CARBAPENEM RESISTANT ENTEROBACTERALES (CRE) AT HELEN JOSEPH HOSPITAL

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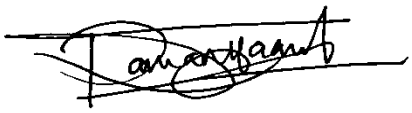
A research report submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine

Johannesburg, 2021

DECLARATION

I, Romana Jassat, hereby declare that this research report is my own, unaided work. It is being submitted for the degree of Master of Medicine in the branch of Internal Medicine, at the University of the Witwatersrand, Johannesburg. It is submitted in the submissible format with a protocol and extended literature review as recognized by the Faculty of Health Sciences. It has not been submitted before for any degree or examination at this or any other university.



Signature: ……

Date: ……15/09/2021……

Place: ……Johannesburg……...

DEDICATION

To my family

PRESENTATIONS ARISING FROM THIS RESEARCH

ETHICAL CONSIDERATIONS

Permission for this retrospective study was obtained from Dr M. Mukhansi (Chairperson of the Ethics and Research Committee, Helen Joseph Hospital), Dr M. Hunter (Acting Head of Department, Internal Medicine, Helen Joseph Hospital) as well as Dr R. Chomba (Clinical Microbiologist, National Health Laboratory Service). Permission was also obtained from the Human Research Ethics Committee of the University of Witwatersrand (Clearance number – M180120).

ABSTRACT

Background: Antimicrobial Resistance (AMR), in particular Antibacterial resistance (ABR) is a growing public health concern. The emergence of resistant Gram-negative bacteria coupled with a dwindling antibiotic armamentarium poses a significant threat. In South Africa, there is an urgent need to evaluate this situation and due consideration should be given to prevent the emergence of multidrug resistant (MDR), extensively drug resistant (XDR), and pandrug resistant (PDR) organisms.

Objectives: The objective of this study was to describe both the clinical and microbiological characteristics of patients at Helen Joseph Hospital (HJH) with confirmed Carbapenem resistant Enterobacterales (CRE) infection and/or colonization. In addition, infection prevention and control practices were highlighted in this study.

Methods: A single centre retrospective descriptive study was undertaken at a tertiary public sector hospital in Johannesburg, South Africa. All patients with a positive CRE culture collected retrospectively in a 12 month study period were included. Microbiological data was obtained from the National Health Laboratory Service (NHLS) database and clinical data from patient records. A quantitative method of data analysis was performed.

Results: A total of 106 patient files were reviewed. Demographically, 52.83% of patients were males while females represented 47.17%. Ethnically, 64.15% of patients were of African descent. The majority of patients were admitted to the medical wards (35.85%), while 34.9% of all CRE's were cultured in an intensive care setting (27 patients in the Intensive care unit (ICU) and 10 patients in High care)**.** The predominant site of culture was urine and blood representing 35.85% and 26.42% respectively. The dominant CRE organism subtype was *Klebsiella pneumoniae* (94/106; 88.68%), followed by *Enterobacter cloacae* (6.6%) and *Escherichia coli* (2.83%). *Bla*$_{\text{OXA-48}}$ & variants represented the predominant CRE genotype (70.75%), followed by *bla*$_{\text{NDM}}$ (10.38%). Significant differences in resistance patterns between *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM}}$

isolates to carbapenems were noted with 66.67% of *bla*$_{NDM}$ isolates being resistant to imipenem, in contrast to *bla*$_{OXA-48}$ with 12% (p<0.001). Seventy-five percent of the *bla*$_{NDM}$ isolates were resistant to meropenem, compared to 21.33% of *bla*$_{OXA-48}$ isolates (p=0.001). Patients with a previous hospital admission in the last six months were two times more likely to demise (p=0.042). Admissions to the ICU/ high care wards were three times more likely to demise than those admitted in other wards (p=0.009).

Conclusion: There was a high prevalence of CRE in our setting with the three predominant bacteria being *Klebsiella pneumoniae* followed by *Enterobacter cloacae* and *Escherichia coli*. Genotypically, *bla*$_{OXA-48}$ & variants predominates, while *bla*$_{NDM}$ represented the second commonest carbapenemase. Significant differences in the resistance patterns between *bla*$_{OXA-48}$ and *bla*$_{NDM}$ isolates to imipenem and meropenem were observed. Previous hospitalization in the last six months and current admission to an intensive care setting independently predicted mortality. In addition, this study highlighted the importance of infection prevention and control measures in ensuring optimum care of CRE patients.

ACKNOWLEDGEMENTS

The support and assistance of the following persons in preparation of this research report is gratefully acknowledged:

My Supervisors: Professor Prudence Ive, Dr Jeremy Nel and Dr Rispah Chomba for their guidance and supervision throughout this research project.

CONTENTS

LIST OF ABBREVIATIONS

AMR	**Antimicrobial resistance**
ABR	**Antibacterial resistance**
ARV	**Antiretroviral**
BC	**Blood culture**
CDC	**Centre for Disease Control**
CD4	**Cluster of differentiation 4**
CHARM	**Centre for Healthcare Associated Infections, Antimicrobial resistance and Mycoses**
CI	**Confidence interval**
CLSI	**Clinical & Laboratory Standards Institute**
CFU	**Colony-forming unit**
CRE	**Carbapenem resistant Enterobacterales**
CP-CRE	**Carbapenemase-producing carbapenem resistant Enterobacterales**
CRP	**C-reactive protein**
CRACKLE	**Consortium on Resistance against Carbapenems in Klebsiella and other Enterobacteriaceae**
CMJAH	**Charlotte Maxeke Johannesburg Academic Hospital**
ESBL	**Extended spectrum β-lactamase**
ED	**Emergency department**
GNB	**Gram-negative bacilli**
GES	**Guiana extended-spectrum β-lactamase**
GERMS-SA	**Group for Enteric, Respiratory and Meningeal Disease Surveillance-South Africa**
GLASS	**Global Antimicrobial Resistance Surveillance System**
HCU	**High care unit**

HIV	**Human immunodeficiency virus**
HJH	**Helen Joseph Hospital**
ICU	**Intensive care unit**
ID	**Infectious diseases**
IQR	**Interquartile range**
IMP	**Imipenemase**
IPC	**Infection prevention and control**
KPC	**K. Pneumoniae carbapenemase**
MDR	**Multidrug resistant**
MIC	**Minimum inhibitory concentration**
MBL	**Metallo-β-lactamase**
MHT	**Modified Hodge test**
NDM	**New Delhi metallo-β-lactamase**
NHLS	**National Health Laboratory Services**
NICD	**National Institute for Communicable Diseases**
Non CP-CRE	**Non-carbapenemase producing CRE**
OXA-48	**Oxacillinase-type carbapenemase**
OR	**Odds ratio**
PCR	**Polymerase chain reaction**
PDR	**Pandrug resistant**
UTI	**Urinary tract infection**
VIM	**Verona integron-encoded metallo-β-lactamase**
VL	**Viral load**
WCC	**White cell count**
WHO	**World Health Organization**
XDR	**Extensive drug resistant**

CHAPTER 1: PROTOCOL WITH EXTENDED LITERATURE REVIEW

1.1 INTRODUCTION

Antimicrobial Resistance (AMR), in particular Antibacterial resistance (ABR) is a growing public health concern. The World Health Organization (WHO), in the latest Global Antimicrobial Resistance Surveillance System (GLASS) report conducted in the period 2017 to 2018, alludes to the serious consequences of increasing antibiotic resistance and recognizes the need for urgent and immediate action (1).

