> , <i>V. vulnificus</i>
Obligate intracellular parasitic	
Chlamydiaceae	<i>Chlamydia trachomatis</i> , <i>Chlamydophila pneumoniae</i> , <i>C. psittaci</i>
Coxiellaceae	<i>Coxiella burnetii</i>
Rickettsiales	<i>Rickettsia prowazekii</i> , <i>R. rickettsii</i> , <i>R. typhi</i> , <i>R. tsutsugamushi</i> , <i>Ehrlichia chaffeensis</i> , <i>Anaplasma phagocytophilum</i>

Infectious pathogens are evolutionarily dynamic, resulting in an ever-changing and continuously growing list of diseases (Fauci & Morens, 2012:455). Minor changes in the dynamism of bacteria have occurred over numerous years. However, the rise in the frequency of infectious diseases and the new challenges associated with this phenomenon can easily be linked to the period between 1939 and 1949, the same time at which sulphonamides and penicillin became available (Weinstein, 1985:S538). An initial ever-increasing number of antimicrobial agents posed a solution to many problems from the pre-biotic era. This has led to an increase in challenges compared to those in the past (Weinstein, 1985:S540). Many bacteria were initially

highly susceptible to the majority of antibiotics that became available about 40 years ago. Susceptible strains of these organisms were gradually replaced by resistant strains as a consequence of persistent exposure to these antimicrobial agents (Saga & Yamaguchi, 2009:103; Weinstein, 1985:S542).

Infections caused by gram-negative bacteria such as *Enterobacter cloacae*, *Pseudomonas* spp. and *Klebsiella oxytoca* were infrequent in the pre-antibiotic era compared to the present and not considered a very important problem (Weinstein, 1985:S540). The only alarming factor associated with infections caused by gram-negative organisms previously was the very high mortality rate. Gram-negative bacteria were also infrequently associated with nosocomial infections (Weinstein, 1985:S539).

Pseudomonas aeruginosa, *Serratia marcescens* and gram-negative bacilli were not considered pathogenic prior to the availability of antimicrobial agents (Ventola, 2015:281; Weinstein, 1985:S539). Data suggest that pathogenic bacteria evolved from related non-pathogenic organisms through the acquisition of genetic material that encode for virulence, rather than through a slow adaptive process of existing genetic material. Essential virulence factors can be disseminated to other bacteria through conjugation, alteration and transduction (De Souza, 2003:27).

Bacteria are currently linked to a continuously growing list of diseases where resistance is an increasing challenge (Fauci & Morens, 2012:455). It is clear from the literature that bacteria have evolved from being unrecognised as the leading cause of many fatal diseases to becoming a growing threat as pathogens become more resistant through continued exposure to antibiotics. This has had an impact on the need for development of the different antibiotic classes.

2.4 The value of the carbapenem class in the treatment of infectious diseases

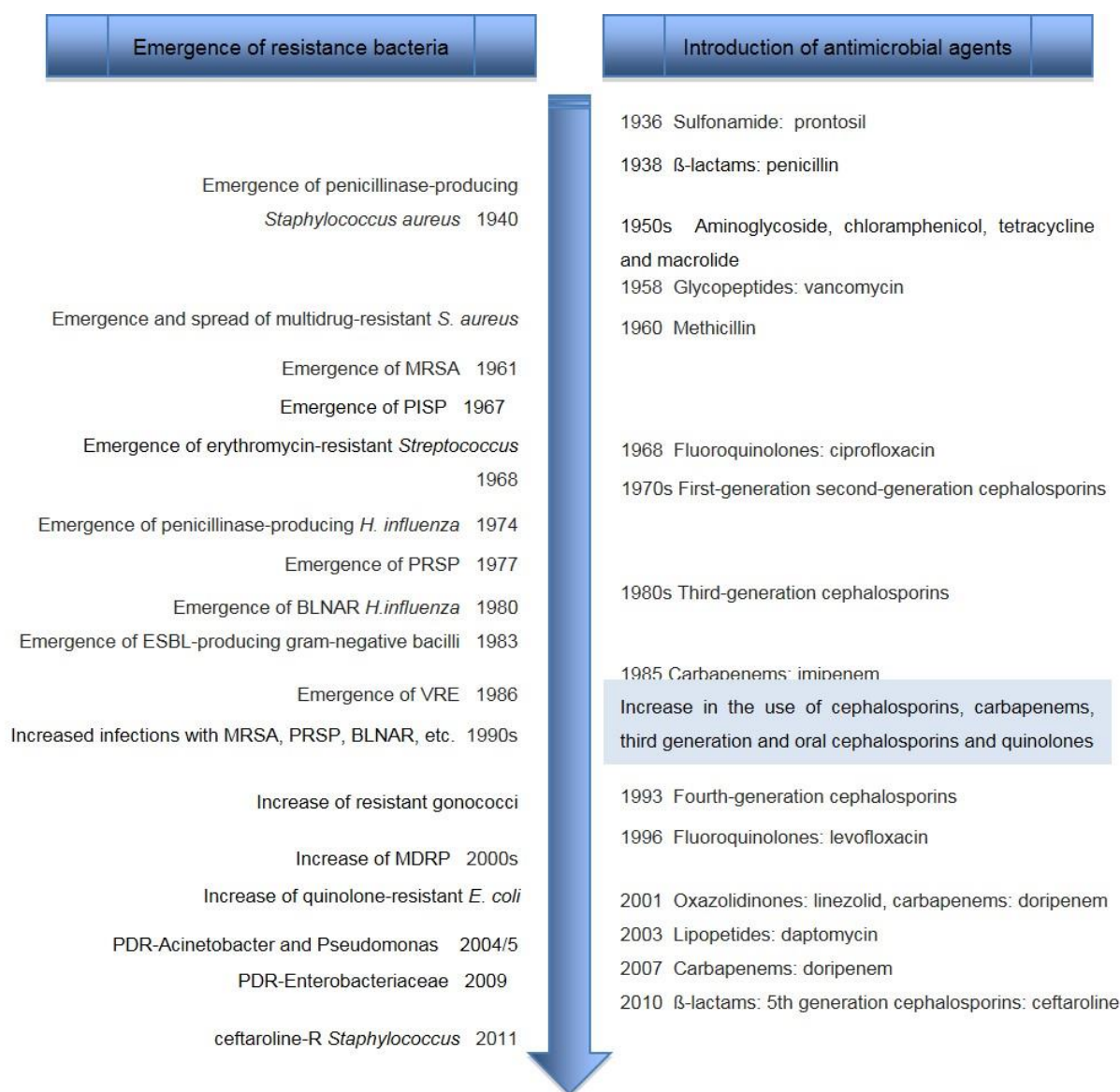
The next section demonstrates the need for the development of the carbapenem class through the chronological illustration of the development of the different antibiotic classes in the context of the global burden of infectious diseases.

2.4.1 History to the development of the carbapenem class

The introduction of the first antimicrobials had a significant impact on the treatment of infectious disease and hence, morbidity and mortality (Zaffiri *et al.*, 2012:67). The 40-year period that followed the discovery of antibiotics in the 1930s and 1940s was characterized by the discovery of new antibiotic classes, modification of existing antibiotics and synthetic chemicals that were

constantly tailored to combat emerging antimicrobial resistance by improving the clinical qualities (Asokan & Kasimanickam, 2013).

The literature demonstrates that the respective classes of antimicrobial agents were developed chronologically (Figure 2-1) to achieve an expanded antimicrobial spectrum and an increased antimicrobial activity (CDC, 2013:28; CDDEP, 2015:51; Lewis, 2013:375; Saga & Yamaguchi, 2009:104; Zaffiri *et al.*, 2013:172). Concurrently, microorganisms developed resistance to antibiotics leading to certain antibiotics previously effective to no longer be of value, necessitating the need for new development (Saga & Yamaguchi, 2009:107).



β -lactamase-negative ampicillin-resistant (BLNAR); extended spectrum β -lactamases (ESBL); methicillin-resistant *S. aureus* (MRSA); multidrug resistant *P. aeruginosa* (MDRP); pan drug-resistant (PDR); penicillin-intermediate *S. pneumoniae* (PISP); penicillin-resistant *S. pneumoniae* (PRSP); vancomycin-resistant enterococci (VRE)

Figure 2.1: Timeline of bacterial resistance and consequent antimicrobial development

The first antimicrobial agent, salvarsan was synthesized in 1910 and used as a remedy for syphilis. Sulfonamides were developed in 1935, but had constraints due to safety and efficacy concerns (Saga & Yamaguchi, 2009:104). Some bacteria rapidly developed resistance towards these drugs (Wright *et al.*, 2014:8845).

Penicillin was already discovered in 1928, but only came into clinical use in the 1940s (Saga & Yamaguchi, 2009:104). It is classified as a beta-lactam antibiotic because it contains a β -lactam ring in its molecular structure (Lakshmi *et al.*, 2014:37). The emergence of resistance has played a major role in the expansion of the penicillin class. The main mechanism of resistance to β -lactam antibiotics is due to the cleavage of the β -lactam ring by a collection of bacterial enzymes, namely the β -lactamases (Zaffiri *et al.*, 2012:71).

The cephalosporins were the second beta-lactam antibiotics class to be developed. This class was discovered in 1945 following observations that certain compounds suppressed the proliferation of specific gram-negative organisms implicated in typhoid fever (Zaffiri *et al.*, 2012:73).

Gram-negative pathogens were most often identified as the cause of hospital-acquired infection between 1960 and 1980 (Zaffiri *et al.*, 2013:168). Second generation penicillins like ampicillin and amoxicillin were introduced in 1961 and 1972 respectively to achieve better coverage against gram-negative bacteria (Kaushik *et al.*, 2014:ME01; Zaffiri *et al.*, 2012:71). The next generation of penicillins include carbenicillin, ticarcillin and piperacillin. These penicillins have an extended-spectrum of activity from gram-positive cocci to anaerobes. In addition, these drugs are able to resist the beta-lactamases produced by organisms like the *Enterobacter* spp. and *Pseudomonas aeruginosa* (Zaffiri *et al.*, 2012:71). The combination of piperacillin and tazobactam was introduced in 1995 and has high efficacy against gram-positive and gram-negative aerobic bacteria, which includes *Streptococcus pneumoniae* and most strains of *Enterococcus faecalis*. It has no activity against methicillin-resistant staphylococci and the main benefit of this combination is its efficacy against gram-negative bacilli like *Klebsiella* spp. and *Enterobacter* spp. and *Escherichia coli*. The combination is also active against *Pseudomonas aeruginosa* (Perry & Markham, 1999:807; Zaffiri *et al.*, 2012:71).

The emergence of multidrug resistance complicated the management of gram-negative bacteria. Resistant organisms such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. have necessitated the development of new antimicrobials with intrinsic resistance to beta-lactamases or with different mechanisms of action (Zaffiri *et al.*, 2013:167).

The quinolone class of antibiotics was introduced in the 1960s. The first generation fluoroquinolones are mainly active against gram-negative like Enterobacteriaceae, *Haemophilus*

influenzae and some gram-positive bacteria. It has good activity in the urinary tract (Appelbaum & Hunter, 2000:12). The activity of the second generation was enhanced to be active against gram-positive bacteria, aerobic gram-negative bacteria and atypical organisms. It has improved tissue penetration compared to the first generation, which enhances its clinical efficacy for the management of infections of the respiratory system, gastrointestinal tract, bone, skin, soft tissue and genitourinary tract (Dougherty & Pucci, 2012:127; Zaffiri *et al.*, 2013:170). The third- and fourth-generation quinolones' activity was improved to include efficacy against aerobic gram-positive bacteria and penicillin-resistant pneumococci with only a moderate activity against anaerobes (Appelbaum & Hunter, 2000:5; Dougherty & Pucci, 2012:127). The fourth generation retained the inherent activity of the class against aerobic gram-negative bacteria with most of the activity of the fourth-generation quinolones against pneumococci and anaerobes. Its spectrum of activity is broader than that of the third generation and its cover includes atypical pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA), streptococci and anaerobes (Zaffiri *et al.*, 2013:170). However, accumulating resistance to the quinolones by organisms like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycoplasma pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus pneumonia* has limited the classes' use (Zaffiri *et al.*, 2013:171).

The development of carbapenems in the 1960s was in reaction to the dramatic increase in resistance against the widely used β -lactam class (Zaffiri *et al.*, 2013:171). The most frequent mechanism of resistance seen in both gram-positive and gram-negative pathogens was the production of bacterial β -lactamases (Bush, 2013:86; Zaffiri *et al.*, 2013:171). The first β -lactamase inhibitors, olivanic acids, were developed in 1976. Olivanic acids possess a "carbapenem backbone" but development was not continued due to chemical instability and poor penetration into the bacterial cell (Papp-Wallace *et al.*, 2011:4944). Two superior β -lactamase inhibitors, clavulanic acid and thienamycin, were discovered shortly thereafter (Zhanel *et al.*, 2007:1028). Thienamycin is seen as the model compound for all carbapenems and exhibited inhibitory microbiological activity against gram-positive cocci, *Pseudomonas aeruginosa* and anaerobes such as *Bacteroides fragilis*. However, chemical instability of thienamycin again necessitated a need for further development of more stable carbapenems (Papp-Wallace *et al.*, 2011:4943; Wright *et al.*, 2014:8854). The constant evolution of cephalosporin-resistant pathogens emphasised the future value of carbapenems developed from thienamycin (Papp-Wallace *et al.*, 2011:4944; Wright *et al.*, 2014:8855).

In 1985, imipenem — a more-stable derivative of thienamycin — was the first carbapenem available for the management of complicated microbial infections. The combination of imipenem with cilastatin was necessary due to its susceptibility to dehydropeptidase-I (DHP-I), an enzyme produced in the kidneys that rendered the antibiotic inactive (Papp-Wallace *et al.*, 2011:4944;

Zhanel *et al.*, 2007:1029). A journey that has led to the discovery of carbapenems with increased stability has resulted in the broader-spectrum carbapenems like meropenem, ertapenem, and doripenem that are currently available (Papp-Wallace *et al.*, 2011:4944).

Carbapenems have an extended antimicrobial spectrum *in vitro* compared to the rest of the β -lactam class (Papp-Wallace *et al.*, 2011:4946; Zhanel *et al.*, 2007:1028). Carbapenems can be classified into one of two groups. The first group includes ertapenem that has limited efficacy against *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Stenotrophomonas maltophilia*. The second group includes imipenem, meropenem, and doripenem with activity against these bacilli (Zaffiri *et al.*, 2013:171).

The *in vitro* activity of imipenem includes most aerobic and anaerobic gram-negative as well as gram-positive bacteria. It is a potent growth inhibitor of the majority of Enterobacteriaceae spp., *Pseudomonas aeruginosa* and the *Enterococcus* spp. (Pastel, 1986:719; Zaffiri *et al.*, 2013:171).

Meropenem is more effective than imipenem against gram-negative bacteria including Enterobacteriaceae, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, *Burkholderia cepacia* and *Neisseria meningitidis* (Holliday & Benfield, 1998:365; Pfaller & Jones, 1997:161). Meropenem has been shown to be effective against Enterobacteriaceae spp. resistant to cephalosporins, piperacillin, amikacin, gentamicin and ciprofloxacin (Holliday & Benfield, 1998:365). This broad range and potent bactericidal activity contributed to meropenem being the preferred treatment for nosocomial infections caused by resistant organisms (Zaffiri *et al.*, 2013:171).

The spectrum of activity of doripenem is comparable to that of meropenem and imipenem. It has *in vitro* activity against vancomycin-resistant enterococci (VRE), extended-spectrum β -lactamase-producing bacteria and common anaerobic bacteria like *Bacteroides fragilis* (Matthews & Lancaster, 2009:45; Zaffiri *et al.*, 2013:172). Doripenem is suitable for the treatment of complicated intra-abdominal infections because of its penetration into several body fluids and tissues like peritoneal fluid, bile as well as its activity against *Bacteroides fragilis*, which is often implicated in these infections. It also penetrates well into the urine making it suitable for complicated urinary tract infections (Matthews & Lancaster, 2009:44; Zaffiri *et al.*, 2013:172).

Ertapenem is active against gram-positive and gram-negative aerobes and anaerobes. It exhibits resistance to extended spectrum β -lactamases, including AmpC cephalosporinases, a class C β -lactamases. However, it has limited activity against *Enterococcus* spp. and non-fermentative gram-negative bacteria like *Pseudomonas aeruginosa*. It is indicated mostly for the

management of community-acquired infections due to its limited activity compared to meropenem (Hawkey & Livermore, 2012; Zaffiri *et al.*, 2013:171; Zhanel *et al.*, 2005:23).

The growing resistance of bacteria has compelled the development of new antibiotics (Zaffiri *et al.*, 2013:172). Vancomycin, the first glycopeptide developed in 1956 was another example of this trend (Finch & Eliopoulos, 2005:ii5; Zaffiri *et al.*, 2013:172). Glycopeptides are classified as first-generation or second-generation. The first generation includes vancomycin and teicoplanin whilst the second-generation consists of oritavancin, dalbavancin and telavancin. This class is used mainly for infections caused by gram-positive bacteria (Zaffiri *et al.*, 2013:172).