Resistant Gram-negative bacteria, in particular, pose a significant threat. Unlike resistant Gram-positive bacteria, where therapeutic options remain available, there is a lack of new, innovative, therapeutic options for Gram-negative bacteria in the near or foreseeable future (2). It is worthwhile mentioning that the last novel drug class effective against Gram-negative organisms (being the antibiotics belonging to the sub-class quinolones) was in fact developed more than 50 years ago, with little in the way of drug innovation since then (3). The consequence, is the emergence of multidrug resistant (MDR), extensive drug resistant (XDR), and pandrug resistant (PDR) organisms in the face of a rapidly dwindling antibiotic armamentarium (4). Coupled with this dilemma, is the knowledge that Gram-negative bacteria already possess at their disposal a wide and extensive range of resistance mechanisms, capable of targeting antibiotics using single or multiple pathways (5).

Furthermore, the development of carbapenem resistance has been fuelled by the worldwide rise in extended spectrum β-lactamases (ESBLs) possessing capabilities of hydrolysing all β-lactams with the exception of the carbapenems. This has resulted in the increased and injudicious utilization of the carbapenem antibiotics from as far back as twenty years ago (6). In a study conducted by Bamford *et al* over a twelve month period (January to December 2010), the documented ESBL prevalence rate for

Klebsiella pneumoniae in South African public sector hospitals ranged from 55% to as high as 74% (7). This highlights the effect of antibiotic selective pressure on the development of resistance.

All things considered, it is evident that the pendulum between resistant organism and effective antibiotic therapy swings precariously, and only time will tell which entity will ultimately emerge victorious.

1.2 EXTENDED LITERATURE REVIEW

Enterobacterales species (also known as Enterobacteriaceae) are rod-shaped in appearance and are classified in the sub-category of Gram-negative organisms. Amongst the dominant species that constitute this group are the *Klebsiella*, *Escherichia coli*, and *Enterobacter* species. *Proteus, Morganella, Providentia* and *Serratia* species also form part of the Enterobacterales group (8). They are considered to be ubiquitous colonizers of the human intestinal tract and are known to cause a wide range of human illnesses. The spectrum of disease is inclusive of, but not limited to, bodily sites involving the respiratory tract, urinary tract, and blood-stream, and disease severity may range from benign to life-threatening infection (6,9). Enterobacterales are capable of spreading via direct or indirect contact of mucosal surfaces with infectious organisms or through contaminated food and water (faecal-oral) route. In particular, transmission via hand carriage is a well-documented mode of spread (9).

Carbapenem resistant Enterobacterales (CRE), formerly known as Carbapenem resistant Enterobacteriaceae (CRE) has been categorized by the Centre for Disease Control (CDC) as those Enterobacterales that are non-susceptible to any carbapenem antimicrobial. Non-susceptibility encompasses both intermediate susceptibility as well as resistant isolates (10). Although carbapenemase production is known to be the most

important mechanism in Enterobacterales resistance (8), this definition is inclusive of both carbapenemase-producing CRE (CP-CRE) as well as non-carbapenemase producing CRE (Non-CP-CRE). Pathogen resistance is interpreted in the context of a minimum inhibitory concentration (MIC), which is a marker of antibiotic efficacy with resistant isolates having MIC's greater than their breakpoints, indicative of therapeutic failure (11). The 2012 CDC surveillance definition for CRE utilizes a phenotypic definition which defines an MIC of ≥2 μg/ml for ertapenem or ≥4 μg/ml for imipenem, meropenem, or doripenem as being a resistant isolate. In addition, any Enterobacterales that produce carbapenemases are considered to fall under the category of resistant CRE. Some Enterobacterales (such as the *Proteus, Providencia* and *Morganella morganii species*),however, are intrinsically less susceptible to the carbapenem drug imipenem and in these cases resistance to another carbapenem aside from imipenem is necessary (10).

The Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines, which were current when the study was conducted, utilized a minimum inhibitory concentration (MIC) of ≤0.5 to define an isolate susceptible to ertapenem. An MIC of ≤1 was used in the case of imipenem and meropenem susceptibility. A MIC of ≥2 for ertapenem and ≥ 4 for imipenem and meropenem defined resistance (11). The intermediate category was not considered as a criteria for defining resistance in this data set. This was the phenotypic definition that was utilized in the National Health Laboratory Service (NHLS) laboratories during the period of the study. Other antimicrobial committees, such as the European Committee on Antimicrobial Susceptibility Testing (EUCAST), utilize alternative break-point criteria for defining carbapenem resistance which was not considered in this study (12).

The mechanisms producing resistance in Enterobacterales are numerous. However, the broad principles governing resistance can be outlined by two distinct pathways, namely,

non-carbapenemase-mediated resistance and carbapenemase-mediated resistance (6). The first pathway (non-carbapenemase mediated resistance) involves a deficiency or modification of porin expression resulting in the inadequate uptake of antibiotics into the bacterium. Porins are essential components of the outer cell membrane of Gram-negative bacteria and provide permeability in the form of hydrophilic channels. These are located within an otherwise hydrophobic cell membrane and permit the differential uptake of certain substances (including antibiotics) into the bacterium. Any alteration or mutations in porin expression, including a modification of the porin protein or loss of porin expression can thus result in decreased membrane permeability and influence antibiotic uptake (6). Coupled to this mechanism is the production of β-lactamase inactivating enzymes which hydrolyse antibiotics as well as the development of efflux pumps within the cell membrane which export therapeutic drugs out of the bacterium (5,6). These mechanisms act synergistically and together contribute toward Enterobacterales resistance (13).

Carbapenemase-mediated resistance is the second mechanism implicated in Enterobacterales resistance. Carbapenemases are β-lactamases which are capable of degrading and hydrolysing all β-lactams, including the carbapenems (13). The molecular classification of the carbapenemases, based on amino acid homology, is called the Ambler classification. It divides the carbapenemases into three molecular classes, namely the Ambler class A, B and D groups (14). The Ambler class C group involves the production of cephalosporinases which although possessing some activity against the carbapenems, have an unclear clinical significance. *Klebsiella pneumonia* carbapenemases (KPCs) and Guiana extended-spectrum β-lactamases (GESs) constitute the Class A carbapenemases, also referred to as the penicillinases. Class B carbapenemases are composed of the Metallo-β-lactamases (MBLs), which include the three sub-types being the Verona integron-encoded MBLs (VIMs), the Imipenemases (IMPs), and the New Delhi MBL (NDM-1). The Oxacillinase-type carbapenemases such

as OXA-48 and its derivatives are included in the class D Ambler molecular classification (6,8,14).

The worldwide distribution of carbapenemase-producing Enterobacteriales (CP-CRE) varies and marked differences are observed across different areas of the globe (9,15). Van Duin and Doi in a 2017 review article, highlight this epidemiological variability and report that the predominant carbapenemase world-wide belongs to the Ambler Class A sub-category. In particular, *Klebsiella pneumonia* carbapenemases (KPC) predominate globally (15). The origin of all the classes of carbapenemases are highlighted with the initial documentation of a Class A KPC-1 isolate being the United States of America in 1996 (16). Ito *et al* describe the first documented Class B MBL isolate in Japan, of the Imipenemase (IMP) subtype in the year 1991, with subsequent prevalence of MBL's predominantly distributed in Asia (17). Within the Metallo-β-lactamase class, the initial detection of an NDM-1 isolate was reported by Yong *et al* in a Swedish patient visiting New Delhi, India, who developed a urinary tract infection in the year 2008 (18). Poirel *et a*l report on the original description of the Class D carbapenemase of the Oxa-48 subtype in a Klebsiella urine sample in Turkey in 2003 (19).

Since the initial description of these isolates current epidemiological data shows the prevalence of KPC isolates to be dominant world-wide. However, one cannot extrapolate these studies to an African context owing to the paucity of available data. In a small cross-sectional study by Mushi *et al* conducted in Tanzania, the prevalence of KPC-producing isolates was small (3/29 isolates; 10.3%) (20). In a South African context, the initial description of a KPC-producing isolate was reported in 2011 in Pretoria, South Africa, taken from a critically ill patient from three sites (respiratory tract, blood culture and invasive venous catheter device). This was the first documented KPC producer in an African context (21).

The predominant carbapenemase in our South African setting appears to be *bla*$_{OXA-48}$ & variants subtype which belongs to Ambler class D. This is supported by the Group for Enteric, Respiratory and Meningeal Disease Surveillance-South Africa (GERMS-SA) report conducted in the year 2017 (22). In this report, the National Institute for Communicable Diseases (NICD) describes the predominant carbapenemase in 923 bacteraemic samples over a 30 month period. *Bla*$_{OXA-48}$ & variants represented the commonest subtype (43%), followed by the *bla*$_{NDM}$ carbapenemase at 38% (22). Further studies, including the one conducted by Thomas and Duse, elaborate on the local epidemiology of CRE's in a South African setting. They undertook a retrospective, descriptive study over a period of two years at a tertiary hospital in Gauteng, South Africa. The study aimed to shed some light on the local patterns of CRE's, with a primary focus on laboratory detection methods (23). This study concluded that the commonest carbapenemase produced was that of the *bla*$_{OXA-48}$ & variants, followed by the *bla*$_{NDM}$ subtype. A smaller study authored by Chibabhai and Perovic, utilizing a smaller sample size of twelve PCR-positive isolates, produced a more diverse range of carbapenemase-producers, namely, five isolates with the *bla*$_{IMP}$ gene (41,6%), four isolates with the *bla*$_{NDM}$ gene (33,3%), two with the *bla*$_{OXA-48}$ gene (16,6%) and one *bla*$_{VIM}$ isolate (8,3%) (24). However, this diversity could be explained by the relatively small sample size. Perhaps of even greater concern, is the emergence of and rapid spread in South African private sector hospitals of *Klebsiella pneumoniae* sequence type (ST) 307 belonging to the *bla*$_{OXA-181}$ subclass, a multidrug-resistant super clone that poses an even greater threat to existing resources (25).

The commonest organism implicated in the production of carbapenemases globally is *Klebsiella pneumonia* (26). This finding is reflected in local studies conducted in South Africa in both the adult and paediatric population groups (22–24,27). Other organisms cultured include *Enterobacter cloacae (E.cloacae), Escherichia coli (E.coli) and Serratia marcescens (S. marcescens)*(22–24,27).

The differentiation between infection and those colonization remains a challenge and is especially problematic in samples cultured from non-sterile sites (28). Physicians rely on a combination of clinical acumen as well as guidance from biochemical and radiological investigations. Tang *et al* proposed criteria to accurately describe infection at various sites and criteria was referenced in this study (29). Urinary tract infection (UTI) was quantified by a positive urine culture with bacterial growth $\geq 10^5$ CFU/ml or the presence of pyuria. Tracheal aspirate/sputum samples were quantified by a positive culture associated with a clinically significant new pulmonary infiltrate. Cather-tip infection was assessed by the presence of a positive tip culture ≥15 CFU. Colonization was defined as the presence of CRE from a body site that is not associated with clinical signs and symptoms (29).

The diagnosis of CRE carbapenemase production relies on a combination of both phenotypic and genotypic laboratory detection methods (23,30). There is currently a lack of consensus on the ideal screening tool, as concluded by Thomas and Duse in comparing various laboratory detection methods. Hence a combination of methods is advocated (23). Phenotypic carbapenem resistance is detected using antibiotic susceptibility testing methods such as manual disc diffusion or automated detection systems like the Vitek 2 system (bioMerieux, France) (12). However, it has been reported by Coetzee and Brink amongst others, that automated detection methods lack sensitivity and specificity (31). Miriagou *et al* discuss the preferred phenotypic method for the detection of carbapenemases in the form of the Modified Hodge test (MHT), the method endorsed by the CLSI in the 2014 guidelines (11,12). This is a useful diagnostic test and is sensitive for the detection of carbapenemase production, but has low specificity (12). This is especially true for the detection of carbapenemase producers belonging to the Ambler Class B MBL groups (10,12). To combat this issue, laboratories utilize another phenotypic detection method, the E-test MBL strip (bioMérieux, Solna, Sweden), an inhibitor-based test, for MBL metalloenzyme identification (12).