The oxazolidinone class currently consists of only one compound, namely linezolid. Linezolid has *in vitro* activity against gram-positive bacteria, which include VRE and MRSA. Other agents belonging to this class are currently being developed (Zaffiri *et al.*, 2013:174).

Daptomycin forms part of the lipopeptide class with activity against aerobic and anaerobic gram-positive organisms. Part of the value of daptomycin is that it has activity against gram-positive pathogens, like MRSA, glycopeptide-intermediate *Staphylococcus aureus* (GISA) and VRE (Carpenter & Chambers, 2004:997; Zaffiri *et al.*, 2013:175). Other examples of its spectrum of cover include the anaerobic strains like *Clostridium* and *Propionibacterium* spp., *Corynebacterium* and certain bacillus species. However, daptomycin has no activity against gram-negative bacteria due of the lack of penetration into the outer membrane of these bacteria (Zaffiri *et al.*, 2013:175).

The continuous development of new classes of antibiotics has been the strategy to combat resistant bacteria, but has effectively come to a halt due to economic and regulatory obstacles (Ventola, 2015:279). Developing antibiotics to treat highly resistant bacterial infections remains challenging, since the number of patients that contract these infections is small and does not necessarily meet the requirements to participate in clinical trials. Approximately 36 new antibiotics designed to treat complicated bacterial infections are currently in clinical development. Only 11 out of the 36 antibiotics are expected to have activity against gram-negative organisms. One in five of these compounds are expected to reach phase three development (Paris, 2015). Gram-negative hospital pathogens are of particular concern due to its efflux-mediated resistance that vitiates a broad spectrum of structural classes (Payne *et al.*, 2007:39).

The above-mentioned factors have contributed to carbapenems being the sole option for the management of resistant gram-negative pathogens considered for the past two decades. This is especially true for those with extended-spectrum beta-lactamase (ESBL) resistance mechanisms (Zaffiri *et al.*, 2013:172). Studies that compared meropenem/metronidazole

therapy to the newly US FDA registered ceftazidime/avibactam in patients with complicated intra-abdominal infections, found similar efficacy rates in ceftazidime non-susceptible isolates where the main mechanism of ceftazidime resistance was ESBL production (Goodlet *et al.*, 2016:1820).

2.4.2 The global burden of clinically relevant bacterial infectious diseases and antimicrobial resistant organisms

Infectious diseases represent a global burden (Leprince *et al.*, 2015:175). This section provides information on skin or skin structure and lower respiratory tract infections (LRTIs) that are ranked at the top of infectious diseases in terms of mortality and morbidity (Leprince *et al.*, 2015:175). Further highlighted in the section are the important pathogens that are linked to antimicrobial resistance (AMR) and increased mortality.

2.4.2.1 Skin or skin structure infections

Complicated skin and soft tissue infections (cSSTIs) can lead to disability or result in fatality if not treated effectively (Leprince *et al.*, 2015:175). *Staphylococcus aureus* was identified as the most frequent pathogen (40.6%) in patients with cSSTIs, followed by the Enterobacteriaceae spp. (29.1%) and lastly, β -haemolytic streptococci (group A and B, 7.1%) (Leprince *et al.*, 2015:178). Gram-negative bacteria that are often cultured in cSSTI infections include the Enterobacteriaceae and *Pseudomonas aeruginosa*. Other relatively common pathogens seen in cSSTIs include anaerobic bacteria like *Bacteroides fragilis*, *Clostridium* spp. and *Peptostreptococcus* spp. Many infections of the skin or skin structures are polymicrobial due to the mixture of aerobic and anaerobic pathogens (Fish, 2006:403).

A recent study that examined economic outcomes in cSSTIs demonstrated that mixed infections with gram-positive and negative pathogens results in a longer length of hospital stay compared to those with gram-positive pathogens (Lipsky *et al.*, 2014:266). Gram-negative bacteria are associated with an increased length of stay despite the fact that gram-positive bacteria are the most common cause of cSSTIs infections (Lipsky *et al.*, 2014:271).

2.4.2.2 Lower respiratory tract infections

A recent nationwide observational study in France found that the distribution of the respiratory pathogens comprised of *Streptococcus pneumoniae* (45.5%), *Haemophilus influenzae* (15.2%), *Staphylococcus aureus* (12.6%), Enterobacteriaceae (12.2%) and *Branhamella catarrhalis* (4.5%) (Leprince *et al.*, 2015:178).

The European REACH study (Retrospective Study to Assess the Clinical Management of Patients With Moderate-to-Severe Complicated Skin and cSSTI or CAP in the Hospital Setting) included patients from 10 countries with community-acquired pneumonia (CAP) (Blasi *et al.*, 2013). *Streptococcus pneumoniae* was ranked as the number one (39.2%) cause of CAP. Enterobacteriaceae and MRSA ranked second and third, respectively and *Pseudomonas aeruginosa* ranked fourth (Blasi *et al.*, 2013; Leprince *et al.*, 2015:177). Another recent review confirmed these findings where *Streptococcus pneumoniae* was most often implicated as the cause of CAP across the different studies *aeruginosa* (Torres *et al.*, 2014:1065). Other bacteria frequently implicated in CAP include *Haemophilus influenzae*, *Mycoplasma pneumoniae* and *Pseudomonas aeruginosa* (Torres *et al.*, 2014:1074).

According to the World Health Organization (WHO), LRTIs are globally the number one cause of mortality with a reported 3.19 million deaths per annum in 2015 and rank third as the reason for all-cause mortality (WHO, 2017b). The Global Burden of Disease Study demonstrated that LRTI continues to be the second biggest contributor to mortality (Murray & Lopez, 2013:451; Prina *et al.*, 2015:1098). The study showed that the age-standardised mortality rate was 41.7 (95% CI, 37.1 - 44.1) per 100 000 population. The short-term mortality of patients with LRTI in an intensive care unit (ICU) was as high as 50% with the average rate in hospitalised patients ranging between 4% and 18%. The incidence of pneumonia is around 1.5 to 14 cases per 1000 person-years and costs related to this disease are high (Prina *et al.*, 2015:1098).

2.4.2.3 Bloodstream infections

Patients with bloodstream infections due to antibiotic-resistant gram-negative bacteria had higher fatality rates in comparison to similar patients with susceptible isolates. Rates of mortality range from 18.9% to 48.0% with carbapenem-resistant Enterobacteriaceae (CRE) infections and may also be associated with significant confounding comorbidity (Harris, Paterson, *et al.*, 2015:244).

The true burden of bacterial infection in South Africa is incompletely documented due to the level of empiric antibiotic prescriptions and an overall paucity of samples sent for laboratory bacterial identification (Mendelson & Matsoso, 2015).

2.4.2.4 Most important pathogens linked to invasive infections and resistant organisms

The European Antimicrobial Resistance Surveillance Network (EARS-Net) identified eight important pathogens in response to the growing threat of AMR in Europe as the cause of invasive infections. These pathogens include *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. (EARS-Net, 2015).

2.4.3 Use of carbapenems in clinically relevant diseases and antimicrobial resistant organisms

The literature confirms that carbapenems remain important for the management of clinically relevant diseases and infections due to resistant organisms, either as monotherapy or as part of a carbapenem-containing combination (Bassetti *et al.*, 2016:366). Carbapenems have already proven in the 1990s to be of value in hospital units where third-generation cephalosporin resistance were present, in patients previously treated with antibiotics and in polymicrobial infections (Bradley *et al.*, 1999:100).

2.4.3.1 Complicated skin and skin structure infections

A review identified aerobic gram-positive bacteria as the causative organism in 57% of cases, followed by aerobic gram-negative pathogens and anaerobes in 26% and 17% of cases (Fish, 2006:409). *Staphylococcus aureus* was the gram-positive pathogen most often isolated and *Pseudomonas aeruginosa* was the most common gram-negative organism seen in especially more serious and chronic infections (Itani *et al.*, 2011:43).

Polymicrobial infections were found in 38% of patients prior to the initiation of treatment (Fish, 2006:407). Thus, empiric antibiotic regimens should include activity against both gram-positive and gram-negative bacteria, with anaerobic cover when needed (Fish, 2006:404). Carbapenems are recommended for the empiric treatment of specific types of cSSTIs especially those likely to involve mixed and/or multidrug-resistant pathogens (Fish, 2006:403).

2.4.3.2 Lower respiratory tract infections

An investigation showed that carbapenems were frequently used for the management of community-acquired and healthcare-associated pneumonia, where meropenem and doripenem were used in 47% and in 26% of patients respectively (Kamata *et al.*, 2015:598).

Practice guidelines for healthcare-associated pneumonia recommend carbapenems as an option for the empiric treatment of in-hospital patients in whom risk factors for resistant pathogens are present (Kohno *et al.*, 2013:117). Carbapenems play an important role in the treatment of nosocomial acquired pneumonia, severe CAP, as well as acute infections in patients with chronic obstructive pulmonary disease (COPD) (Bradley *et al.*, 1999:100). The target of the treatment is against these resistant pathogens as well as microorganisms like *Pseudomonas aeruginosa* and *Acinetobacter*. Extended-spectrum beta-lactamase producing *Klebsiella* spp. are a key group of pathogens that has a higher prevalence in patients with healthcare acquired pneumonia with carbapenems being the only β -lactam that is effective against these organisms (Kohno *et al.*, 2013:117).

2.4.3.3 Most important pathogens linked to invasive infections and resistance

Table 1-1 confirms the *in vitro* activity of the respective carbapenems against the eight clinically important pathogens. Carbapenems are usually reserved as the “last-line” treatment for infections due to resistant Enterobacteriaceae, including those producing ESBLs. Extended spectrum β -lactamases confer resistance to several β -lactams like the penicillins, cephalosporins and the monobactam, aztreonam (Morris *et al.*, 2015). A further benefit of the class is its inherent resistance to hydrolysis by ESBLs and AmpC, a class C β -lactamases. The class has a clear defined role as first-line empirical therapy and as definitive treatment in a variety of serious infections (Bradley *et al.*, 1999:100).

2.4.4 Infection threat with the elimination of the carbapenem class

Pathogens that are resistant to carbapenems exhibit cross-resistance to other β -lactam antibiotics, quinolones and aminoglycosides resulting in limited or no effective therapeutic options (Harris, Paterson, *et al.*, 2015:245; Xu *et al.*, 2015:377). The rate of death amongst patients with bacteraemia caused by CRE was 2-fold higher compared to patients with bacteraemia caused by carbapenem-sensitive Enterobacteriaceae (CSE) (Falagas, Tansarli, *et al.*, 2014:1173). Few treatment options for multidrug-resistant pathogens exist. Mortality rates in patients with CRE vary from 18% to 60% depending on therapy (Morris *et al.*, 2015). A systematic review found that patients on a tigecycline-gentamicin had a mortality rate of up to 50% compared to 67% for a carbapenem-colistin combination. The mortality for monotherapy-treated patients was up to 57% for colistin and up to 80% for tigecycline (Falagas, Lourida, *et al.*, 2014:654). Patients receiving a carbapenem-containing regimen showed a lower mortality rate (12%) compared to a non-carbapenem regimen (41%). It is postulated that pharmacologic limitations of available treatment options contribute to this high mortality rate (Morris *et al.*, 2015).

Infections due to multidrug resistant bacteria are a daily challenge to infectious diseases physicians and the patients they treat. Research to combat multidrug resistant pathogens has focused mainly on gram-positive pathogens in the past and several novel antimicrobial agents were developed as a consequence of this. However, the increasing problem of multidrug resistance in gram-negative bacteria has not seen the same effort in the development of new treatment. This has resulted in an increased number of diseases caused by gram-negative organisms with no adequate treatment options (Souli *et al.*, 2008).

2.5 Risk factors for acquired carbapenem resistance

Insight into the especially modifiable risk factors linked to acquired carbapenem resistance, is important to aid the preservation of the carbapenem class and the spread of resistant organisms.

The literature showed that risk factors for carbapenem-resistant gram-negative bacteraemia due to *Acinetobacter baumannii* and *Pseudomonas aeruginosa* included an increased symptom severity with admission, additional intravascular devices, history of diabetes mellitus and renal impairment (Routsis *et al.*, 2013:1255). An increased exposure to the majority of classes of antimicrobial agents also contributes to gram-negative bacteraemia and thereby increasing the risk for carbapenem resistance (Akinci *et al.*, 2005:320). The development of ventilator-associated pneumonia (VAP) and the use of additional intravascular devices are independent risk factors for the acquisition of carbapenem resistance. A significant relationship was found between the duration of exposure to carbapenems, colistin and acquired carbapenem resistance in gram-negative isolates (Ling *et al.*, 2015; Routsis *et al.*, 2013:1255).

A meta-analysis showed that nine risk factors are linked to carbapenem-resistant *Pseudomonas aeruginosa* (Voor In 't Holt *et al.*, 2014:2629). The risk factors include in order of statistical significance; the administration of a carbapenem, use of medical apparatus, use of other antibiotic classes, admission to ICU, quinolone use, concomitant diseases, vancomycin use, patient characteristics and duration of stay (Voor In 't Holt *et al.*, 2014:2631). Colonisation with imipenem-resistant gram-negative bacteria is more likely where a carbapenem was used previously, even in the case of a brief exposure (Harris, Tambyah *et al.*, 2015:475; Ling *et al.*, 2015).

A retrospective analysis found that there was a strong correlation between meropenem use and carbapenem-resistant *Klebsiella pneumonia* (CRKP) (Ulu *et al.*, 2015:220). Other risk factors identified in this analysis include third-generation cephalosporin use, immunosuppression, presence of a nasogastric catheter, peripheral arterial catheter and being admitted to the neurosurgical unit (Ulu *et al.*, 2015:219). Another retrospective study further detected older age as an independent risk factor for CRKP-induced deaths (Jiao *et al.*, 2015:71). Exposure to cefoperazone plus sulbactam, glycopeptides, concomitant disease (renal insufficiency) and tracheostomy were other independent risk factors for colonisation or infection with CRKP (Jiao *et al.*, 2015:72).

Common risk factors associated with CRE include patients requiring long-term care, admission to an intensive care unit, use of indwelling devices and antibiotic exposure (Perez & Van Duin, 2013:228).

Brink, Coetzee, Clay, Corcoran, *et al.* (2012:600) reviewed evidence on the risk factors for the acquisition of carbapenem resistance. The article refers to a publication on case reports in patients admitted to private hospitals, where it was observed that these patients had typical risk factors associated with carbapenemases producing bacteria (Brink, Coetzee, Clay, Corcoran, *et al.*, 2012:601). Risk factors mentioned were prolonged hospitalisation, ICU stay, invasive devices and therapy with multiple antibiotics. The case reports however, were not designed to investigate the risk factors associated with carbapenem resistance (Brink, Coetzee, Clay, Sithole, *et al.*, 2012:526). The other South African publications referenced in this article contained no information on risk factors (Bamford, Bonorchis, *et al.*, 2011; Brink, Botha, *et al.*, 2012; Brink, Coetzee, Clay, Corcoran *et al.*, 2012; Brink, Coetzee, Clay, Sithole, *et al.*, 2012:526).

The available evidence discussed in this section shows that the most common risk factors for carbapenem resistance are exposure to antimicrobial agents, admission to ICU and the use of indwelling devices. These factors can be addressed through appropriate infection control measures and dedicated antibiotic stewardship (Brink, Coetzee, Clay, Corcoran, *et al.*, 2012:600).

2.6 Mechanisms of carbapenem resistance

Gram-negative bacilli have been identified as the most important emerging threat in antibiotic resistance, proving to be tough adversaries for both research and clinical teams (Kanj & Kanafani, 2011:250; Pop-Vicas & Opal, 2014:206). This is particularly true for extended-spectrum β -lactamase-producing Enterobacteriaceae, CRE and multidrug-resistant *Pseudomonas aeruginosa* (Kanj & Kanafani, 2011:250). Non-Enterobacteriaceae gram-negative bacilli that have conferred resistance to the carbapenem class include *Pseudomonas* spp. and *Acinetobacter* spp. (Public Health Agency, 2015:24).

Table 2-2: Scale of measurement classification

Ambler class	Enzyme type	Host pathogens	Substrate for the enzyme
A	Broad-spectrum β -lactamases (TEM, SHV)	Enterobacteriaceae and nonfermenters e.g. <i>Acinetobacter</i> spp., <i>Pseudomonas</i> spp., <i>Stenotrophomonas maltophilia</i>	Ampicillin
	ESBL (TEM, SHV, CTX-M)		Penicillins, 3rd-generation cephalosporins ^a
	Carbapenemases (KPC, GES, SME)		All β -lactams ^b
B	Carbapenemases (NDM, VIM, IMP)	Enterobacteriaceae and nonfermenters	All β -lactams
C	AmpC cephamycinases (AmpC)	<i>Enterobacter</i> spp. <i>Citrobacter</i> spp.	3rd-generation cephalosporins ^b
D	Broad-spectrum β -lactamases (OXA)	Enterobacteriaceae and nonfermenters	Ampicillin
	ESBL (OXA)		Penicillins, 3rd-generation cephalosporins ^c
	Carbapenemases (OXA)		All β -lactams
	AmpC cephamycinases (CMY, DHA, MOX, FOX, ACC)	Enterobacteriaceae	3rd-generation cephalosporins
^a = except cephalosporin/ β -lactamase inhibitor combination; ^b = inhibited by ceftazidime/avibactam ^c = variable inhibition by ceftazidime/avibactam ACC = Ambler class C; AmpC = Class C β -lactamases; CMY = Cephamycins; CTX-M = Cefotaxime hydrolysing capabilities; DHA = Dhahran hospital; ESBL = Extended spectrum β -lactamase; FOX = Cefoxitin; GES = Guyana extended-spectrum-lactamase; IMP = Imipenemase; KPC = <i>Klebsiella pneumoniae</i> carbapenemase; MOX = Moxalactam; NDM = New Delhi Metallo- β -lactamase; OXA = Oxacillin hydrolysing capabilities; SHV = Sulfhydryl variable; SME = <i>Serratia marcescens</i> enzyme; TEM = Temoneira; VIM = Verona integron-encoded metallo- β -lactamase			

The carbapenem resistance is conveyed through changes in outer bacterial membrane proteins, carbapenem-hydrolysing enzymes and mutations in efflux pumps that lead to altered PBPs as well as altered porin function and/or expression (Papp-Wallace *et al.*, 2011:4946; Perez & Van Duin, 2013:257). The production of β -lactamase enzymes are the most frequent resistance mechanism encountered in pathogens. β -lactamases are commonly classified according to its molecular structure (Ambler classification) as summarised in Table 2-2 (Goodlet *et al.*, 2016:1813; Kanj & Kanafani, 2011:251). This table shows that carbapenemases have the greatest spectrum of activity of all the β -lactamase enzymes. These enzymes not only inactivate carbapenems through hydrolysis but also have the ability to render most other members of the β -lactam family inactive (Kanj & Kanafani, 2011:251; Sharma *et al.*, 2016:434).

The *Klebsiella pneumoniae* carbapenemases (KPCs) are the most common and widely spread carbapenemases (Kanj & Kanafani, 2011:250). These enzymes render resistance to all β -lactams except ceftazidime/avibactam, are plasmid-based and capable of inter-species transfer, which in turn can be conferred to other gram-negative bacteria such as *Escherichia coli*, *Enterobacter*, *Pseudomonas* and *Salmonella* (Goodlet *et al.*, 2016:1813; Pop-Vicas & Opal, 2014:207).

Clinically relevant enzymes in class B (metallo- β -lactamases) are imipenemase (IMP), Verona integron-encoded metallo- β -lactamase (VIM), São Paulo metallo- β -lactamase (SPM) and the New Delhi metallo- β -lactamase (NDM) type. The enzyme that received most of the attention recently, NDM-1, transfers resistance to all β -lactams except aztreonam. Most metallo- β -lactamases are found on mobile gene cassettes that can be inserted into integrons that already contain resistance genes to other antibiotic classes. Transfer of this multidrug resistance to other species is via transposons and plasmids. This places severe limitations on available treatment options for serious infections caused by Class D carbapenemases which consist of mostly of the oxacillinase-48 (OXA-48) type carbapenemase family and produced mostly in *Pseudomonas aeruginosa* and *Acinetobacter* spp. (Pop-Vicas & Opal, 2014:207).

Resistance to carbapenems is also mediated through mechanisms such as impermeability due to changes in the size and number of porins as well as multidrug efflux pumps, especially in *Pseudomonas* spp. (Meletis *et al.*, 2012:305). The combination of cephalosporinases like AmpC and the decrease in antibiotic diffusion across bacterial membranes due to genetically altered porins can convey carbapenem resistance in gram-negative bacteria (Pop-Vicas & Opal, 2014:207). Organisms like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* might have increased levels of resistance to carbapenems through a combination of the resistance mechanisms (Papp-Wallace *et al.*, 2011:4946).

2.7 Prevalence of carbapenem resistance

The threat of carbapenem resistance on our ability to treat common infectious diseases and the associated debilitating effects can only be halted if the true prevalence and extent of carbapenem resistance is known (WHO, 2016a, WHO, 2017a:7). The WHO (2016a) acknowledge the importance in understanding the prevalence of AMR through their continuous global efforts to assist countries in developing their own surveillance systems. A panel of experts recently identified a global priority pathogens list of antibiotic-resistant bacteria. All the pathogens that were given a critical priority were linked to carbapenem resistance and include *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae (WHO, 2017a:5). A further concern is the spread of carbapenem resistance not only in humans, but also to food-producing animals despite the prohibited use the class in these animals (EFSA, 2013:54;

ECDC, 2016c:163; Michael *et al.*, 2015:431; Department of Health and Human Services, 2014:15). These facts emphasise the particular importance to evaluate the current literature on the prevalence of carbapenem resistance not only globally but more specifically in South Africa.

Table 2-3: Global antimicrobial resistance stakeholder mapping

Category	Stakeholders	Organisation	Focus
Policy	United Nations (UN) institutions	World Health Organization (WHO) - secretariat	H, A, E
		Food and Agriculture Organization of the United Nations (FAO)	A, E
		Tripartite Collaboration: WHO, FAO and World Organization for Animal Health (OIE)	H, A, E
		CODEX Alimentarius Commission	A
		World Bank (WB)	H
		Every Woman Every Child (EWEC)	H
	Intergovernmental & international institutions	World Organization for Animal Health (OIE)	A, E
		Global Health Security Agenda (GHSA)	H, A, E
		Organisation for Economic Co-operation and Development (OECD)	H, A
		Trans Atlantic Task Force on AMR (TATFAR)	H, A
		International Federation of Pharmaceutical Manufacturers & Associations (IFPMA)	H, A, E
		Consultative Group for International Agricultural Research (CGIAR)	A, E
Advocacy		ReAct - Action on Antibiotic Resistance	H, A
		Antibiotic Resistance Coalition (ARC)	H, A, E
		South Centre	H, A, E
		Third World Network (TWN)	H
		Center for Disease Dynamics, Economics and Policy (CDDEP)	H, A, E
		Alliance for the Prudent Use of Antibiotics (APUA)	H, A
		Médecins Sans Frontières (MSF) Access Campaign	H
		Health Action International (HAI)	H
		World Alliance Against Antibiotic Resistance (WAAAR)	H, A, E
		Consumers International	H, A
Innovation & Research		Global Antibiotic Research and Development (GARD)	H
		Partnership Incubation (formerly known as Drugs for Neglected Diseases <i>initiative</i> (WHO-DNDi) Collaboration)	H
		Foundation for Innovative New Diagnostics (FIND)	H
		Community for Open Antimicrobial Drug Discovery (CO-ADD)	H
Surveillance	World Health Organization	Global Antimicrobial Resistance Surveillance System (GLASS)	H
		WHO Advisory Group on Integrated Surveillance of AMR (AGISAR)	H, A
		The Gonococcal Antimicrobial Surveillance Program (GASP)	H
A = Animal health; AMR – Anti-microbial resistance; E = Environmental effects; H = Human health			

The literature search revealed a substantial list of potential stakeholders involved in the field of AMR (ReAct, 2016b). The indeterminate role of the respective organisations has prompted the WHO to commission a global network of antibiotic resistance experts, the Action of Antibiotic Resistance (ReAct) to map their current involvement across a number of sectors (ReAct, 2016b:6). These sectors include policy, advocacy, surveillance, research and funding (ReAct, 2016b). Table 2-3 provides an overview of the mapping and shows the involvement of the organisations in the respective sectors and in the environment, human and/or animal health (Grundmann *et al.*, 2011:82; ReAct, 2016b; WHO 2014a; WHO, 2015a:10). It is clear from this classification that only a handful of organisations are involved in the surveillance of the prevalence of AMR at a global level (ReAct, 2016b).

A recent publication by the World Bank stated that Europe and the Americas have the best surveillance systems, whereas South and Southeast Asia and Sub-Saharan Africa have the least developed systems (World Bank Group, 2016:42). The biggest contributors to the discrepancies between countries and regions are weak infrastructures and laboratory surveillance systems (World Bank Group, 2016:42). A list of organisations (Table 2-4) involved in AMR at a regional and supranational level in the respective sectors was compiled from the most recent available publications (CDDEP, 2015; Grundmann *et al.*, 2011:82; ReAct, 2016b; Shaban *et al.*, 2014; WHO, 2014a; WHO, 2015a:9).

Supranational programmes such as the SENTRY programme were established in 1997 through funding from a pharmaceutical company to monitor AMR patterns. The programme collects data from Asia-Pacific, which includes Asia, Australia and South Africa, as well as North America, Latin America and Europe (Shaban *et al.*, 2014). The most recent publication on carbapenem resistance from this group was in 2014 in patients hospitalised with pneumonia in US and European hospitals (Sader *et al.*, 2014:328). The last publication for Africa was in 2002 (Bell *et al.*, 2002:125). One of the Center for Disease Dynamics, Economics and Policy (CDDEP) projects, the Global Antibiotic Resistance Partnership (GARP) partners with eight countries (South Africa, Mozambique, Kenya, Tanzania, Uganda, India, Vietnam, Nepal and India) to develop and analyse policy on antimicrobial resistance (CDDEP, 2015). The organisation has not published data on carbapenem resistance to date.

Table 2-4: Regional antimicrobial resistance stakeholder mapping

Stakeholders: Surveillance	Organisation	Focus
European Region	European Centre for Disease Prevention and Control (ECDC) Surveillance Programs:	H
	European Antimicrobial Resistance Surveillance Network (EARS-NET) measures antibiotic resistance European Surveillance of Antimicrobial Consumption Network (ESAC-NET) measures antibiotic consumption	H
	Central Asian & Eastern European Surveillance of Antimicrobial Resistance (CAESAR)	H
	European Medicines Agency - European Surveillance of Veterinary Antibiotic Consumption (ESVAC)	A
Eastern Mediterranean Region	Antibiotic Resistance Surveillance and Control in the Mediterranean Region (ARMed): Activity ceased	H
South-East Asian & Western Pacific Region	Asian Network for Surveillance of Resistant Pathogens (ANSORP) (project of Asia Pacific Foundation for Infectious Diseases (APFID))	H
	Chinese Ministry of Health National Antimicrobial Resistant Investigation Net (MOHNARIN)	H
	Western Pacific Regional Antimicrobial Resistance Surveillance (WePARS) (proposed)	
Region of the Americas	Latin American Antimicrobial Resistance Surveillance Network (ReLAVRA) (administered at the Pan American Health Organization (PAHO))	H
Supranational	SENTRY antimicrobial surveillance programme	H
	Global Antibiotic Resistance Partnership (GARP)	H
A = Animal health; E = Environmental effects; H = Human health		

Only a few low- and middle-income countries have national surveillance systems in place to monitor AMR as seen in Table 2-5 (CDDEP, 2015:24; World Bank Group, 2016:36). Some countries are in the process to develop surveillance systems, which is critically important to complete the global picture of AMR (CDDEP, 2015:24). The WHO (2013a) found that the understanding of the current situation in Africa is lacking due to limited drug resistance surveillance in a few countries only, resulting in inadequate information regarding the magnitude of this problem. No data on the prevalence of carbapenem resistance were found in this publication (WHO, 2013a). This information already adds to the observation by the WHO that community-acquired infections are in most cases underrepresented, leading to underreporting of resistance in important patient groups (WHO, 2014a:70).

Table 2-5: National surveillance programmes

Country	Region	Programme	Focus
Australia	Western Pacific	Australian Group on Antimicrobial Resistance (AGAR)	H
Cambodia	Western Pacific	United States Naval Medical Research Unit 2 Phnom Penh (NAMRU-2 PP)	H
Canada	Americas	Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS)	H, A, F
China	Western Pacific	China Antimicrobial Resistance Surveillance Study (CHINET)	H
China, Hong Kong	Western Pacific	Hong Kong Antibiotic Stewardship Program (ASP)	H
Denmark	European	Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP)	H, A, F
Federated States of Micronesia	Western Pacific	Federated States of Micronesia Surveillance Network	H
Finland	European	The Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents report (FINRES-VET)	A, F
France	European	l'Observatoire National de l'Epidémiologie de la Résistance Bactérienne aux Antibiotiques (ONERBA)	H, A
Germany	European	German National Veterinary Antibiotic Resistance Monitoring (GERM-VET)	H
Italy	European	Italian Veterinary Antimicrobial Resistance Monitoring (ITAVARM)	H, A
Japan	Western Pacific	Japan Nosocomial Infections Surveillance (JANIS) Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM)	H, A
Malaysia	Western Pacific	National Surveillance of Antimicrobial Resistance Program (NSAR)	H
Netherlands	European	Consumption of Antimicrobial Agents and Antimicrobial Resistance among Medically Important Bacteria in the Netherlands/ Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (NETHMAP/MARAN)	A, F
New Zealand	Western Pacific	New Zealand Institute of Environmental Science and Research (ESR)	H
Norway	European	Norwegian Surveillance System for Antimicrobial Drug Resistance (NORM/NORM-VET)	H, A, F
Republic of Korea	South-East Asia	Korean Antimicrobial Resistance Surveillance Program (KARMS) Korean Nationwide Surveillance of Antimicrobial Resistance (KONSAR)	H

Country	Region	Programme	Focus
Singapore	Western Pacific	The Network for Antimicrobial Resistance Surveillance (NARS-Singapore)	H
Sweden	European	Swedish Veterinary Antimicrobial Resistance Monitoring programme/ Swedish utilisation and resistance in human medicine (SWEDRES/SVARM)	A
Thailand	South-East Asia	National Antimicrobial Resistance Surveillance Center, Thailand (NARST)	H
Taiwan	Western Pacific	Taiwan Surveillance of Antimicrobial Resistance (TSAR)	H
United Kingdom	European	English Surveillance Programme for Antimicrobial Utilization and Resistance (ESPUAR)	H, A
United States	Americas	National Antimicrobial Resistance Monitoring System (NARMS) Emerging Infections Program (EIP) National Healthcare Safety Network (NHSN) Gonococcal Isolate Surveillance Program (GISP) National Tuberculosis Surveillance System	H
Vietnam	Western Pacific	Viet Nam Resistance Project (VINARES)	H
A = Animal health; E = Environmental effects; H = Human health			

2.7.1.1 Prevalence of specific carbapenem-resistant organisms at an international and regional level

2.7.1.1.1 Prevalence of carbapenem-resistant Enterobacteriaceae

Reporting of the prevalence of carbapenem-resistant Enterobacteriaceae is not done by all organisations. Canada, the United States and Asia had the most reports on the prevalence thereof.

The Government of Canada has realised the importance of leading activities that will minimise the emergence and spread of AMR. Its first report, which included the epidemiology of carbapenem resistance, was compiled in 2015. It reported on the incidence of carbapenemase-producing organisms (CPO) and CRE. While the incidence of CPO remained stable, the incidence of CRE increased almost two-fold from 2010 to 2013 (0.15 per 10 000 patient-days vs. 0.26 per 10 000 patient-days). The biggest contributor to the change in the rate of CRE in 2013 was due to an outbreak at one hospital (Public Health Agency, 2015:24). The CRE level in has remained stable in 2014 (0.22 per 10 000 patient days) compared to 2010 (0.19 per 10 000 patient days). The CPO remained stable over the same period, 0.10 per 10 000 patient days in 2010 vs. 0.11 per 10 000 patient days in 2014 (Public Health Agency, 2016:22). These stable results are encouraging given the fact that the Canadian Nosocomial Infection Surveillance Program (CNISP) experienced an almost 50% increase in the number of participating hospitals

in the programme since 2010 (Public Health Agency, 2016:21). The Canadian Ward Surveillance Study (CANWARD) is a surveillance study endorsed by the Canadian Public Health Agency. Resistance data collected over the period 2007 to 2015 was presented at the American Society for Microbiology (ASM) congress held in June 2016. It reported observed carbapenem resistance in individual organisms but not on total prevalence of carbapenem resistance (Zhanel *et al.*, 2016).