Other phenotypic tests include, but are not limited to, the use of the Carba NP test (bioMérieux, France), pioneered in 2012 (10), as well as the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) detection method which detects carbapenem degradation products (30). Genotypic detection methods such as multiplex polymerase chain reaction (PCR) assays remain the gold standard for the detection of carbapenem resistance genes (31). However, this test is also limited by its ability to detect pre-determined carbapenemase genes and the risk therefore exists of missing some of the novel genes (30).

The risk factors predisposing an individual to developing CRE have been enunciated in the literature (4,5,32), and are comparative to the risks associated with the development of ESBL infections (4) . Admission to an intensive care setting, with the associated implications of critical illness and severe disease have been particularly highlighted, both as a risk factor for developing CRE, as well as a factor contributing to patient mortality (4,33,34). A Turkish study (34) consisting of fifty-four patients, documented that the majority of nosocomial CRE isolates was to be found in an ICU setting (49/54; 90.75%), while only five isolates (9.25%) were cultured in other areas of the hospital. This was independently identified as a risk factor for nosocomial CRE infection. In addition in the above mentioned study, prior hospitalization in the last six months was also identified as a risk factor for CRE acquisition (34).

Estimating the degree of morbidity and mortality directly attributed to CRE infection can be particularly challenging. The baseline characteristics of patients, including their pre-morbid condition, concomitant illnesses and level of functioning are important determinants and are contributory in predicting mortality outcomes (28). The findings in the Consortium on Resistance against Carbapenems in Klebsiella and other Enterobacteriaceae (CRACKLE) affirm that mortality should be assessed in totality. Due consideration should be given to both the infectious and non-infectious determinants

when determining mortality (35).

There is no definitive consensus on the optimal therapeutic approach to the management of CRE infection and treatment should be individualized and tailored to the clinical context (36–38). When considering a therapeutic regimen, multiple variables should be considered. Amongst the considered variables, host factors, mode of action and penetration of the drug, as well as identification and susceptibility of the pathogen should be evaluated before reaching a therapeutic consensus (39). However, the benefits of combination therapy over monotherapy has been illustrated in numerous studies (40–42). Tumbarello *et al* and Qureshi *et al* both concluded after investigating the efficacy of combination therapy versus monotherapy on KPC bacteraemic isolates, that the use of two or more drugs was superior to a single drug regime (40,41). However, these studies were done on KPC isolates only. Further studies that consider the varied carbapenemase epidemiology in a South African setting need to be done.

An extensive systematic review conducted by Falagas *et al* conducted with twenty nonrandomized trials concluded that combination therapy may be advantageous over monotherapy in the setting of critically ill patients and may confer a mortality benefit in this subset of patients (43). The INCREMENT study published in the Lancet Infectious Diseases in 2017, assessed the effect on mortality of combination versus monotherapy on bloodstream CPE infections utilizing and concluded that combination therapy was preferred in the setting of critically ill patients with high mortality scores (44). The use of monotherapy may still be considered in uncomplicated infections and its use has been particularly described in the treatment of uncomplicated urinary tract infections with aminoglycosides (37). Monotherapy can also be considered in less critically ill patients and should be considered in those with lower mortality risk (44). However, based on the literature reviewed, further research is needed before definitive conclusions are advocated.

Drugs available for the treatment of CRE's include older agents like the polymyxin class of antibiotics (39). Doi highlights the issue of potential toxicity with the use of either colistin or polymixin B and they should best be used in combination therapy with other agents (39). The use of colistin as monotherapy was associated with an increased mortality risk in the INCREMENT study as compared to combination regimens incorporating aminoglycosides, colistin or tigecycline (44). However, this class of antibiotics is currently considered our last line of defence, and concern about resistance to colistin has been described in a South African setting (45). Other options to include in a combination regimen include tigecycline, aminoglycosides or fosfomycin (36–39).

The decision to incorporate a carbapenem into a combination therapeutic regimen is largely guided by the carbapenem MIC and should be considered if the MIC ≤8 (23). The clinical benefit of incorporating a carbapenem into a combination regimen was not definitively proven in the INCREMENT study, but this finding could be attributed to a small subgroup of the sample size being placed on carbapenems and hence does not provide definitive answers on the use of carbapenems in a therapeutic regimen (44).

The use of dual carbapenem therapy (DCT) has been described, (36), but was largely evaluated in the setting of KPC isolates and further research is required in this field.

Newer agents described by both Doi and Sheu in different literature, include ceftazidime-avibactam, meropenem-vaborbactam, plazomicin and eravacycline. Novel drug with future potential include cefiderocol as well as the combination drug imipenem/cilastatin and relebactam (36,39).

The prevention of CRE spread is a key aspect of holistic management and is accomplished by rigorous surveillance practice (2,9). This is accomplished by screening methods for detection of CRE carriers via rectal swabs, cohorting protocols, as well as

effective antimicrobial and device management stewardship practices (10). As always, it is essential to remember that in dealing with CRE's as with other multi-drug resistant organisms, that prevention is ultimately always better than cure!

1.3 STUDY AIM AND OBJECTIVES

1.3.1 AIMS:

The aim of this study was to describe both the clinical and microbiological characteristics of patients at Helen Joseph hospital with confirmed Carbapenem resistant Enterobacterales (CRE) infection and/or colonization.

1.3.2 OBJECTIVES:

In order to achieve this aim, the following parameters were sought and described:

1) Identification of the baseline demographic characteristics of patients with CRE infection and/or colonization.
2) Identification of the clinical characteristics and tabulation of risk factors associated with CRE infection and/or colonization
3) Description of the microbiological parameters of patients with CRE infection and/or colonization.
4) Reported on the antibiotic treatment as well as duration of treatment in the management of CRE.
5) Reported on the infection control practices employed in the care of these cases.
6) Analysed the patient outcome including death or discharge from the hospital.

1.4 METHODS

1.4.1 RESEARCH ASSUMPTIONS

Case definitions:

1) Infection was defined as CRE isolated from anybody site (sterile and non-sterile) associated with clinical signs and symptoms.
2) Colonization was defined as the presence of CRE from a body site that is not associated with clinical signs and symptoms.
3) Hospital acquired infection was defined a CRE isolated ≥48 hours after hospital admission. Community acquired infection was defined as CRE isolated ≤48 hours after hospital admission.
4) MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial classes.
5) XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial classes (i.e., susceptible to one or two categories alone).
6) PDR was defined as non-susceptibility to all agents in all antimicrobial classes.

1.4.2 STUDY DESIGN

A single centre retrospective descriptive study was undertaken at Helen Joseph hospital which is a tertiary public sector hospital based in Johannesburg, South Africa. All patients with a positive CRE culture (cultured from both sterile and non-sterile sites) in a retrospective twelve month study period (1 January 2017- 31 December 2017) were included in the study sample.