The last formal report by the Centers for Disease Control (CDC) on carbapenem resistance in humans was in 2013. Its website has an interactive antibiotic resistance patient safety atlas application that shows the average prevalence of CRE for the entire US population as 3.5% for the years 2011 to 2014 (CDC, 2016). Other publications on the topic reported that the prevalence of carbapenem resistance has increased more than five-fold in the US from 2008 to 2012 (Thaden *et al.*, 2014:981). The latest major activity of the Healthcare-Associated Infections Community Interface (HAIC), was launched in 2009 in eight of the Emerging Infections Program (EIP) states (Magill *et al.*, 2015:1537). This initiative reported that the overall crude annual CRE incidence during the 2-year period, 2012 to 2013 was 2.93 from seven EIP states (95% CI, 2.65 - 3.23) per 100 000 population (Guh *et al.*, 2015:1482). Other AMR patterns reported to the NHSN and the CDC detect that the proportion of CRE (7.1%) was highest in central line-associated bloodstream infections (CLABSI), particularly in *Klebsiella pneumoniae* and *Klebsiella oxytoca* (Weiner *et al.*, 2016:1291). This is in contrast to catheter-associated urinary tract infections (CAUTI) Enterobacteriaceae, where 4.0% were resistant to carbapenems and surgical site infections (SSI) Enterobacteriaceae were resistant in 1.8% of cases (Weiner *et al.*, 2016:1296).

European surveillance found that resistance to carbapenems was more than 10% in 19 of 29 reporting countries (ECDC, 2014:8). The prevalence of carbapenem-resistant CRE in the Netherlands is rare and stable (NETHMAP/MARAN, 2016:12).

Recent publications and information on carbapenem resistance in New Zealand appear to be lacking. A report by the Royal Australasian College of Physicians (RACP) mentions that the majority of CRE detected, were contracted overseas (ReAct, 2016b:6). The number of CREs has increased dramatically since 2009 and 41 isolates were identified in 2015 compared to 35 isolates between 2009 and 2014 (RACP, 2016:10).

A meta-analysis published in 2015 described the epidemiology of CRE over the period of 2000-2012 in Asia (Xu *et al.*, 2015:376). This meta-analysis found that Enterobacteriaceae exhibited resistance rates of 0.8% and 1.0% respectively, to imipenem and meropenem (Xu *et al.*, 2015:378). The analysis also reported that Indonesia (5.8%), Vietnam (3.0%) and Philippines (3.7%) were the countries with the highest rates of resistance (Xu *et al.*, 2015:379). The most

recent published data on carbapenem resistance in Hong Kong was for the period 2009 to 2011. The publication determined that resistance to CRE was 0.6% (Lai *et al.*, 2014:229).

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) system has identified the surveillance of carbapenem-resistant *Acinetobacter baumannii* (CRAB) spp. and Enterobacteriaceae as a first order priority. None of the animals monitored for resistant organisms, transferred bacteria that exhibited carbapenem resistance (Public Health Agency, 2016:8). However, it has to be kept in mind that there is limited data on AMR in animals due to gaps in the surveillance of AMR in animals and the food chain (Public Health Agency, 2016:12). A report by the German Antimicrobial Resistance Strategy (Deutsche Antibiotika-Resistenzstrategie – DART) commented that rates of resistance to carbapenems for Enterobacteriaceae are still less than one percent (DART, 2015:6). The report mentions that there are early indications of CRE in livestock in Germany (DART, 2015:8). No publication on carbapenem resistance by the German National Veterinary Antibiotic Resistance Monitoring (GERM-VET) could be found.

A meta-analysis commented on the considerable detail in which the epidemiology of CPO is described in Europe, North America and Asia with relatively little known about the spread in Africa. A systematic review of carbapenemase-producing bacteria in African countries was done to determine the epidemiology thereof (Manenzhe *et al.*, 2015:24). The majority of studies were conducted in North Africa (74%), followed by Southern Africa (12%), of which South Africa published the majority (nine studies) (Manenzhe *et al.*, 2015:24). The prevalence of carbapenemase-producing isolates ranged from 2.3% in North Africa to between 9% and 60% in sub-Saharan Africa (Manenzhe *et al.*, 2015:23). A recent meta-analysis of 30 publications demonstrated that the median carbapenem resistance was 9% in adult neutropaenic patients (Righi *et al.*, 2017:668).

2.7.1.1.2 Prevalence of *Escherichia coli*

The ECDC annual report detected that the population-weighted mean percentage of carbapenem resistance in *Escherichia coli* isolates was less than 0.1% for the region and the national prevalence ranged from 0 to 2.6% in 2012. The surveillance system reported ICU-acquired carbapenem resistance in isolates of *Escherichia coli* was 1.1% in 2012 (ECDC, 2014:23). Their latest report indicated that *Escherichia coli* prevalence is still below 0.1% in the Europe (ECDC, 2016b:5). A review on AMR commissioned by the UK Government and the Wellcome Trust found that carbapenem-resistant *Escherichia coli* more than doubled between 2008 and 2013 in the UK (O'Neil, 2016:63).

The Antibiotic Resistance Surveillance and Control in the Mediterranean Region (ARMed), a four-year project that ran from 2003–2007, conducted relevant scientific studies on the surveillance of antibiotic resistance in the Mediterranean until the project was terminated due to a lack of funds. Malta, Morocco, Cyprus, Egypt, Jordan, Tunisia and Turkey participated in this project (WHO 2014a). Carbapenem resistance in *Escherichia coli* was low, all countries showed resistance levels below 5%, except for Egypt with a 9% prevalence (Borg *et al.*, 2008:793).

Denmark introduced their surveillance system twenty years ago and was the first country in the world to recognise the overlapping reservoirs of AMR in humans, foods and animals. Carbapenem resistance levels are low in the country. The Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) reported one carbapenem-resistant *Escherichia coli* isolate (DANMAP, 2015). The Norwegian Surveillance System for Antimicrobial Drug Resistance (NORM/NORM-VET) found in 2013 that all *Escherichia coli* isolates cultured from humans were susceptible to meropenem (NORM/NORM-VET, 2013:57). Carbapenem resistance remained low in bloodstream infections in England where carbapenem resistance was detected in 0.2% of *Escherichia coli* (Public Health England, 2016:6). Carbapenem resistance was confirmed in 0.01% of *Escherichia coli* isolates (NETHMAP/MARAN, 2016:12). The Canadian report has not detected any *Escherichia coli* isolates (Public Health Agency, 2015:42).

Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) was established in 2012 as initiative includes all the countries of the WHO European Region (19 in total), which are not part of EARS-Net (WHO, 2016b). Five countries collected and submitted AMR data in 2014 for the programme's first annual report and seven countries for the second annual report published in October 2016. Slightly elevated resistance proportions of *Escherichia coli* were seen in Belarus (2%), Serbia (1%) and Turkey (2%) (WHO, 2016b:95). The data are mainly level B, indicating that the magnitude of resistance presented is biased but can still provide an indication of the resistance patterns present in the clinical settings (WHO, 2016b:26). It should be interpreted with caution due to indications that the data are not representative for the population (WHO, 2014b:19; WHO, 2016b:26). The COMParative Activity of Carbapenem Testing (COMPACT II study) investigated carbapenem susceptibility in the Asia-Pacific region in 2010. The survey found that only 2.8% of Enterobacteriaceae were non-susceptible (Kiratisin *et al.*, 2012:313).

Escherichia coli exhibited the lowest resistance rates against imipenem and meropenem amongst all the Enterobacteriaceae with resistance levels of 0.2% and 0.5% respectively during the period 2000-2012 in Asia (Xu *et al.*, 2015:380). Data from the Japan Nosocomial Infections Surveillance (JANIS) and Korean Antimicrobial Resistance Monitoring System (KARMS) surveillance programmes showed the level of carbapenem resistance for *Escherichia coli* was

0.1% and 0.2%, respectively for Japan and Korea in 2012 (Shibayama *et al.*, 2015:113). Carbapenem resistance in *Escherichia coli* remained stable at 0.1% in Japan from 2012 to 2014 (Ministry of Health, Labour and Welfare, 2016:65; Shibayama *et al.*, 2015:114). Surveillance data from the China Antimicrobial Resistance Surveillance Study (CHINET) over the period 2005 to 2014 reported that *Escherichia coli* resistance levels to imipenem and meropenem resistance rates fluctuated around 1.0 and 2.2% respectively (Hu *et al.*, 2015:S10). The national surveillance network in Malaysia found that resistance levels in *Escherichia coli* isolates isolated from blood were low, 0.8% and 0.7% resistance to imipenem and meropenem, respectively (Ministry of Health, 2015:16).

The first report in carbapenem resistance was published in mid-2012 carbapenemase-producing *Escherichia coli* was detected on a pig farm in Germany. Other reports identified carbapenemase-producing *Acinetobacter* spp. in horses from Belgium and dairy cattle from France (Michael *et al.*, 2015:4310). Resistant bacteria in these animals can spread to humans via food-borne-routes, water and other environmental contamination, as well as through direct animal contact (ECDC, 2016c:22). Norway appears to not have detected carbapenem resistance in animals yet because there is no mention of it in the 2015 report (Ministry of Health and Care Services, 2015). The production of carbapenemases was not detected in any Enterobacteriaceae sampled from animals in Sweden (SWEDRES/SVARM, 2016:46).

2.7.1.1.3 Prevalence of carbapenem-resistant *Klebsiella pneumoniae*

A report by the WHO comments on the knowledge gaps in carbapenem resistance in *Klebsiella pneumoniae*. The two regions where longstanding regional surveillance and collaboration exist, Europe and the Americas, submitted the greatest number of reports (Table 2-6) (WHO, 2014a:x). The organisation also found that carbapenem-resistant *Klebsiella pneumoniae* is found in all regions with two of the regions showing resistance of more than 50% as seen in Table 2-6 (WHO, 2014a:18). Data from Eastern Mediterranean region showed that *Klebsiella pneumoniae* isolates exhibited resistance to carbapenems from 0% to 54% for the period 2008 to 2013 (WHO, 2014a:18). Surveillance by CAESAR found that *Klebsiella pneumoniae* isolates showed resistance in up to 36% of isolates in Serbia, whilst the prevalence was equal or less than 11% in the other countries (WHO, 2014a).

Latin American Antimicrobial Resistance Surveillance Network (ReLAVRA) (administered at the Pan American Health Organization (PAHO)) coordinate AMR surveillance and consists of 19 countries in Latin America plus Canada and the USA that record resistance data annually (Grundmann *et al.*, 2011:82; WHO, 2014a:3). A search of their website revealed that their publications are mostly in Spanish and no English publication on carbapenem prevalence could be found on their site. The 2014 WHO report included data collected from 15 countries from this

region. It only noted resistance of *Klebsiella pneumoniae* to carbapenems in this region (WHO, 2014a:18).

Table 2-6: Global prevalence of carbapenem-resistant *Klebsiella pneumonia*

Region	Reported range of resistance (%)
Africa (n=4)	0–4
Americas (n=17)	0–11
Eastern Mediterranean (n=4)	0–54
Europe (n=31)	0–68
South-East Asia (n=4)	0–8
Western Pacific (n=9)	0–8
n = number of reports	

Carbapenem resistance was detected in 1.1% of *Klebsiella pneumoniae* isolates in England (Public Health England, 2016:6). The Danish monitoring programme reported no cases of CRKP (DANMAP, 2015:110). The Netherlands confirmed carbapenem resistance in 0.21% cases *Klebsiella pneumoniae* in 2015, compared to levels of 0.16% in 2013 to 2014 (NETHMAP/MARAN, 2016:95). The vast majority of *Klebsiella* spp. isolates in humans from Norway were still susceptible to meropenem (99.9%) in 2013 (NORM/NORM-VET, 2013:63)

Japan reported a carbapenem resistance of 0.2% for *Klebsiella pneumoniae* in 2014 (Ministry of Health, Labour and Welfare, 2016:65). Two patients presented with CRKP in a surveillance study of 12 092 patients in Phnom Penh, Cambodia, between July 2007 and December 2014 (Phe *et al.*, 2016, 110). Resistance levels of *Klebsiella pneumoniae* in China rose from 2.4% to 10.5% for imipenem and 2.6% to 13.4% for meropenem (Hu *et al.*, 2015:S11). *Klebsiella pneumonia* resistance to imipenem increased from 2.2% to 3.2% from 2014 to 2015 in Malaysia (Ministry of Health, 2015:19). Resistance to meropenem increased from 3.2% to 3.7% over the same period (Ministry of Health, 2015:19). A search on the website of the National Antimicrobial Resistance Surveillance Center, Thailand (NARST) showed no official publications on the prevalence of total carbapenem resistance. Many of the links on the website resulted in an error message. An antibiogram on their website showed the susceptibility of individual organisms to carbapenems for the period of January to June 2016 (NARST, 2016). The antibiogram showed that *Klebsiella pneumoniae* (92.5%) and *Pseudomonas aeruginosa* (79.3%) isolates were still susceptible to meropenem (NARST, 2016).

2.7.1.1.4 Prevalence of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is one of the most relevant nosocomial pathogens in especially more serious and chronic infections (Gilarranz *et al.*, 2013:E424; Itani *et al.*, 2011:43).

This pathogen exhibited resistance in 87% of isolates in Belarus, 47% in Serbia and 33% in Turkey (WHO, 2014b). Surveillance of AMR in Sweden detected that *Pseudomonas aeruginosa* resistance to imipenem continued to be higher (7.5%) than to meropenem (4.7%) in 2015 (SWEDRES/SVARM, 2016:78). The CANWARD study demonstrated *Pseudomonas aeruginosa* isolates collected for the period January 2008 to December 2015 in the same study demonstrated resistance to meropenem in 12.1% of samples (Walkty, 2017:61).

The COMPACT II survey found that an average of 29.8% of *Pseudomonas aeruginosa* samples were not susceptible to any carbapenems in Asia-Pacific region in 2010 (Kiratisin *et al.*, 2012:313). *Pseudomonas aeruginosa* imipenem resistance rates were 19% in Japan and 28% in Korea in 2012 (Shibayama *et al.*, 2015:114). Data from Japan showed carbapenem resistance by *Pseudomonas aeruginosa* at 20% in 2014 (Ministry of Health, Labour and Welfare, 2016:65). In 2015, *Pseudomonas aeruginosa* samples were resistant to imipenem and meropenem in 7.5% and 7.4% of cases in Malaysia respectively, whilst 79.3% of cultures were still susceptible to meropenem in Thailand (Ministry of Health, 2015:23; NARST, 2016). Data collected by the Taiwan Surveillance of Antimicrobial Resistance (TSAR) ascertained that the overall prevalence of carbapenem-resistant *Pseudomonas aeruginosa* was 10.2% over the period 2000 to 2010. Only 2.0% of carbapenem-resistant *Pseudomonas aeruginosa* isolates were susceptible to imipenem, whereas 38.1% were susceptible to meropenem (Lin *et al.*, 2016:54). The Network for Antimicrobial Resistance Surveillance (NARS-Singapore) did its first comprehensive survey in 2006 to 2008. The network detected imipenem resistance rates of 7.5% in *Pseudomonas aeruginosa*. No data for resistance rates of these organisms have been published data for resistance rates since this survey by the network (Teo *et al.*, 2016). Australia observed resistance against meropenem in 8.1% of *Pseudomonas aeruginosa* isolates (AGAR, 2015).

2.7.1.1.5 Prevalence of *Acinetobacter* spp.

Acinetobacter spp. have evolved over time from a non-pathogenic bacteria to one of the most problematic pathogens that is the frequent cause for nosocomial infection (Abbot *et al.*, 2013:395; Evans *et al.*, 2013:223).

The ECDC found that half of *Acinetobacter* spp. were resistant to all antimicrobial groups under surveillance (carbapenems, fluoroquinolones and aminoglycosides). The percentage of carbapenem-resistant isolates was high and showed in most cases combined resistance to the three antimicrobial groups under surveillance (ECDC, 2014:8). More than 40% of EU/EEA (European Union/European Economic Area) countries demonstrated carbapenem resistance of 50% or higher in *Acinetobacter* spp. from 2012 to 2015 (ECDC, 2016a:7).

Acinetobacter spp. resistance towards a carbapenem ranged between 71% and 93% of samples in the central Asian and eastern European region, except in Switzerland where it was 11% (WHO, 2014b). The surveillance network in France, l'Observatoire National de l'Epidémiologie de la Résistance Bactérienne aux Antibiotiques (ONERBA), found that 91.9% of *Acinetobacter baumannii* strains were susceptible to imipenem (ONERBA, 2016:100). Susceptibility of *Acinetobacter baumannii* has decreased by 7.1% over the last ten years from 99% in 2000 to 91.9% in 2013. Antimicrobial resistance patterns reported to the NHSN and the CDC had the highest occurrence in *Acinetobacter* spp. (64%) isolated from CAUTI in 2014 (Weiner *et al.*, 2016:1291).

An average of 73.0% of *Acinetobacter baumannii* were not susceptible to any carbapenems in the Asia-Pacific region (Kiratisin *et al.*, 2012:313). Surveillance by CHINET detected marked changes in resistance levels by *Acinetobacter baumannii* for imipenem with increases from 31% in 2005 to 62.4% in 2014 and meropenem from 39% in 2005 to 66.7% in 2014 (Hu *et al.*, 2015:S12). The most recent report by the Chinese National Antimicrobial Resistance Investigation Net (MOHNARIN) published in 2011, reported resistance to imipenem in 56.8% of *Acinetobacter baumannii* strains and resistance to meropenem in 58.7% of strains (MOHNARIN, 2012:3). The most recent published data in Hong Kong determined that resistance to *Acinetobacter* spp. was 40.2% for the period of 2009 to 2011 (Lai *et al.*, 2014:229). Resistance to meropenem and imipenem was observed in one of three *Acinetobacter* spp. isolates in 251 specimens in collected between 2011 and 2013 in Phnom Penh, Cambodia (Hout *et al.*, 2015). The prevalence of imipenem-resistant *Acinetobacter baumannii* isolates was 2% in Japan and 70% in Korea (Shibayama *et al.*, 2015:115). *Acinetobacter* spp. isolates from all samples collected in 2015 by the national surveillance network in Malaysia demonstrated resistance levels of 54.9% and 56% to imipenem and meropenem, respectively (Ministry of Health, 2015:13). The antibiogram by NARST showed that only 35.2% *Acinetobacter* spp. isolates were susceptible to meropenem, and *Pseudomonas aeruginosa* (79.3%) isolates were still susceptible to meropenem (NARST, 2016).

The report on bacterial resistance in France makes reference of the prevalence of resistance of bacteria in animal populations to other antibiotic classes, but not to carbapenems (ONERBA, 2016:102).

2.7.1.2 Prevalence of specific carbapenem-resistant organisms in South Africa

Surveillance of carbapenem resistance forms part of the AMR monitoring systems of the public and private sectors of the healthcare system in South Africa. The National Institute for Communicable Diseases (NICD) collects data through the Johannesburg Antimicrobial Resistance Laboratory Culture Collection (AMRL-CC) and the Group for Enteric, Respiratory

and Meningeal Disease Surveillance in South Africa (GERMS-SA) (NICD-NHLS, 2016b:5; NICD-NHLS, 2017b). GERMS-SA compiles carbapenem resistance data from 12 public sector academic hospitals (NICD-NHLS, 2016b:12; NICD-NHLS, 2017b). AMRL-CC originally collected data from the National Health Laboratory System (NHLS) only, but has recently included private sector data. Private surveillance data were initially conducted for the SENTRY programme by one private participating laboratory (Bamford, Brink, *et al.*, 2011:580). The South African Society for Clinical Microbiology (SASCM) collates data from the public sector and five private laboratory groups, which do not cover the entire population and are therefore not representative of the national burden (CDDEP, 2015:23; FIDSSA, 2016c:5). AMR data from smaller healthcare facilities and rural areas are not represented (Bamford, Brink, *et al.*, 2011:580). Reports can be accessed on the website of the Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) (Bamford, Brink, *et al.*, 2011:580). The data sets from the public and private sector are consolidated through GARP and the CDDEP's Resistance Map online tool (World Bank Group, 2016; CDDEP, 2015:23).

The South African National Department of Health (NDoH) has prepared a national AMR strategy document, the Antimicrobial resistance national strategy framework, to prioritise surveillance and early detection of AMR (National Department of Health, 2014:10). The department identified the weaknesses to determine the national burden of microbial resistance as prescribers' inability to send appropriate clinical samples for sensitivity testing prior to antimicrobial initiation, low numbers of trained microbiologists outside of major urban centres as well as limited or no access to laboratories (National Department of Health, 2014:14). None of the publications by the NDoH contains data regarding the epidemiology of resistance in general or carbapenem resistance (National Department of Health, 2014; National Department of Health, 2015).

2.7.1.2.1 Public and private sector

A review of the literature that delineated the current level of carbapenem resistance in South Africa found that there was an increase in carbapenem resistance but did not provide actual figures on the prevalence thereof (Osei-Sekyere, 2016). The NICD found in 2015 that 76 out of a total of 80 Enterobacteriaceae isolates received were carbapenem-resistant and 79% of these isolates produced carbapenemases (NICD-NHLS, 2015b:10). The report also cautioned that these figures only partially represent the current burden of carbapenemase-producing Enterobacteriaceae (CPE) in South Africa (NICD-NHLS, 2015b:10). The most recent reports detected that 85% of Enterobacteriaceae isolates received by the NICD expressed the carbapenemases that were screened for (NICD-NHLS, 2017a:9). A study conducted over a five-year period at a public sector referral hospital in KwaZulu Natal, a province in South Africa found that carbapenem-resistant non-Enterobacteriaceae (CRNE) fluctuated between 39%

(368/941) to 48% (331/689) during the period of 2010 to 2014. The prevalence of the CRE increased from 0% (0/1110) to 2% (20/1265) over the same period in this hospital (Swe-Swe-Han *et al.*, 2015). An investigation into presence of carbapenemases in Enterobacteriaceae obtained samples from the National Health Laboratory Service and some private laboratories for the period of 2012 to 2015 (Perovic *et al.*, 2016:975). Submission practice from public laboratories was based on NICD guidelines and private laboratories submitted samples on a voluntary basis (Perovic *et al.*, 2016:975). One or more carbapenemase-producing genes were isolated in 68% of Enterobacteriaceae samples (Perovic *et al.*, 2016:976). Carbapenemases were confirmed in 68% of *Klebsiella pneumoniae* isolates (Perovic *et al.*, 2016:976). The publication noted that the prevalence of carbapenem resistance could not be estimated due to the voluntary referral practice (Perovic *et al.*, 2016:976).

Samples collected for the ESKAPE pathogens — *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa* and *Enterobacter cloacae* complex and *Escherichia coli* — from mostly tertiary academic hospitals, showed that resistance was the highest for *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (NICD-NHLS, 2015a:33). *Acinetobacter baumannii* complex displayed resistance to imipenem and meropenem in 82% and 81% of isolates respectively, in 2015 (FIDSSA, 2016b). *Pseudomonas aeruginosa* resistance levels were 31% and 29% to imipenem en meropenem, respectively (FIDSSA, 2016b). *Klebsiella pneumoniae* cases demonstrated non-susceptibility in 5% of isolates, which has remained stable compared to the previous year (NICD-NHLS, 2016c:19). Table 2-7 gives an overview of the carbapenem resistance of *Klebsiella pneumoniae*, *Enterobacter cloacae* complex and *Escherichia coli* (FIDSSA, 2016b). The reports also caution that these figures only partially represent the current burden of CPE in South Africa (FIDSSA, 2016b; NICD-NHLS, 2016c:19; NICD-NHLS, 2017a:7). Data from the CDDEP report showed that carbapenem resistance in South Africa was 2% and 0.8% for *Klebsiella pneumoniae* and *Escherichia coli* isolates, respectively for 2013 (CDDEP 2015:22).

Table 2-7: Comparison of susceptibility profiles at public-sector sentinel sites

Organism	Ertapenem		Imipenem		Meropenem	
	2014	2015	2014	2015	2014	2015
<i>Klebsiella pneumoniae</i>	96%	97%	97%	94%	97%	94%
<i>Acinetobacter baumannii</i> complex	-	-	23%	18%	22%	19%
<i>Pseudomonas aeruginosa</i>	-	-	71%	69%	73%	71%
<i>Enterobacter cloacae</i> complex	89%	92%	98%	97%	98%	98%
<i>Escherichia coli</i>	99%	99%	100%	99%	100%	99%

According to Bisi-Johnson and Obi (2015), 23.7% of *Salmonella typhi* isolates exhibited resistance or intermediate resistance to carbapenems in a tertiary hospital in the Eastern Cape province of South Africa (Bisi-Johnson & Obi, 2015).

2.7.1.2.2 Private sector

The latest available report on carbapenem resistance on the SASCM website was for the period of January to December 2014. The data were reported separately for Johannesburg, Pretoria, Durban, Cape Town, Port Elizabeth, East London and Bloemfontein (SASCM, 2014). Large variances were seen in *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* susceptibility between the different cities (SASCM, 2014). Data from the city of Pretoria are shown in a separate column in Table 2-8 as the study hospital falls within the borders of the city (SASCM, 2014). Private sector carbapenem resistance data seems to not be reported on a regular basis. Searches of published data did not reveal data in the last three years and the websites of SASCM, FIDSSA and GERMS-SA did not appear to have data for the last two years.

Table 2-8: Comparison of susceptibility profiles at private sector sites (blood cultures)

Organism	Ertapenem		Imipenem/ Meropenem/ Doripenem	
	Pretoria	All cities	Pretoria	All cities
<i>Klebsiella pneumoniae</i>	94%	90 - 100%	96%	90 - 100%
<i>Acinetobacter baumannii</i> complex	-	-	28%	27 - 100%
<i>Pseudomonas aeruginosa</i>	-	-	46 – 52%	53 - 91%
<i>Enterobacter cloacae</i> complex	94%	72 -100%	97%	92 - 100%
<i>Escherichia coli</i>	100%	99.9 - 100%	100%	99.9 - 100%
Carbapenem-resistant Enterobacteriaceae	No data	No data	18%	7 - 22%

2.8 Approaches, methods and data extraction instruments

The literature search aimed to find research that followed the same design, approach and methods as the investigation undertaken in this dissertation and to compare data extraction instruments used in these investigations to the one used in this study. It appears from the literature research that no other investigations followed the same approaches and methods, so data extraction instruments could therefore not be compared.

2.9 Appraisal of literature findings

The literature search consistently confirmed the importance of the carbapenem class and the mechanism and risk factors for the acquisition of resistance. The search for the prevalence of carbapenem resistance has proven to be very challenging.

A search for literature by the stakeholders and programmes as listed in section 2.7 found that certain regions and countries had a substantial amount of information compared to others. The WHO (2013a) found that the understanding of the current situation in especially Africa is lacking due to limited drug resistance surveillance in a few countries only, resulting in inadequate information regarding the magnitude of this problem. No data on the prevalence of carbapenem resistance were found in this publication.

The literature view found a number of organisations that used to be active as well as an encouraging number of organisations that are beginning work on antibiotic resistance. The websites of some organisations resulted in error messages or have no clear indication whether these programmes are still active. Other programmes that were previously active had periods of inactivity before surveillance was commenced again. The collaboration of the fourteen member countries of the WHO Western Pacific region was active in the past and has been re-established in 2015 with the proposed name Western Pacific Regional Antimicrobial Resistance Surveillance (WePARS). The aim of this initiative is to improve surveillance and share findings on the prevalence of resistance by 2020 (Grundmann *et al.*, 2011:82; WHO, 2015a:5). The ARMed project ran from 2003–2007 and its activity ceased when funding was terminated at the end of the four-year period. Malta, Morocco, Cyprus, Egypt, Jordan, Tunisia and Turkey participated in this project (WHO, 2014a). Malta, Cyprus, and Turkey have subsequently joined EARS-Net (WHO, 2011:12). The current status of surveillance in the balance of the countries is unclear.

Many organisations that appear to be involved and active in the surveillance of carbapenem resistance have no publications on their findings or no recent publications (ReAct, 2016b; CDDEP, 2015; World Bank Group, 2016). A few examples include the CDC, the Asia Pacific Foundation for Infectious Diseases (APFID), The Viet Nam Resistance Project (VINARES) and the Federated States of Micronesia. The CDC tracks resistance patterns through five tracking networks, namely, the National Antimicrobial Resistance Monitoring System (NARMS), the National Healthcare Safety Network (NHSN), the Gonococcal Isolate Surveillance Program (GISP), the National Tuberculosis Surveillance System (NTSS) and EIP in the US (CDC, 2013:39; Sievert *et al.*, 2013). The CDC tracks CRE through the EIP and NHSN systems (CDC, 2013:39). NARMS focuses on resistance in the enteric bacteria, *Shigella*, *Campylobacter* and *Shigella* transmitted commonly through food (CDC, 2013:39, Department of Health and Human

Services, 2014:2). The CDC has not published a formal report on carbapenem resistance in humans since 2013. Its website has an interactive antibiotic resistance patient safety atlas application where data can be accessed. Its site however, cautions that data presented in the atlas are not representative of the entire US population (CDC, 2016). The Surveillance Network (TSN) in the US is an electronic repository of antimicrobial drug susceptibility data from a national network of >300 microbiology laboratories in the United States. It has a closed website with no access except for users only. The Asian foundation, APFID, was founded to initiate and support research in infectious diseases. The Asia Network for Surveillance of Resistant Pathogens (ANSORP) forms part of this foundation with the specific focus to conduct research AMR and infectious disease. It is an independent and non-governmental research network active in 14 countries in the Asia region (APFID, 2016). The foundation appears not to have any formal publications on the prevalence of carbapenem resistance and no data could be found on its website either. The CDDEP report does refer to this project, but only provides information on carbapenem resistance prevalence in Vietnam (CDDEP, 2015). The Federated States of Micronesia are part of the Western Pacific region of the WHO and no publications on carbapenem resistance could be found for this country (WHO, 2015a:9).

Gaps still exist in the surveillance of bacterial pathogens that cause common infections, despite the fact that many surveillance programmes have been gathering data over many years (WHO, 2015c:1). Community-acquired infections are in most cases underrepresented, leading to underreporting of resistance in important patient groups (WHO, 2014a:70). Comprehensive data from the African region especially, is scarce because of limited surveillance in a few countries only (WHO, 2013a:28; WHO, 2015b:13). The FIDSSA newsletter comments that CRE continues to be an on going challenge in South Africa and that the NICD has requested the submission of all isolates in order to provide an accurate representation of the situation in South Africa (FIDSSA, 2016a:3).

A critical evaluation of available data resulted in the use of information from the WHO, CDDEP and mapping by the ReAct as it appeared to be the most recent sources of information on the prevalence of carbapenem resistance that could be found (CDDEP, 2015:9; ReAct, 2016b; WHO 2014a). The Center for Disease Dynamics, Economics and Policy does not actively survey resistance, but record information on carbapenem resistance for specific organisms based on surveillance data from EARS-Net, PAHO, Public Health Agency of Canada and the CDC in its state of the world's antibiotics report (CDDEP, 2015:17). The data set on the CDDEP Resistance Map online tool references SASCM as the source of the information. It appears to be a useful tool to get a view of the current trends (CDDEP, 2015:15). Caution should however be undertaken when comparing resistance rates between countries as the breadth of specimen sources varies between countries (CDDEP, 2015:17; WHO, 2015b:37). However, the format in

which data is presented in the website lacks the principle of rigour, the detail, meticulous accuracy, order and precision that quantitative peer-reviewed research would provide (Brink, van der Walt, *et al.*, 2012:97). Data often show large fluctuations over time, indicating that it is important to perform antibiotic resistance surveillance studies over longer time periods (Hu *et al.*, 2015:S13). Another example of an organisation that does not have its own surveillance system is ReAct, which uses information collected through existing systems, like those of the WHO and CDC to populate an interactive toolbox (CDC, 2013; ReAct, 2016a; WHO, 2014a).

Information on the prevalence of carbapenem resistance in animals is limited. Surveillance systems that focus on the control of AMR in livestock have only recently been identified as critical to reduce the rising global threat of detrimental infectious diseases at the animal-human-ecosystem interface (WHO, 2013c:8; WHO, 2015b:4; World Bank Group, 2016). The Tripartite Collaboration between the WHO, Food and Agriculture Organization of the United Nations (FAO) and World Organization for Animal Health (OIE) has recognised the importance of this interface for the control of AMR through its global 'One health' approach (ReAct, 2016b). A comprehensive surveillance system has therefore become vital to address the problem in not only clinically ill humans but also in foods and animals raised to produce food (WHO, 2013c:8; WHO, 2015b:4). The literature search focused on data available in animals through the official programmes in place. It is unknown whether the scarcity of information is a result of a lack of surveillance, reporting or the fact that carbapenem resistance have not been detected in livestock in certain regions or countries. For example, the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) aims to ensure the global containment of foodborne antimicrobials in animal, food and human sectors (WHO, 2013c:24). The group has not yet published a report on their findings and is in the process of selecting pilot projects for this surveillance initiative that will start early 2017 (WHO, 2016c). The European Surveillance of Veterinary Antibiotic Consumption (ESVAC) reports on veterinary antibiotic use within the EU and the EEA and does not report on carbapenem resistance (ReAct, 2016b:62). The last published report by the Italian Veterinary Antimicrobial Resistance Monitoring (ITAVARM) was in 2003 (ITAVARM, 2003). Carbapenem resistance are rare in NARMS isolates because carbapenems are not approved for use in animals raised to produce food. This program has, to date, reported only two cases of carbapenemase-producing *Salmonella*, one in 1998 and the other in 2011 (Department of Health and Human Services, 2014:15).

The WHO has acknowledged an urgent need for a more coordinated surveillance system at global, regional and national level with a formal call for country enrolment in this surveillance system (WHO, 2015c:2). The Global Antimicrobial Resistance Surveillance System (GLASS) was adopted in May 2015 at the World Health Assembly to enable the collection of standardised, validated and comparable data on AMR. The project aims to publish its first report

on the consolidated baseline data on antibiotic resistance of samples collected from humans by the WHO Member States in 2018 (WHO, 2015c:4). The UN General Assembly has for the fourth time in history only, taken up a health issue. A commitment for a broad, coordinated approach to address the root causes of AMR has for the first time ever been made by Heads of State (UN, 2016).