Cases were identified from the NHLS database at Helen Joseph hospital before collection of patient records from the hospital archives. Additional information about infection control practices was obtained from the Infection Prevention and Control (IPC) department at Helen Joseph hospital. Microbiological parameters were sought from the

NHLS at Helen Joseph hospital. Identification and susceptibility testing carried out with the Vitek 2 system (bioMerieux, France) was recorded. The results of the Modified Hodge test (MHT), together with the E-test (bioMerieux) and imipenem EDTA test where required were investigated. In addition, detection of specific carbapenemase genes was carried out by the Centre for Healthcare Associated Infections, Antimicrobial Resistance and Mycoses (CHARM) of the National Institute for Communicable Diseases (NICD), using PCR assays. In particular, the *bla*$_{KPC}$, *bla*$_{GES}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM}$, and *bla*$_{OXA-48}$ genes were screened for. A quantitative method of data analysis was undertaken.

Demographic and clinical characteristics recorded included the following:

a) Gender and age.
b) Baseline presence or absence of co-morbid disease.
c) Admission ward with particular mention of admission to an ICU or a high care unit at the time of or prior to culture of the CRE.
d) Transfer in from a different institution.
e) The reason for the admission i.e. the primary diagnosis at the time of admission.
f) Duration of hospitalization prior to the positive culture of the CRE.
g) Total length of hospital stay.
h) Antimicrobial usage prior to culture of the CRE.
i) Presence of intravenous devices at the time of, or prior to the identification of the CRE.
j) History of any surgical procedure during admission but prior to the culture of the CRE.

Microbiological characteristics recorded:

a) Tabulated site of recovery of the organism: sterile vs non-sterile sites- bacteraemic (blood) as well as non-bacteraemic (urine, pus, tracheal aspirate, catheter tip, fluid, tissue) sites.

b) Tabulated the number and genus, the identified resistance mechanisms of the organisms and the where available, recorded in table format, the average carbapenem Minimum inhibitory concentration (MIC) for each organism recovered during the study.
c) Differentiated between colonization versus infection. This relied on the clinical acumen of the treating doctor and well as the opinion of specialist ID involvement/consultation with the use of clinical, biochemical and radiological parameters.
d) Biochemical parameters: White cell count (WCC) and C-reactive protein (CRP) at the time of culture.
e) Eradication of the organism and/or the control of the infection: negative culture post treatment or clinical parameters of recovery or the failure of treatment.

Infection control practices recorded:

a) Admission to an isolation cubicle vs admission to a general ward.
b) Duration from culture of the CRE to patient placement under contact precautions.
c) Contact precautions viz. use of disposable gowns, gloves, hand washing on entry and exit of unit/ward.
d) Active screening of patient contacts via rectal swabbing.

1.4.3 STUDY POPULATION

All patients' ≥18 years with a positive CRE culture from 1st January 2017 until 31st December 2017 were included in the study. These patients were identified from the NHLS database.

Inclusion criteria

1) All male and female patients' ≥18 years that has had a CRE culture confirmed between the study period 1st January 2017 until 31st December 2017.

2) CRE cultured from all sites (sterile and non-sterile sites) were included in the study.

Exclusion criteria

1) Male and female patients <18 years were excluded from the study sample.
2) Samples obtained from the Obstetrics and Gynaecology wards were excluded as this service is based at Rahima Moosa hospital and does not fall within the ambit of Helen Joseph hospital.
3) Samples obtained from rectal swabs for secondary screening processes of CRE were *not* considered in this study.

1.5 STATISTICAL ANALYSIS

All extracted data was transferred to Microsoft Excel 2007 (Microsoft, USA) spreadsheet for further analysis. Data was analysed using descriptive statistics with the assistance of a statistician. Confidentiality was maintained by assigning a study number to each identified case. Normally distributed numeric variables were described using the mean and standard deviation. The Student's T-test for equality of means was used to compare means of normally distributed variables. Non-normally distributed variables were described using the median and interquartile range. The two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare medians for non-normal data. For categorical variables, Pearson's chi-squared test was used to compare proportions. If $n<5$ in any of the cells, the Fischer's exact test for comparison of proportions was used. P values <0.05 were considered statistically significant and are shown in bold font.

1.6 ETHICAL CLEARANCE

Ethical approval was sought from the University of the Witwatersrand Human Research Ethics Committee. Permission from the CEO of Helen Joseph hospital was also

obtained for access to patient records and clinical data. Permission from the Chairperson of the Ethics and Research Committee, Helen Joseph hospital was also obtained. Further permission was obtained from the NHLS Lab service based at Helen Joseph hospital for access to microbiological data.

1.7 TIMELINE

	Sep 2017	**Oct 2017**	**Nov 2017**	**Dec 2017**	**Jan 2018**	**Feb 2018**	**Mar 2018**	**Apr 2018**	**May 2018**	**Jun 2018**	**July 2018**	**Aug 2018**
Protocol/ Literature Review												
Protocol Submission												
Ethics Approval												
Data Collection												
Data Analysis												
Write up												
Final Submission												

1.8 FUNDING

The costs incurred in conducting this research were expected to be minimal and hence no funding was sort from any association/committee.

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CHAPTER 2: SUBMISSIBLE ARTICLE

Title: Antibiotics under siege: Carbapenem resistant Enterobacterales in a South African Hospital

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Conflicts of interest: None

Key words: Carbapenem resistant Enterobacterales, carbapenemase, antibiotics, resistance

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Abstract word count: 472. **Total word count:** 4319.

*Article has been prepared for submission to The South African Medical Journal

ABSTRACT

Background: Antimicrobial Resistance (AMR), in particular Antibacterial resistance (ABR) is a growing public health concern. The emergence of resistant Gram-negative bacteria coupled with a dwindling antibiotic armamentarium poses a significant threat. In South Africa, there is an urgent need to evaluate this situation and due consideration should be given to prevent the emergence of multidrug Resistant (MDR), extensively drug resistant (XDR), and pandrug resistant (PDR) organisms.

Objectives: The objective of this study was to describe both the clinical and microbiological characteristics of patients at Helen Joseph Hospital (HJH) with confirmed Carbapenem resistant Enterobacterales (CRE) infection and/or colonization. In addition, infection prevention and control practices at HJH were highlighted in this study.

Methods: A single centre retrospective descriptive study was undertaken at a tertiary public sector hospital in Johannesburg, South Africa. All patients with a positive CRE culture collected retrospectively in a twelve month study period were included. Microbiological data was obtained from the NHLS database and clinical data from patient records. A quantitative method of data analysis was performed.