The way that the prevalence of carbapenem resistance is reported has an impact on the comparability of data. Publications record carbapenem resistance as the percentage susceptible, percentage resistant, patient-days per 10 000 patient-days or just provide number of cases. Some publications record resistance to the carbapenem class, whilst others do so in reference for each individual carbapenem. Differences between terminology and microbiological methods add to the difficulties to assess the burden of resistance (WHO, 2014a:23). Several older studies applied Clinical and Laboratory Standards Institute (CLSI) breakpoints that are no longer current resulting in data not being comparable (Walkty *et al.*, 2017:60). A lack of specificity for carbapenem resistance could be the result of a carbapenemase not included in the test panel (Grundmann *et al.*, 2017:160). Statistical analysis, methods, ethical considerations and rigour are more comprehensive recorded in certain publications. The prevalence of carbapenem resistance across countries and time-periods showed many differences, which influences the generalizability and comparability of publications.

It has also been identified that there is a lack of formal collaboration between existing surveillance programmes to track AMR (Grundmann *et al.*, 2011:82). Continuous surveillance programs for AMR, where data to some extent can be compared, currently only exist in most of the EU-countries, USA and Canada (WHO, 2014a:60; WHO, 2016b:1). Inadequate sources of information, the lack of homogeneity in the methodology and collection of data of AMR, contributed to a situation where comparability of data becomes problematic (WHO, 2015c:1). The WHO also experienced similar findings in 2014, which prompted the development of a strategy for the collection of standardised, validated and comparable data on AMR (WHO, 2014a:23; WHO, 2015c:2).

The common thread in all publications was that the preservation of the carbapenem class was critical for continued effective treatment of multidrug-resistant organisms. All publications in one way or another underpinned the need for all role-players in the field of AMR to be involved in the investigation of carbapenem resistance to increase the database. This confirms the importance of objective and results of the empirical investigation of the dissertation.

2.10 Chapter summary

The specific objectives of the literature review have been met. The literature search has confirmed the importance of the carbapenem class in the treatment of clinically important organisms and infectious diseases. However, the search has also, unexpectedly so, revealed that the extent of resistance is unknown due to the underreporting and differences in reporting systems across the world. Africa and South Africa has, in particular, a lack of data that represents the burden of carbapenem resistance. The literature search revealed the need for a more complete investigation on the situation not only in South Africa but also the rest of the world. The results of the empirical investigation on the prevalence of carbapenem resistance in the study population are presented in chapter 3.

CHAPTER 3: RESULTS

3.1 Introduction

This chapter presents the results and analysis of the collected data of the empirical investigation in the form of a manuscript, which will be submitted for consideration for publication. The data-collection protocol and analysis of data with the ethical implications as set out in the first chapter have been adhered to in this manuscript.

3.2 Proposed journal for publication

The manuscript is titled “A retrospective study on the prevalence of carbapenem resistance in adult patients admitted to a private hospital in Tshwane” and will be submitted to the journal “*South African Family Practice Journal*”. The author guidelines for this journal are included in Annexure 4. The article was language edited as part of the mini-dissertation (Annexure 5) and will again be language edited once it is finalised for submission. Editor permission has not been obtained for inclusion of the article into the mini-dissertation, since the article has not been submitted for publication.

3.3 Individual manuscript according to journal guidelines

A retrospective study on the prevalence of carbapenem resistance in adult patients admitted to a private hospital in Tshwane

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ABSTRACT

Background: Growing reports of carbapenem resistance and the association with multidrug resistance question the true spread of this difficult-to-treat occurrence. Current surveillance in South Africa, especially in the private sector of the health system is representative of a minority of healthcare facilities. A lack of data on the prevalence of carbapenem resistance has the implication that resistant organisms can spread unimpeded and cause serious infections. This is exacerbated by the inappropriate use of antibiotics and more notably, the carbapenem class. The objective of this investigation was to gain an understanding into the prevalence of carbapenem resistance and the organisms that exhibit acquired resistance in a private hospital setting.

Methods: A retrospective, cross-sectional design was followed to determine the prevalence of carbapenem resistance in patients admitted to a private hospital in the Gauteng province of South Africa. Male and female patients were eligible for inclusion if they were admitted during the period 1 January 2014 to 31 December 2014, were 18 years and older, treated with a carbapenem and had a microbiology report with susceptibility data towards a carbapenem in their file.

Results: The prevalence of carbapenem resistance in a sample of 71 patients was 12.7% (95% CI, 4.9 - 20.4). Carbapenem-resistant organisms detected in this investigation included *Pseudomonas putida* and *Enterobacter cloacae*.

Conclusions: The results from this investigation should prompt other private healthcare facilities to increase surveillance activities, as it is likely that carbapenem resistance is present in their hospitals. Carbapenem-resistant organisms observed in this study form part of the group of priority pathogens where resistance should be prevented in the interest of community health.

Keywords: carbapenem, resistance, prevalence

INTRODUCTION

Emerging reports of carbapenem resistance and their increasing association with resistance to all currently available antibiotics, have raised concerns about the management of infections caused by these bacteria.^{1,2} A clear correlation has been observed between increasing carbapenem use and the increasing carbapenem resistance in Enterobacteriaceae.^{3,4} A recent meta-analysis of 30 studies from 21 countries demonstrated that the overall carbapenem resistance ranged from 2% to 53% (median 9%) in adult neutropaenic patients.⁵ The risk of carbapenem resistance is no longer limited to humans only, because resistant organisms have

recently been observed in livestock despite the prohibited use of carbapenems in these animals.^{6,7,8,9}

Infections caused by multidrug resistant bacteria are difficult to treat and the rising incidence of carbapenem-resistant Enterobacteriaceae (CRE) suggests a precipitous increase in the clinical and economic burden thereof.^{2,10} The median cost of a single CRE infection arising from current prevalence rates, vary from US \$10 440 to US \$66 031 for third-party payers and hospitals.¹⁰ The added costs are due to an increase in hospitalisation, drug treatment and associated tests.¹⁰ Societal costs are mainly caused by productivity losses, a result of fatality, followed by direct medical costs, which can range from US \$58 026 to US \$83 512.¹⁰ Mortality rates due to carbapenem-resistant infections range from 33% to 71% (median 50%).⁵

There are nevertheless, despite increased global surveillance efforts, still significant limitations to the current available evidence related to resistance in both community and healthcare settings.¹¹ High-quality data are by and large missing from community-acquired infections and low-income countries.¹¹ The World Bank Group established that Europe and the Americas have the best surveillance systems, whereas South and Southeast Asia and Sub-Saharan Africa have the least developed systems.¹²

The surveillance system in South Africa involves a few organisations that focus their efforts in either the healthcare system's private or public sector, or both. The Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA), at the National Institute for Communicable Diseases (NICD) collects carbapenem resistance data for hospital pathogens from public sector academic hospitals only.^{13,14} The Johannesburg Antimicrobial Resistance Laboratory Culture Collection (AMRL-CC) at the NICD collates anti-microbial resistance data annually from the National Health Laboratory System (NHLS) and more recently from the private sector.¹³ The South African Society for Clinical Microbiology (SASCM) monitors antimicrobial resistance patterns in both sectors.¹⁵ The initial shortcoming of the current surveillance approach was that the magnitude of antimicrobial resistance across the country was not reflected due to limited surveillance in a smaller group of healthcare facilities only.¹⁶ Increased efforts in surveillance by certain healthcare facilities has given rise to resistance patterns not necessarily reflective of the true prevalence and spread of carbapenem resistance.^{17,18} The entire burden of carbapenem resistance in South Africa still remains largely undetected, especially in the private sector hospitals.^{18,19}

The National Department of Health (NDoH) has prioritised the optimisation of surveillance and early detection of antimicrobial resistance (AMR) in the Antimicrobial Resistance National Strategy Framework.²⁰ Part of this effort is to benchmark and publish data through a partnership with the Global Antibiotic Resistance Partnership (GARP) and Center for Disease Dynamics,

Economics and Policy (CDDEP).^{19,21} These publications are based on data received from the current surveillance system in South Africa.¹⁹ A meeting held in June 2016 by SASCM confirmed the strengthening of surveillance as a key deliverable and identified CRE as a priority surveillance area.¹⁵ The burden of carbapenem resistance will be more representative when all hospitals and laboratories report their carbapenem-resistant cases. It was therefore important given this context, to investigate the prevalence of carbapenem-resistant organisms in patients admitted to a private hospital known for its high antibiotic prescription practices.

METHODS

Study design and setting

A quantitative, cross-sectional, descriptive, retrospective design was followed to investigate the current prevalence of carbapenem-resistant organisms in patients treated with a carbapenem during their hospital stay. The investigation was undertaken in a private hospital situated in the Tshwane municipality in the Gauteng province of South Africa. This hospital was a suitable choice since it had at the time of the investigation, the highest antibiotic consumption in the hospital group that it belongs to. The major contributor to this antimicrobial consumption was the frequent prescription of carbapenems in this hospital.

Participants

The population consisted of all patients that were admitted during the defined study period of 1 January 2014 to 31 December 2014, which received treatment with a carbapenem during their stay. A non-randomised consecutive sampling approach was used to select the sample representative of the study population. It was assumed that patients presented for admission in a random order and could therefore be selected for inclusion using this approach. Male and female patients were eligible for inclusion if they were 18 years and older, admitted during the study period and treated with a carbapenem. Furthermore, patients could only be considered for inclusion if their hospital file contained at least one microbiology report from a pathology laboratory with susceptibility data. Carbapenem resistance was defined as an acquired resistance to at least one of the carbapenems i.e. doripenem, ertapenem, imipenem or meropenem or documented evidence of the presence of a carbapenemase-producing gene. Exclusion criteria were evidence of HIV-positive status or pregnancy. The hospital-dispensing programme was used to identify patients for inclusion into the study.

Variables

The dependent variable investigated were the percentage of carbapenem-resistant organisms in the total study population. Independent variables investigated comprised of the prevalence of

acquired carbapenem resistance by all bacteria, per individual species, which of the carbapenems resistance was acquired to, resistance per gender group and across age groups.

Data sources/measurement

Data were retrospectively extracted from the patient hospital files onto a hardcopy of the data extraction form by the main author of the article, on completion of the sampling process. Information recorded during the extraction process included the relevant patient demographic data (age and gender), prescribed carbapenem and organism-specific resistance to any of the carbapenems. Threats to validity and reliability of the data collection method were minimised through assessment and evaluation of the data extraction form by the co-authors of the article. The test was done prior to the onset of the formal investigation to assess whether the correct information were to be extracted through the data extraction sheet.

Study size

The planned study sample size of 120 was calculated based on a Chi-square statistical significance test with a significance level of $\alpha \leq 0.05$ and the power of the test set to 0.8. Fewer patients could be selected for inclusion mainly due to lower hospital occupancy trend towards the latter part of the study period and a greater number of files without culture results. The calculated margin of error for the observed prevalence in the study was 7.74%, which falls within the accepted margin of error (5 to 10%) for descriptive studies.²² This information confirmed that the data set is sufficient to generalise findings from the sample back to the population.

Statistical methods

Data were analysed by means of SAS[®] version 9.4. Descriptive statistics were used to describe and summarised the data. An independent *t*-test was executed to determine if differences exist in the mean age of patients on the respective antibiotics. The Cohen's *d*-value was used to interpret the practical significance of this difference (practical significance was interpreted as follows: 0.2 ~ small practical significance; 0.5 ~ medium practical significance; 0.8 ~large practical significance). A 2-tailed *p*-value of < 0.05 was considered statistically significant. The Pearson's Chi-square test was performed to determine the association between gender and the resistance status of the patients.

Ethical considerations

Ethical approval for this study was obtained from the Health Research Ethics Committee from the North-West University (Ethical number NWU-00004-15-A1) and the research operations

committee of the private hospital group granted goodwill permission. In order to ensure the privacy and confidentiality of the study subjects, anonymised data were collected.

RESULTS

The study comprised of 175 patient files of patients admitted to the private hospital during the study period 1 January 2014 - 31 December 2014. They all received a carbapenem during their stay. Of these, information of 76 patient files met the inclusion criteria. A further five patient files were excluded due to the use of more than one carbapenem (four patients received ertapenem at admission and were later switched to meropenem and the fifth was initiated on ertapenem and later changed to doripenem). The final amount of patient files that were included for statistical analysis was then 71 (see Fig.1).

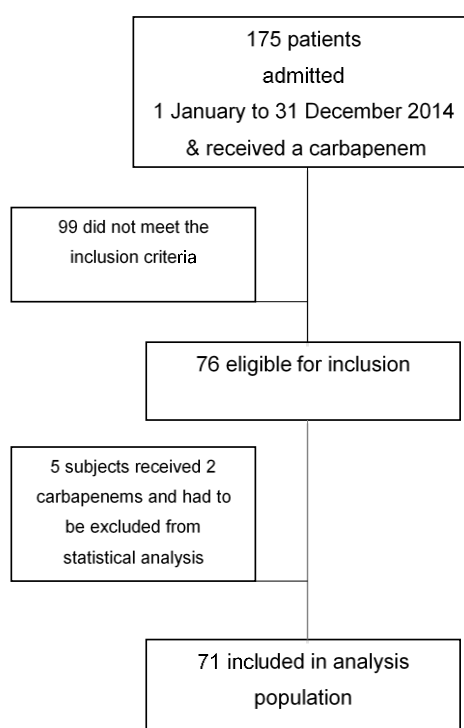


Figure 1: Selection of the study population

The demographic characteristics of the patient files analysed are summarised in Table 1. The majority of the study population were female (70.4%) with the mean age of the study population being 60.21 years. The ages ranged from 19 to 88 years.

Table 1. Patient demographics of the analysed cases

Age mean $\pm$ SD	60.21 $\pm$ 18.52
Gender n (%)	
Male	21 (29.6)
Female	50 (70.4)
	65

Table 2 represents the carbapenem treatment analysis. Ertapenem (70.4%) and meropenem (29.6%) were the drugs of choice with doripenem and imipenem not featuring at all. The independent *t*-test found no statistically significant difference in the mean age between patients on ertapenem (58.94 ± 17.64 years) and meropenem (63.24 ± 20.62 years) ($p=0.376$, $d=0.21$). Pearson's Chi-square test determined that there was no statistically significant association between gender and the prescribed medication ($p=0.653$, $V=0.05$).

Table 2. Treatment and carbapenem resistance of the analysed cases

Prescribed medication n (%)	
Ertapenem	50 (70.4)
Meropenem	21 (29.6)
Carbapenem resistance n (%)	
Yes	9 (12.7)
No	62 (87.3)

Carbapenem-resistant organisms were cultured in 9 of the patients (12.7%) (95% CI, 4.9 - 20.4) and the margin of error for this result was 7.74%. The independent *t*-test determined no statistically significant difference in the mean age of patients infected with resistant organisms (68.78 ± 22.57 years) versus those infected with carbapenem-susceptible bacteria (58.97 ± 17.73 years) ($p=0.139$). The difference between these two groups tended to an effect size of medium clinical significance ($d=0.43$). Furthermore, the Pearson's Chi-square test found no statistically significant association between gender and the resistance status of the patients ($p=0.792$, $V=0.031$).

Two resistant organisms, *Pseudomonas putida* and *Enterobacter cloacae* were detected in the resistant patient group. *Pseudomonas putida* was cultured in the one specimen and showed resistance towards imipenem, meropenem and doripenem. This specific organism is inherently resistant to ertapenem. The other specimen cultured *Enterobacter cloacae* (extended spectrum β -lactamase (ESBL) positive), which exhibited resistance towards ertapenem only. Seven of the nine patients exhibited resistance through carbapenemase production based on a rectal polymerase chain reaction (PCR) swab. No carbapenemase-producing Enterobacteriaceae (CPE) were found in these patients. The microbiology report stated in these cases that the absence of a viable culture to support the PCR result suggests that the risk for horizontal transmission of CPE was low. The contribution of each organism to the total prevalence could not be calculated.

DISCUSSION

The study provides evidence that carbapenem resistance is prevalent in this population of the private sector of the health system. The organisms that exerted carbapenem resistance in this study, *Enterobacter cloacae* and *Pseudomonas putida* form part of the group of bacteria that is a growing concern in a nosocomial healthcare environment.

The carbapenem resistance prevalence detected in the study shows similarities to published evidence from similar settings around the world. The CRE prevalence was 10.9% (95% CI, 7.7 - 14.7) from January to April 2014 in a Mexican hospital.²³ A study that investigated CRE in Israeli post-acute care hospitals detected that 12.5% of patients were carriers of CRE in 2011.²⁴ A hospital in Uganda showed a prevalence of 10.3% for the period September 2013 to June 2014.²⁵ This is in contrast to another setting in Uganda where a higher prevalence of resistance to meropenem (18.4%) was observed from January 2013 to March 2014.²⁶ Analysed samples in a community-based hospital Korea reported much lower levels with a CRE prevalence of 1.6%.²⁷ An analysis of 30 studies from 21 countries found significant heterogeneity in the reported resistance levels, where the overall carbapenem resistance ranged from 2% to 53%, with an average of 9%.⁵ Surveillance programmes of the general population in the United States found the incidence of CRE to be 2.93 per 100 000 population (95% CI, 2.65 - 3.23).²⁸

Table 3. Prevalence of carbapenem-susceptible organisms

Organism	Percentage of carbapenem-susceptible isolates/ carbapenem			
	Ertapenem		Imipenem/ Meropenem/ Doripenem	
	Pretoria	All cities	Pretoria	All cities
<i>Klebsiella pneumoniae</i>	94%	90 - 100%	96%	90 - 100%
<i>Acinetobacter baumannii</i> complex	-	-	28%	27 - 100%
<i>Pseudomonas aeruginosa</i>	-	-	46 - 52%	53 - 91%
<i>Enterobacter cloacae</i> complex	94%	72 - 100%	97%	92 - 100%
<i>Escherichia coli</i>	100%	99.9 - 100%	100%	99.9 - 100%
<i>Klebsiella pneumoniae</i>	94%	90 - 100%	96%	90 - 100%
<i>Acinetobacter baumannii</i> complex	-	-	28%	27 - 100%
Carbapenem-resistant Enterobacteriaceae	No data	No data	18%	7 - 22%

South African private sector data collected by SASCM found that only 7% to 22% of Enterobacteriaceae isolates were susceptible to imipenem, meropenem and/or doripenem during January to December 2014.²⁹ When considering data collected from the same geographical area as where the study hospital is located, a mere 18% of Enterobacteriaceae were susceptible to a carbapenem (Table 3).²⁹

Carbapenem resistance can be acquired by a variety of species. The carbapenem-resistant organisms detected in the study falls into the group of priority pathogens recently identified by the World Health Organization's (WHO) in the fight against antibiotic-resistant bacteria.¹¹ All carbapenem-resistant pathogens were given the critical priority ranking and include amongst others *Enterobacter* spp. and *Pseudomonas aeruginosa*.¹¹

Enterobacter cloacae has been one of the most common *Enterobacter* spp. causing nosocomial infections in recent years.³⁰ The species form part of the ESKAPE pathogens (*Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa* and *Enterobacter cloacae* complex and *Escherichia coli*), which were described as the main contributor to the health human infection problem.^{29,31} *Enterobacter cloacae* belong to the Enterobacteriaceae family and the prevalence of carbapenem resistance is therefore normally recorded as part of the CRE grouping.²⁸ An active surveillance of CRE conducted among individuals living in one of seven US metropolitan areas showed that 75/599 CRE cases (12.5%; 95% CI, 9.8 - 15.2) were *Enterobacter cloacae*.²⁸ Data collected in South Africa during 2014 found that 9.6% of CRE were carbapenem-resistant *Enterobacter cloacae* and these isolates' susceptibility rates ranging from 72% up to 100% (Table 3).²⁹ Separate data on the species found that the majority (96%) of *Enterobacter cloacae* strains isolated from 24 patients in the Eastern Cape province showed resistance to ertapenem, but not to imipenem and meropenem.³¹

The second carbapenem-resistant pathogen detected in the study, *Pseudomonas putida*, has over time become an increasingly encountered threat to humans.³² A study conducted at two Korean hospitals in 2012 reported eighteen cases of *Pseudomonas putida* bacteraemia with high rates of carbapenem resistance and mortality at two Korean hospitals. It found that 22% and 23% of the *Pseudomonas putida* isolates were resistant to imipenem and meropenem respectively.³² The 30-day mortality rate of these patients infected with carbapenem-resistant *Pseudomonas putida* bacteraemia was 39% to 40% compared to 38% in patients with carbapenem-susceptible resistant *Pseudomonas putida*.³² The prevalence of carbapenem resistance in *Pseudomonas putida* in South Africa is unknown at this stage as no publications on the prevalence thereof in human isolates could be found.

The simultaneous emergence of carbapenemase-producing *Pseudomonas putida* and *Pseudomonas aeruginosa* in Spain suggest an underestimated role of *Pseudomonas putida* as a nosocomial reservoir of transferable resistance determinants.³³ *Pseudomonas aeruginosa* is one of the most relevant nosocomial pathogens, especially in intensive care settings.³³ A striking feature of this pathogen is its capacity for the acquisition of antimicrobial resistance through horizontal transfer of class B carbapenemases genes, which has the capacity to hydrolyse all β -lactams.^{33,34} The presence of carbapenem-resistant *Pseudomonas putida* is therefore important when these qualities of *Pseudomonas aeruginosa* are kept in mind. *Pseudomonas putida* have the potential as a long-lasting reservoir of transferable β -lactamase.^{33,34} Carbapenem resistance genetic elements are not only transferred from *Pseudomonas putida* to *Pseudomonas aeruginosa* recipients, but these genetic elements are then shared between *Pseudomonas aeruginosa* isolates.³⁵ The relevant role of *Pseudomonas putida* as a nosocomial reservoir of resistance determinants is also underestimated due to the lack of active surveillance in environmental *Pseudomonas* species and their misidentification as *Pseudomonas aeruginosa*.³³ A retrospective bacterial re-identification of *Pseudomonas aeruginosa* isolates found that some of these isolates were in fact *Pseudomonas putida* isolates.³³

Antibiotic consumption is one of the contributing factors to carbapenem resistance and a clear correlation has been observed between increasing carbapenem use and increasing resistance in Enterobacteriaceae.^{3,4} Patients in this investigation were treated with either ertapenem or meropenem and none of the analysed population received doripenem or imipenem. The attending physician, based on best evidence, clinical expertise and patient circumstances, makes the choice of a carbapenem for an individual patient. The most noticeable difference between ertapenem and the rest of the carbapenem class is the limited efficacy of ertapenem against *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Stenotrophomonas maltophilia*.³⁶ A previous review suggested a lack of association between ertapenem use and changes in pseudomonal susceptibility to carbapenems.³⁷ However, a recent large case-control study found that carbapenem resistance was increased in patients who received ertapenem after the index specimen date and not prior to that. This pattern was not observed with other antibiotics such as imipenem or meropenem.³⁸ Findings as such are in contrast to previous analyses, which compared collections of isolates to grouped antibiotic usage instead of directly correlating the individual specimen and the antibiotic received by that specific patient.³⁸

The increase carbapenem-resistant *Acinetobacter baumannii* (CRAB) has on the other hand, been strongly correlated with an increased annual use of antipseudomonal carbapenems.³⁹ The use of other classes analysed in this study, antipseudomonal cephalosporins, antipseudomonal fluoroquinolones and β -lactam- β -lactamase inhibitor combinations with an antipseudomonal

effect, showed no significant association between increased use and the annual increase in CRAB.³⁹

The isolation of carbapenemases has been associated with therapy that included β -lactam- β -lactamase inhibitor combinations or with a combination of fluoroquinolone and a carbapenem.² Antibiotics can influence resistance through several mechanisms like changes in cell permeability, efflux or alterations in the antibiotic target, and horizontal transfer of resistance genes.

LIMITATIONS

The study has several limitations. Selective information bias might have occurred due to the retrospective design. A substantial percentage (47%) of patients screened for initial inclusion had to be excluded due to a lack of microbiology results. This could have been as a result of microbiology reports that were not filed in the patient hospital files or due to third party insurance restrictions that prevent attending physicians to submit species for laboratory analysis. The design of the study to include patients treated with a carbapenem and no other antibiotics was based on the high use of carbapenems in the hospital and the established link of carbapenem use and resistance. The literature search revealed that other antibiotic classes might also be implicated in carbapenem resistance. Patients infected with carbapenem-resistant bacteria might have been excluded based on the above-mentioned factors. These factors have the potential to influence the reported size of the prevalence of carbapenem resistance in the study population.

CONCLUSION AND RECOMMENDATIONS

Study results strengthen the fact that carbapenem resistance is likely to be prevalent at all private sector hospitals in South Africa. The heterogeneity in the current carbapenem prevalence between local and international publications in South Africa, confirm recent observations that resistance patterns are not necessarily reflective of the true prevalence of carbapenem resistance in South Africa. The question of whether a prevalence of 12.7% as seen in this study, would be observable in a similar private hospital setting can therefore only be speculated on.

The main future research direction suggested by this study is the expansion of the study population through the inclusion of patients treated with all antibiotic classes and not limited to carbapenems only. The lack of published surveillance data in the private sector necessitates that the study setting should include a larger geographical area of the private sector. A prospective design, rather than a retrospective design can exclude the confounder of possible information bias.

Conflict of interest

Ms M de Kock is an employee at the hospital where the study was conducted. The authors do not have any financial or personal conflict of interest to declare.

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3.4 Chapter summary

This chapter discussed the significance of the results of the collected data of the empirical investigation and made recommendations based on the findings and limitations of the study.

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

4.1 Introduction

This chapter will conclude on the key findings from the literature search and empirical investigation to provide recommendations for future research.

4.2 Dissertation objectives

The objective of the dissertation was to assess the prevalence of carbapenem-resistant bacteria in patients admitted to a private hospital in Daspoort, Tshwane. Specific objectives were set for the literature review and the empirical investigation to meet this aim and answer the research questions, as set out in the first chapter.

The formulated objectives for the literature review were aimed at:

- Analysing the value of the carbapenem class in the treatment of infectious diseases.
- Determining the prevalence of carbapenem resistance at national and international level.
- Investigating the prevalence of specific carbapenem-resistant organisms and the mechanism through which resistance is exerted.
- Investigating the risk factors for acquired carbapenem resistance.
- To evaluate the types of data extraction instruments utilised in quantitative, descriptive (non-experimental), cross-sectional retrospective investigations using the same approaches and methods as in the empirical investigation to determine carbapenem resistance.

The formulated objectives for the empirical study were firstly, to determine the prevalence of carbapenem resistance and secondly, to determine the specific organisms that exhibited resistance to carbapenems in patients where a carbapenem was prescribed during their stay in the hospital.

4.3 Key findings

4.3.1 Literature review

4.3.1.1 Analysis of the value of the carbapenem class

The literature search revealed that carbapenems still remain an important class of antibiotics and is currently referred to as the last-line of antimicrobials because it has the broadest

spectrum of activity. The literature review identified gram-negative organisms as a common source of invasive infections, which is associated with a higher burden on morbidity. A meta-analysis investigating the epidemiology of carbapenem-resistant Enterobacteriaceae (CRE) in Asia noted the increasing prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae, which resulted in carbapenems becoming a drug of choice for the management of multidrug-resistant (MDR) Enterobacteriaceae. The efficacy and safety of the class has made it suitable as the last resort option for the treatment of MDR Enterobacteriaceae (Xu *et al.*, 2015:376).

A recent report with the aim to assess antibiotic consumption between 2000 and 2010 showed that carbapenem prescriptions had a significant increase of 45% (Kamata *et al.*, 2015:596; Van Boeckel *et al.*, 2014:745). A global rise in the burden of methicillin-resistant *Staphylococcus aureus* and ESBL gram-negative bacteria partly explain this increase in carbapenem use (Van Boeckel *et al.*, 2014:747). The antibiotic consumption for the management of five gram-negative bacteria *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* was analysed for the period of 2003 to 2011. Carbapenem consumption by year increased from 4.9 to 44.1 DDD/1000 patient-days over this period (Lee *et al.*, 2015:650).

Development of new antibiotics for the use against gram-negative hospital pathogens is also problematic due to its mechanism of resistance that negates a broad spectrum of structural classes. The first report of resistance against the recently licensed cephalosporin/ β -lactamase inhibitor combination, ceftazidime/avibactam, has already emerged (Goodlet *et al.*, 2016:1814; Shields *et al.*, 2017). The preservation of the carbapenem class as treatment option remains a priority.

The literature review met the first objective and established that the carbapenem class remains an important treatment option especially in the management of infections caused by gram-negative (including MDR) pathogens and in light of the difficulty to develop new antibiotics.

4.3.1.2 Prevalence of carbapenem resistance at national and international level

The search for publications on the prevalence has proven to be particularly challenging. The major theme uncovered in many publications is the lack of representative data, the consequence of many factors.

The difference in the amount of evidence between different regions and countries worldwide was also noticeable. The majority of information was found in Europe and the Americas, with Africa lacking the most in publications on this topic. Many organisations appear to be involved in antimicrobial resistance (AMR), but recent mapping of stakeholders revealed that few are

actually concerned in actual collection of carbapenem resistance statistics (ReAct, 2016b). Many programmes that are listed to be involved in surveillance have no recorded data, either on their website or in scientific journals. This highlights viewpoints by the World Health Organization (WHO) that community-acquired infections are in most cases underrepresented, leading to underreporting of resistance in important patient groups (WHO, 2014a:70). The heterogeneity of data presented in the literature review impacted on the comparability of the prevalence rates across continents and countries.

South Africa has had a good start in surveillance, but has much to do to increase its efforts (Bamford, Brink, *et al.*, 2011:579). The National Department of Health (NDoH) has already prioritised the optimisation of surveillance in 2014 (National Department of Health, 2014:10). A meeting held in 2016 confirmed the strengthening of surveillance in CRE as a priority surveillance area and the standardisation of susceptibility reporting (FIDSSA, 2016c:5). The magnitude of carbapenem resistance can be better managed, should all hospitals and laboratories report their carbapenem-resistant cases more often (Osei-Sekyere, 2016).

The literature search on the prevalence of carbapenem resistance was lengthy and sifted through an innumerable amount of data to ensure an objective reflection of the evidence available. The full picture of global and national prevalence of carbapenem resistance could not be reflected in the literature review due to a lack of recorded data and not because the objective was not met.

4.3.1.3 Prevalence of specific carbapenem-resistant organisms

Searches of databases with the keywords and phrases create the impression of a myriad of information. However, further analysis of these sources found that the biggest shortcoming in many publications were the unsubstantiated claims of the prevalence of carbapenem resistance without any actual data. Articles that did report on the prevalence of resistance did not do so in uniform units of measure (ECDC, 2014:8; Guh *et al.*, 2015:1482; NICD-NHLS, 2015a:33; Public Health Agency, 2016:22; Righi *et al.*, 2017:668; WHO, 2015c:2). Some publications reported in percentages while others reports in patient days (ECDC, 2014:8; Guh *et al.*, 2015:1482; Public Health Agency, 2016:22; Righi *et al.*, 2017:668). Some organisations only reported the prevalence of organism-specific carbapenem resistance (ECDC, 2014; NICD-NHLS, 2015a; Public Health Agency, 2015; WHO, 2014a; Xu *et al.*, 2015:380). Most members of the European Union (EU) have well established observation systems in place to monitor invasive infections caused by the eight clinically important bacterial pathogens. The European surveillance report therefore, contained the most detailed information on the prevalence of specific carbapenem-resistant organisms (ECDC, 2014). The lack of a uniform internationally standardised method on the reporting of data resulted in some organisations giving preference to investigate the

prevalence of certain resistant bacteria, whilst others did not. The Global Antimicrobial Resistance Surveillance System (GLASS) was adopted in May 2015 at the World Health Assembly to enable the collection of standardised, validated and comparable data on AMR (WHO, 2015c:1). The project aims to publish its first report on the consolidated baseline data antibiotic resistance of samples collected from humans by the WHO Member States in 2018 (WHO, 2015c:4).

The objective for the prevalence of specific carbapenem-resistant bacteria was achieved. It again underscored the need for the implementation of the globally agreed collection and reporting of data.

4.3.1.4 Mechanisms and risk factors of acquired carbapenem resistance

The mechanisms of acquired carbapenem resistance and the ease in the transfer of these mechanisms between bacteria are well described in the literature. A variety of publications were found that investigated the risk factors for carbapenem resistance and carbapenem resistance mechanisms (Akinci *et al.*, 2005:320; Ling *et al.*, 2015; Routsis *et al.*, 2013:1255; Ulu *et al.*, 2015:220; Voor In 't Holt *et al.*, 2014:2631).

4.3.1.5 Approaches, methods and the types of data extraction

The literature search found no research that followed the same design, approach and methods as the investigation undertaken in this dissertation. Data extraction instruments could, therefore, not be compared.

4.3.2 Empirical investigation

4.3.2.1 Prevalence of carbapenem resistance

The study has determined the prevalence of carbapenem resistance in the study subjects. The mean age of patients that were infected with resistant organisms compared to those infected with carbapenem-susceptible isolates did not show a statistical significant difference. The effect size of the difference seen between the two groups is of medium clinical significance. No statistically significant association between gender and the resistance status of the patient were found. The objective of this part of the study was fulfilled.