Results: A total of 106 patient files were reviewed. Demographically, 52.83% of patients were males while females represented 47.17%. Ethnically, 64.15% of patients were of African descent. The majority of patients were admitted to the medical wards (35.85%), while 34.9% of all CRE's were cultured in an intensive care setting (27 in the Intensive care unit (ICU) and 10 in High care)**.** The predominant site of culture was urine and blood representing 35.85% and 26.42% respectively. The dominant CRE organism subtype was *Klebsiella pneumoniae* (94/106, 88.68%), followed by *Enterobacter cloacae* (6.6%) and *Escherichia coli* (2.83%). *Bla*$_{\text{OXA-48}}$& variants represented the predominant CRE genotype (70.75%), followed by *bla*$_{\text{NDM}}$ (10.38%).Significant differences in resistance patterns between *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM}}$ isolates to carbapenems were noted with 66.67% of *bla*$_{\text{NDM}}$ isolates being resistant to imipenem, in contrast to *bla*$_{\text{OXA-48}}$ with

12% (p<0.001). Seventy-five percent of the *bla*$_{NDM}$ isolates were resistant to meropenem, while only 21.33% of the *bla*$_{OXA-48}$ isolates were resistant (p=0.001). Patients with a previous hospital admission in the last six months were more two times more likely to demise (p=0.042). Admissions to the ICU/ high care wards were three times more likely to demise than those admitted in other wards (p=0.009).

Conclusion: There was a high prevalence of CRE at HJH with the three predominant bacteria in our setting being *Klebsiella pneumoniae* followed by *Enterobacter cloacae* and *Escherichia coli*. Genotypically, *bla*$_{OXA-48}$ & variants predominates, while *bla*$_{NDM}$ represents the second commonest carbapenemase. Significant differences in the resistance patterns between *bla*$_{OXA-48}$ and *bla*$_{NDM}$ isolates to imipenem and meropenem was observed. Previous hospitalization in the last six months and current admission to an intensive care independently predicted mortality. Infection prevention and control measures are essential to combat the spread of CRE infection, and the lack of these practices in twenty-five percent of our sample size needs to be urgently addressed.

Introduction

Antimicrobial Resistance (AMR), in particular Antibacterial resistance (ABR) is a growing public health concern. The World Health Organization (WHO), in the latest Global Antimicrobial Resistance Surveillance System (GLASS) report conducted in the period 2017 to 2018, alludes to the serious consequences of increasing antibiotic resistance and recognizes the need for urgent and immediate action (1).

Resistant Gram-negative bacteria, in particular, pose a significant threat. Unlike resistant Gram-positive bacteria, where therapeutic options remain available, there is a lack of *new*, innovative, therapeutic options for Gram-negative bacteria in the near or foreseeable future. The consequence, is the emergence of multidrug resistant (MDR), extensive drug resistant (XDR), and pandrug resistant (PDR) organisms in the face of a rapidly dwindling antibiotic armamentarium (2).

Furthermore, the development of carbapenem resistance has been fuelled by the worldwide rise in Extended spectrum β-lactamase (ESBL) producing Enterobacterales possessing capabilities of hydrolysing all β-lactams with the exception of the carbapenems. This has resulted in the increased and injudicious utilization of the carbapenem antibiotics from as far back as twenty years ago (3).This highlights the effect of antibiotic selective pressure on the development of resistance.

All things considered, it is evident that the pendulum between resistant organism and effective antibiotic therapy swings precariously, and only time will tell which entity will ultimately emerge victorious.

Aim

The aim of this study was to describe both the clinical and microbiological characteristics of patients at Helen Joseph hospital with confirmed Carbapenem resistant Enterobacterales (CRE) infection and/or colonization. In addition, infection prevention and control practices at HJH were highlighted in this study.

Methods

A single centre retrospective descriptive study was undertaken at Helen Joseph hospital which is a tertiary public sector hospital based in Johannesburg, South Africa. All patients >18 years with a positive CRE culture (cultured from both sterile and non-sterile sites) collected retrospectively in a 12 month study period (1 January 2017- 31 December 2017) were included in the sample size. All patients were inpatients at the time of conducting this study. These sites included both bacteraemic as well as non-bacteraemic sites. Samples obtained from the Obstetrics and Gynaecology wards were excluded as this service is based at Rahima Moosa hospital and does not fall within the ambit of Helen Joseph hospital. Samples obtained from rectal swabbing during secondary screening processes for CRE were not considered in the study sample.

Clinical characteristics recorded included gender, age, co-morbid disease and ward of admission. Prior hospitalization within the past six months as well as prior exposure to antibiotic therapy was noted. The presence of invasive devices documented on a standardized nursing admission form was recorded.

Microbiological characteristics recorded the organism subtype as well as the carbapenemase produced. Carbapenem minimum inhibitory concentrations (MIC) were assessed utilizing the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines. A MIC of ≤0.5 defined a susceptible isolate to ertapenem, while A MIC of ≤1 was used for imipenem and meropenem. A MIC of ≥2 for ertapenem and ≥4 for

imipenem and meropenem defined resistance. The intermediate category was not considered as a criteria to define resistance in this study. Identification and susceptibility testing was carried out with the Vitek 2 system (bioMerieux, France). The results of the Modified Hodge test (MHT), together with the E-test (bioMerieux) where required were investigated. Detection of carbapenemase genes was carried out by the Centre for Healthcare Associated Infections, Antimicrobial Resistance and Mycoses (CHARM) of the National Institute of Communicable Diseases (NICD), using PCR assays. In particular, the *bla*$_{KPC}$, *bla*$_{GES}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM}$, and *bla*$_{OXA\text{-}48\text{-like}}$ genes were screened for.

Treatment and antibiotic practices were documented. Outcome was measured by the eradication of the organism and/or the control of the infection: negative culture post treatment (where available) or clinical parameters of recovery or the failure of treatment was noted. Infection control practices employed were also documented.

Case definitions

Carbapenem resistant Enterobacterales (CRE), formerly known Carbapenem resistant Enterobacteriaceae (CRE) is defined by the Centre for Disease Control (CDC) as those Enterobacterales that are non-susceptible to any carbapenem antimicrobial. This phenotypic definition utilizes a minimum inhibitory concentration (MIC) of ≥2 µg/ml for ertapenem or ≥4 µg/ml for imipenem or meropenem. In addition, any Enterobacterales that produce a carbapenemase are considered to fall under the category of CRE. For those Enterobacterales that have reduced susceptibility to imipenem- such as the *Proteus, Providencia* species and *Morganella morganii*,-resistance to a carbapenem other than imipenem is required. This definition includes both carbapenemase-producing Enterobacterales (CP-CRE) and non-carbapenemase producing CRE (Non-CP-CRE).