4.3.2.2 Prevalence of carbapenem-resistant bacteria

The second formulated objective for the empirical study was to determine the specific organisms that exhibited resistance to carbapenems in patients where a carbapenem was prescribed during their stay in the hospital. The investigation uncovered two bacteria,

Pseudomonas putida and *Enterobacter cloacae* exhibiting resistance towards a carbapenem. *Pseudomonas putida* showed resistance towards imipenem, meropenem and doripenem and *Enterobacter cloacae*, exhibited resistance towards ertapenem. The majority (78%) of isolates exhibited resistance through carbapenemase production based on a rectal polymerase chain reaction (PCR) swab. No carbapenemase-producing Enterobacteriaceae (CPE) were found in these patients. The contribution of each organism to the total prevalence could not be calculated. The study sample size was calculated on the estimation that the study would at least have 80% power to detect the expected effects in the total prevalence of carbapenem resistance ($\alpha \leq 0.05$). It would be recommended to do a literature search first to gain an understanding of current available data on the contributions of individual resistant bacteria to the total prevalence of carbapenem resistance. This data can be used to calculate the sample size needed to achieve this objective. The second objective of the study was partially achieved.

4.4 Study strengths

4.4.1 Validity

Information bias was avoided through the extraction of information using the extraction instrument in the same manner for all patient files. Only one investigator, the researcher extracted the information and thereby minimised variability of extraction by more than one investigator. The data-collection instrument used for this study was assessed for face and content validity prior to commencement of the study. The threat to content validity was limited by ensuring that the measuring instrument listed all bacteria that have *in vitro* sensitivity (Table 1-1) against the carbapenems to minimise omission of information during the extraction process (Cunha & Cunha, 2013; Zhanel *et al.*, 2007).

The study was evaluated for confounding bias. No confounders were identified for the study population investigated. However, should a study be designed to include patients receiving other antibiotics, it should be kept in mind that the risk factors for acquired carbapenem resistance identified during the literature review could be confounders, mediators or effect modifiers of carbapenem resistance (Section 2.5). The design of such a study should aim to use a scoring system such as the Acute Physiology Assessment and Chronic Health Evaluation (APACHE II) to measure admission characteristics such as age and pre-existing organ dysfunction and routine physiological measurements such as temperature, blood pressure (BP) and Glasgow Coma Scale (GCS) to assess the severity of disease. Other factors to consider include concomitant disease, presence of indwelling devices, length of exposure to antibiotic treatment and the antibiotic class (Perez & Van Duin, 2013:228; Ulu *et al.*, 2015:219; Voor In 't Holt *et al.*, 2014:2631; Walker *et al.*, 2014:204).

4.4.2 Precision

The non-randomised consecutive sampling approach of the investigation was adhered to. A random sampling error is not expected as it was assumed that the patients were admitted to the hospital in a random manner between the two defined dates of the study period. Fewer participants (n=76) were included than the planned study sample size of 120 calculated for a significance level of $\alpha \leq 0.05$ and the power of the test set to 0.8. The calculated margin of error for the observed prevalence in the study was 7.74%, which is within the accepted margin of error (5 to 10%) for descriptive studies (Rodríguez Del Águila & González-Ramírez, 2014:487).

The threat of random measurement error were minimised before commencement of the study through an assessment by the co-authors of the study as well an evaluation of the data extraction form. The validity of the instrument was tested for consistency of data collection from the different files during the protocol evaluation of the data extraction form to minimised inconsistent validity.

4.5 Study limitations

4.5.1 Validity

Selection bias potentially occurred because 47% of patients that might have been suitable for inclusion, had to be excluded due to absence of a microbiology report in their file. The study was designed to include patients treated with a carbapenem based on the recognised link between carbapenem resistance and carbapenem use. The literature review identified that the treatment with majority of antibiotic classes increases the risk for carbapenem resistance.

The retrospective design poses a threat to the validity because of selective information bias.

4.5.2 Precision

The reliability of the data source (patient file) is unknown and might have contributed to the exclusion of patients that were actually eligible. This has the potential to influence the size of the prevalence of carbapenem resistance in the study population.

4.6 Conclusions on the empirical investigation

The strengths and limitations discussed in the previous section provide a good starting point to conclude firstly on the validity of the findings of the study and then the reproducibility of these results.

The strengths of the study support the fact that it will be likely to detect carbapenem resistance in adult patients treated with a carbapenem in a similar private hospital. The limitation regarding the reliability of the information in the patient file begs the question on how true the findings are on the proportion of carbapenem-resistant bacteria in the sample (12.7%) versus the study population. The heterogeneity in the prevalence of carbapenem resistance in not only South Africa but globally as well, creates an obstacle to validate the findings from this study. It is therefore only possible to conclude that carbapenem-resistant organisms are likely to be present in a similar population in other private hospitals, but not what the scope of the prevalence would be if a similar investigation were launched. The lack of association between the mean age and gender and resistance might be influenced by the factors just mentioned.

The carbapenem-resistant organisms detected in this investigation are part of the global critical priority list of clinically important bacteria identified by the WHO and EARS-Net (EARS-Net, 2015; WHO, 2017a:5). It is likely that these organisms will be detected in other private hospitals.

The contribution of each organism to the total prevalence could not be calculated. The study sample size was calculated to detect the expected effects in the total prevalence of carbapenem resistance. It is recommended to undertake a literature review to get an understanding of the contributing proportions to overall carbapenem resistance first, before an attempt can be made calculate the sample size.

4.7 Conclusions on the literature review

The most compelling aspect of the literature search was the amount of available evidence on the subject of carbapenem resistance and its influence on society, at both a social and financial level. The heterogeneity and disparities of the current surveillance efforts and published data have mandated world leaders and experts in infectious diseases to ensure that all role-players in this field urgently address these gaps. The literature is in unison on the importance of firstly, understanding the extent of the problem before the most effective solutions to the problem can be found. The literature has also clearly indicated that the threat of carbapenem resistance does not only eliminate the carbapenem class as an effective treatment, but also other alternative treatments, including newly launched non- β -lactam/ β -lactamase inhibitors. The study has highlighted the need for more investigations and publications on the topic of carbapenem resistance at all levels of healthcare in South Africa. Data collection and reporting should be according to nationally and internationally determined standards to ensure the comparability of data.

4.8 Recommendations for future research

The results from the study and the literature search have provided valuable insight on the topic of carbapenem resistance and future direction for research in this field.

The focal point from this critique suggests the expansion of the study population to select patients that received not only carbapenems but other classes as well. The methodology should be developed to record baseline characteristics of patients that include demographic information, disease and medical history and previous exposure to antibiotics. A scoring system such as the *APACHE II* can be used to assess acute physiological measurements and pre-existing organ dysfunction. Known risk factors for carbapenem resistance should be recorded and form part of the variables. The available evidence suggest that many common risk factors for carbapenem resistance can be addressed through appropriate infection control measures and dedicated antibiotic stewardship (Brink, Coetzee, Clay, Corcoran, *et al.*, 2012:600). This should also be considered as a mediator in the prevalence of carbapenem resistance.

A prospective rather than a retrospective design will increase the validity of the results. It is expected that a much larger sample size would be needed to account for confounding variables. It is therefore advisable to have a multicentre approach involving more private hospitals to ensure that the sample size can be reached and to increase the validity and precision of results. A more rigorous approach to the collection of specimens and dissemination of culture results are needed to avoid the exclusion of patients due to a lack of microbiology susceptibility data.

It is recommend that more hospitals in South Africa, whether in the private or public sector start to collect data based on these internationally agreed standards to establish a database to monitor where South Africa stands in relation to the rest of the world. It is clear that more publications are needed to evaluate the extent of resistance to ensure better antibiotic prescription practices to preserve the carbapenem class as a treatment option.

4.9 Chapter summary

The chapter has concluded on the key findings centred on the objectives set out at the start of this project. Valuable information was gained from this project to direct future research into this important topic. The research question could only be answered in part due to the absence of representative data on the spread and prevalence of carbapenem resistance in South Africa.

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ANNEXURE 1

STUDY PARTICIPANT ELIGIBILITY FORM PATIENT HOSPITAL CASE NUMBER:

FACTORS	ASSESSMENT			COMMENTS
Was the patient admitted between 1 May 2014 and 31 December 2014?	Yes  Include	Unclear  Exclude	No  Exclude	
Was patient treated with any of the following: imipenem or ertrapienem or meropenem or doripenem	Yes  Include	Unclear  Exclude	No  Exclude	
Is the patient 18 years and older?	Yes  Include	Unclear  Exclude	No  Exclude	
Is the patient pregnant or HIV positive?	Yes  Include	Unclear  Exclude	No  Exclude	
Does the file contain a microbiology report?	Yes  Include	Unclear  Exclude	No  Exclude	
Does the microbiology report contain sensitivity data for any of the carbapenems e.g. imipenem or ertrapienem or meropenem or doripenem	Yes  Include	Unclear  Exclude	No  Exclude	
FINAL DECISION 1 X "No" = EXCLUDE / 1 X "Unclear" = EXCLUDE				INCLUDE: ASSIGN PATIENT STUDY IDENTIFICATION

ANNEXURE 2

CODE SHEET STUDY TITLE			
	HOSPITAL CASE NUMBER	MEETS INCLUSION CRITERIA YES/NO	ASSIGNED PATIENT STUDY IDENTIFICATION
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ANNEXURE 3

Patient study
identification
Data abstractor
Date extracted

Rita de Kock

Study Title

Prevalence of carbapenem resistance in adult patients admitted to a private hospital in Daspoort, Tshwane

Researcher name Rita de Kock

CLINICAL DATA COLLECTION FORM

1. RELEVANT DEMOGRAPHIC DATA

1.1. Subject age	Years old	
1.2. Gender	Male (0) Female (1)	

2. PRESCRIBED MEDICINE

2.1. Doripenem (0)	
2.2. Ertapenem (1)	
2.3. Imipenem- cilastatin (2)	
2.4. Meropenem (3)	

MICROBIOLOGY RESULTS

Please tick the box according to the microbiology report results

3. Any organism resistant to any of the carbapenems

Yes (0)	No (1)

4. ORGANISM SPECIFIC RESISTANCE TO ANY CARBAPENEM

AEROBIC GRAM-POSITIVE BACTERIA

- 4.1. *Enterococcus faecalis*
- 4.2. *Enterococcus faecium*
- 4.3. *Staphylococcus aureus* (MSSA)
- 4.4. *Staphylococcus aureus* (MRSA)
- 4.5. *Staphylococcus epidermidis*
- 4.6. *Streptococcus pyogenes*
- 4.7. *Streptococcus agalactiae*
- 4.8. *Streptococcus pneumoniae*
- 4.9. *Listeria monocytogenes*

Yes (0)	No (1)	If yes, specify resistance to which carbapenem Doripenem (0), Ertapenem (1), Imipenem- cilastatin (2), Meropenem (3)

AEROBIC GRAM-NEGATIVE BACTERIA

- 4.10. *Acinetobacter* spp. (Please specify organism)

- 4.11. *Citrobacter freundii*
- 4.12. *Enterobacter aerogenes*
- 4.13. *Enterobacter cloacae*
- 4.14. *Escherichia coli*
- 4.15. *Haemophilus influenza*
- 4.16. *Haemophilus influenza* (ESBL)

AEROBIC GRAM-NEGATIVE BACTERIA cont'

- 4.17. *Klebsiella pneumoniae*
- 4.18. *Klebsiella pneumoniae* (ESBL)
- 4.19. *Klebsiella oxytoca*
- 4.20. *Moraxella catarrhalis*
- 4.21. *Morganella morganii*
- 4.22. *Neisseria gonorrhoeae*
- 4.23. *Neisseria meningitidis*
- 4.24. *Proteus mirabilis*
- 4.25. *Proteus vulgaris*

Yes (0)	No (1)	If yes, specify resistance to which carbapenem Doripenem (0), Ertapenem (1), Imipenem- cilastatin (2), Meropenem (3)

Patient study
identification
Data abstractor
Date extracted

Rita de Kock

Study Title

Prevalence of carbapenem resistance in adult patients admitted to a private hospital in Daspoort, Tshwane

Researcher name Rita de Kock

CLINICAL DATA COLLECTION FORM

- 4.26. *Pseudomonas aeruginosa*
4.27. *Salmonella* spp.
4.28. *Serratia marcescens*
4.29. *Shigella* spp. (Please specify organism _____)
4.30. *Stenotrophomonas maltophilia*

ANAEROBIC BACTERIA

- 4.31. *Bacteroides fragilis*
4.32. *Clostridium difficile*
4.33. *Clostridium perfringens*
4.34. *Fusobacterium* spp. (Please specify organism _____)
4.35. *Lactobacillus* spp. (Please specify organism _____)
4.36. *Peptostreptococcus* spp. (Please specify organism _____)
4.37. *Prevotella* spp. (Please specify organism _____)

Yes (0)	No (1)	If yes, specify resistance to which carbapenem

4.38. ANY ORGANISM NOT SPECIFIED ABOVE (Please specify)

	Yes (0)	No (1)	If yes, specify resistance to which carbapenem

ANNEXURE 4

Author Guidelines – South African Family Practice

Submissions can only be made online at www.editorialmanager.com/safpj. Authors need to register online with the journal prior to submitting a manuscript. Once registered, simply log in and begin an easy 5 step process to upload your manuscript. All manuscripts must be submitted in MS Word®, Open Office, or RTF format using Times New Roman font size 10 and single-spacing. Headings must be in Bold.

The author must always retain a copy. All the named authors must have approved the final manuscript. Pages should be numbered consecutively in the lower right corner. Please note that the Original Research section will follow a ";print-short, web-long"; policy, which means that only the abstracts will be published in print, with the full article published on the web. Some review articles may also be published under these provisions.

The following contributions are accepted (word counts exclude abstracts, tables and references):

1. Original research (Between 1000 and 3500 words).
2. Letters to the Editor (Up to 400 words).
3. Scientific Letters (Less than 600 words): A short abstract is required (125-150 words) and should be structured under the following headings: background, methods, results and conclusion. One table or graph and not more than 5 references.
4. Review/CPD articles (Up to 1800 words): Most review articles are published as part of the continuous professional development (CPD) programme of SAFP. A scientific editor is appointed to approve topics, invite authors and to review the articles before they are independently peer-reviewed. All articles are reviewed by a family physician as well a topic specialist. Review articles outside the CPD programme are welcomed. Once accepted they may be published in full in the printed journal OR a 250 word abstract will be published in print with the full article available online.
5. Opinions (Open Forum) (Between 1000 and 3500 words)
6. Editorials (Between 600 - 800 words): Scientific editorials can be used to highlight progress in any scientific field related to family medicine.

Please consult the [Section Policies](#) for more details regarding CPD articles.

Format

Title page. All articles must have a title page with the following information and in this particular order: Title of the article; surname, initials, qualifications and affiliation of each author; The name, postal address, e-mail address and telephonic contact details of the corresponding author; at least 5 keywords. Please do not use capital letters only for headings and names, but stick to the normal use of capital letters.

Abstract. All articles should include an abstract. The structured abstract for an Original Research article should be between 200 and 250 words and should consist of four paragraphs labelled "Background, Methods, Results, and Conclusions".

Only the abstract of Original Research articles will be published in print, and the abstract with the full article will be published online. It should briefly describe the problem or issue being addressed in the study, how the study was performed, the major results, and what the authors conclude from these results.

The abstracts for other types of articles should also be no longer than 250 words and need not follow the structured abstract format.

Keywords. All articles should include keywords. Up to five words or short phrases should be used. Use terms from the Medical Subject Headings (MeSH) of Index Medicus when available and appropriate. Key words are used to index the article and may be published with the abstract.

Acknowledgements. In a separate section, acknowledge any financial support received or possible conflict of interest. This section may also be used to acknowledge substantial contributions to the research or preparation of the manuscript made by persons other than the authors.

References. Cite references in numerical order in the text, in **superscript** format. Do not use brackets. In the References section, references must be numbered consecutively in the order in which they are cited, not alphabetically.

The style for references should follow the format set forth in the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals"; prepared by the International Committee of Medical Journal Editors.

Abbreviations for **journal titles** should follow Index Medicus format. Authors are responsible for the accuracy of all references. Personal communications and unpublished data should not be referenced. If essential, such material should be incorporated in the appropriate place in the

text. List all authors when there are six or fewer; when there are seven or more, list the first three, then ";et al.";

When citing URLs to web documents, place in the reference list, and use following format: Authors of document (if available). Title of document (if available). URL. (Accessed [date]).

The following are sample references:

1. London L, Baillie R. Notification of Pesticide Poisoning: Knowledge, Attitudes and Practices of Doctors in the Rural Western Cape. S A Fam Pract 1999;20(1):117-20.
2. FDA Talk Paper: <http://www.fda.gov/bbs/topics/ANSWERS/2002/ANS01151.html> (Accessed 04/10/2002).

[Click here](#) for more sample references.

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**Modified from: Davidoff F, et al. Sponsorship, Authorship, and Accountability. (Editorial) JAMA 2001; 286(10)*

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