Infection was defined as CRE isolated from anybody site (sterile and non-sterile) associated with clinical signs and symptoms. Clinical, biochemical and radiological investigations aided in making this assessment .To clarify the site of infection, urinary tract infection (UTI) was quantified by a positive urine culture with bacterial growth $\geq 10^5$ colony forming units/millilitre (CFU/ml) or the presence of significant pyuria. Tracheal aspirate/sputum samples were quantified by a positive culture associated with a clinically significant new pulmonary infiltrate. Cather-tip infection was assessed by the presence of a positive tip culture ≥15 CFU.

Colonization was defined as the presence of CRE from a body site that is not associated with clinical signs and symptoms.

Hospital acquired infection was defined a CRE isolated ≥48 hours after hospital admission.

Inappropriate treatment was defined by the use of antimicrobials injudiciously without evidence of infection.

Outcome was defined as death of the patient (during current hospital admission) or discharge (the patient was still alive at the time of discharge from HJH).

Statistical analysis

All extracted data was transferred to a Microsoft Excel 2007 datasheet (Microsoft, USA) spreadsheet for further analysis. Confidentiality was maintained by assigning a study number to each identified case. Normally distributed numeric variables were described using the mean and standard deviation. The Student's T-test for equality of means was used to compare means of normally distributed variables. Non-normally distributed

variables were described using the median and interquartile range. The two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare medians for non-normal data. For categorical variables, Pearson's chi-squared test was used to compare proportions. If n<5 in any of the cells, the Fischer's exact test for comparison of proportions was used. P values <0.05 were considered statistically significant and are shown in bold font.

Results

There were 133 patients with culture positive CRE in the retrospective period 1st January 2017 to 31st December 2017. Twelve cultures obtained from rectal swabs during secondary screening processes were excluded from the sample size. Fifteen files contained inadequate data for analysis and were also excluded from this study. This left 106 patient files for review and analysis.

There were 56 male patients (52.83%) with culture positive CRE, and 50 female patients (47.17%). The ethnicity profile demonstrated that 65.15% of patients were of African descent. The mean age of presentation was 52.63 years (SD 18.76) *(Table 1)*. The majority of patients with culture positive CRE were admitted to the medical wards (35.85%). The percentage of patients admitted to an intensive care setting was 34.9% (27 in ICU and 10 in High care). 33.96% of patients (n=36) had a prior hospitalization within the preceding six months. The predominant isolates were*: Klebsiella pneumoniae* representing the majority culture in 94/106 samples (88.68%), followed by *Enterobacter cloacae* (6.6%), and *Escherichia coli* (2.83*%) (Figure 1)*. Genotypically, *bla*$_{\text{OXA-48}}$ and variants represented the predominant CRE genotype (70.75%), while *bla*$_{\text{NDM}}$ represented the second most common genotype (10.38%) *(Figure 2).*

Sixty-four of all samples represented infection (60.38%), while 42 (39.62%) represented colonization. The predominant site of culture of CRE organisms was urine and blood representing 35.85% and 26.42% respectively. Of note is that all blood samples represented infection (n=28; 100%). Twenty-two bacteraemic isolates were associated with an invasive device, while the remainder had no device in-situ. Of the urine specimens, 29/38 urine samples represented colonization (76.31%). True urinary infection was only found in 9/38 samples (23.68%). A large percentage (73.68%) of the urine colonization samples was associated with urinary catheterization, while only 18.42% of urinary infection was associated with a catheter *(Table 2).*

Positive PCR results were obtained in 87/106 samples. There were 75 *bla*$_{\text{OXA-48}}$ & variants isolates, 11 *bla*$_{\text{NDM}}$ isolates and 1 sample containing both the *bla*$_{\text{OXA-48}}$ & variants as well as the *bla*$_{\text{NDM}}$ carbapenemase. *Klebsiella pneumoniae* accounted for 71 (75.5%) of the *bla*$_{\text{OXA-48}}$ isolates, 6 (6.38%) of the *bla*$_{\text{NDM}}$ isolates as well as being responsible for the single isolate with dual carbapenemases. *Enterobacter cloacae* accounted for 4 (57.14%) of the *bla*$_{\text{OXA-48}}$ and variants isolates and 2 (28.57%) of the *bla*$_{\text{NDM}}$ isolates. All 3 of the *Escherichia coli* isolates contained the *bla*$_{\text{NDM}}$ carbapenemase. No PCR testing was done on the single *Enterobacter aerogenes* specimen, neither was it done on the single *Serratia marcescens* sample.

With regard to imipenem susceptibility testing, there were significant differences in resistance patterns between *bla*$_{\text{OXA-48}}$ & variants and *bla*$_{\text{NDM}}$ isolates, with 66.67% of *bla*$_{\text{NDM}}$ isolates being resistant to imipenem. In contrast, only 12% of the *bla*$_{\text{OXA-48}}$ isolates were resistant to imipenem. These differences were statistically significant ($p<0.001$). With regard to meropenem susceptibility testing, a large proportion (75%) of the *bla*$_{\text{NDM}}$ isolates were resistant to meropenem, while only 21.33% of the *bla*$_{\text{OXA-48}}$ & variants were resistant ($p=0.001$). With regard to ertapenem susceptibility testing, all

bla$_{NDM}$ isolates were resistant to ertapenem, while 88% of *bla*$_{OXA-48}$ & variants samples were resistant. However, this was not statistically significant (p=1.000) *(Table 3).*

Infection control measures were instituted in the majority (75.47%) of patients. In those treated with antibiotic therapy, two strategies were employed-monotherapy (n=25; 23.58%), or combination therapy (n=30; 28.30%). Monotherapy involved either a single carbapenem or the use of colistin only. Combination therapy utilizing an aminoglycoside with a carbapenem was used in 16.98%, while the combination of colistin with a carbapenem was used in 11.32%. The commonest carbapenem utilized in our setting in both mono and combination therapy was imipenem.

Half of the patients demised in the study sample . A total number of fifty-three patients demised which included 29 patients (54.7%) not on treatment at the time of death, 7 patients (13.2%) on combination therapy with an aminoglycoside and carbapenem, 6 patients (11.3%) on a carbapenem-colistin regimen, 10 patients (18.8%) on carbapenem monotherapy and a single patient (1.8%) on colistin monotherapy. Forty patients out of the total number of fifty-three that demised were of the *bla*$_{OXA-48}$ & variants genotype (75.5%), while only 6 were of the *bla*$_{NDM}$ genotype (11.3%).One patient that demised had both the *bla*$_{OXA-48}$ & variants and well as the *bla*$_{NDM}$ genotype (1.8%). The remaining 6 samples had no documented PCR (n=4; 7.5%), or negative PCR results (n=2; 3.7%). Of those that survived, a negative culture post treatment was only recorded in 9.43%. Cure based on clinical parameters was documented in 20.75%. The remainder of those that survived had no clear record of a clinical or microbiological cure *(Table 4).*

In the univariable analysis, patients admitted to an intensive care setting (ICU/ High care) wards were three times more likely to demise than those admitted to other wards [OR 95% CI 3.05 (1.32 – 7.06) p=0.009]. In the multivariable model, this remained significant (p=0.012). In the univariable analyses, patients with a previous hospital

admission in the last six months were more two times more likely to demise [OR 95%CI 2.36 (1.03 – 5.40) p=0.042] than those who had not had a recent hospital admission. In the multivariable model, these associations remained significant (p=0.022). Previous hospital admission and admission into ICU/high care independently predicted mortality *(Table 5).*

Discussion

There was no gender predilection found in this study. This is expected as gender is not an identifiable risk factor for the development of CRE infection or colonization. The majority of patients were of African descent reflecting the profile of patients seen in HJH and in our South African context. The mean age at presentation was 52.62 years. Older age and its association with poorer functional status has been identified as a risk factor for infection with multi-drug resistant organisms, and by extension of CRE infection/colonization (3-6). Multiple hypotheses alluding to the reasons for this has been identified, amongst them being age-related physiological changes in the elderly as well as the presence of multiple co-morbidities as compared to a younger population group (6). However, despite the mean age at presentation being >50 years in our study, the number of patient co-morbidities in both the univariable and multivariable analysis had no statistical effect on mortality outcome. Other contributing factors include the fact that older patients may also reside in long-term care facilities such as nursing homes and frail-care centres and this has been recognized as an independent risk factor for the development of drug-resistant infections (4).

The number of identified isolates in our study is in stark contrast to a study conducted by Chibabhai et al in another South African Hospital (CMJAH) during an earlier ten month study period (7). Only 37 CRE isolates were identified between December 2012 and October 2013. However, a study conducted in this same institution over a two year period in the later years 2015 to 2016 by Thomas and Duse described an alarming

increase in identified isolates with 259 identified cases over these two years (8). It will be interesting to do a comparative study between all hospitals in our setting to assess the prevalence of CRE infection/colonization over an extended time period and document trends on a yearly basis.

The majority of the isolates were identified in the medical wards with 34.9% of all cases identified in an intensive care setting (ICU and HC). Possible explanations for this include that these subsets of patients are critically ill having warranted an admission to a critical care setting. This finding was confirmed in the local literature in a study by Thomas and Duse conducted in Charlotte Maxeke Hospital that highlighted the ICU amongst the wards with the highest CRE prevalence over a two year period (8). Admission to an ICU setting is a well-documented risk factor for the development of CRE infection/colonization as these wards may serve as a reservoir for the transmission of infection (3,5,9). Other explanations include that admission to an ICU ward is associated with mechanical ventilation as well as the use of other invasive devices-all recognized independent identifiable risk factors for the development of CRE infection/colonization (3,10). Furthermore, prior antibiotic usage may promote the development of resistance and antibiotic usage and infection control practices in our ICU setting will need to be evaluated (3,10,11). Admission to the ICU/HC in our study also had a significant impact on mortality confirmed in the univariable analysis, where patients admitted to the ICU/ High Care wards were three times more likely to demise than those admitted to other wards. In the multivariable model, this remained statistically significant (p=0.012).This further highlights the critical nature of patients admitted to these wards. However, other factors concerning antibiotic practices, infection control measures and invasive device usage relative to an ICU setting will need evaluation.

Previous hospitalization in the last six months was also identified in the univariable analyses as a factor contributing to mortality. Patients with a previous hospital admission

in the last six months were more two times more likely to demise (p=0.042) than those who had not had a recent hospital admission. Factors contributing to this could be inadequate resolution of their medical condition during the first admission, possibly due to the complicated nature of their condition and/or inadequate treatment resulting in a second admission and exposure to a healthcare setting. Previous hospitalization is a recognized risk factor for the development of hospital-acquired drug-resistant organisms as highlighted in a study by Peleg and Hooper where hospitalization for more than two days in the preceding 90 days was identified as risk factor for nosocomial drug-resistant infections (4). Furthermore, previous hospitalization as a risk factor for CRE acquisition in particular, has been highlighted in the literature (5,9).

The three predominant organism isolates identified in our study were*: Klebsiella pneumoniae* followed by *Enterobacter cloacae* and *Escherichia coli*. This is consistent with the global picture as the commonest carbapenemase producer identified world-wide is *Klebsiella pneumonia* (12)*.* This finding is also reflected in local studies conducted in South Africa in both the adult and paediatric population groups (7,8,13). Genotypically, *bla*$_{\text{OXA-48}}$ and variants, belonging to the Ambler Class D classification, represented the predominant CRE genotype, followed by the *bla*$_{\text{NDM}}$ genotype. This is in keeping with the national surveillance data conducted GERMS-SA in the year 2017 (14). This is however, in contrast to what is noted world-wide. Van Duin and Doi in a 2017 review article reported that the predominant carbapenemase world-wide belongs to the Ambler Class A sub-category, being of the *bla*$_{\text{KPC}}$ subtype (15).

The predominant site of culture of CRE organisms was urine and blood. All blood samples represented infection while the majority of urine samples represented colonization. Possible reasons for this could be the use of urinary catheters which serves as a nidus of infection and promotes colonization of the urinary tract with drug-resistant organisms (16). The use of invasive devices, in particular the injudicious use

thereof will need to be evaluated. However, in the logistic regression model of factors affecting mortality, there was no statistical difference noted in mortality outcomes between bacteraemic versus non-bacteraemic cultures nor was there any difference in mortality outcome between samples representing infection versus those representing colonization.

With regard to the management of CRE infection, two strategies were employed- monotherapy or combination therapy. Combination therapy was marginally preferred (28.30%) over the use of monotherapy (23.58%). Combination therapy utilizing and aminoglycoside with a carbapenem was preferred (16.98%) over the combination of colistin with a carbapenem (11.32%). The reason for the choice of therapy (combination versus monotherapy) was not clear given the retrospective nature of the data acquired and is a recognized limitation of this study. Antibiotic selection in our setting is largely guided by clinician judgment, together with infectious disease consultation and microbiologist/laboratory input where necessary. In addition, a shortage of colistin during the study period could have influenced prescribing practices.

There is no definitive consensus in the literature on the optimal therapeutic approach to the management of CRE infection and treatment should be individualized and tailored to the clinical context (17-19). Multiple variables should be considered when evaluating a therapeutic regimen, amongst them being host factors, mode of action and penetration of the drug, as well as identification and susceptibility of the identified pathogen (20).However, the benefits of combination therapy over monotherapy has been demonstrated in numerous studies (21–23). Tumbarello *et al* and Qureshi *et al* both concluded after investigating the efficacy of combination therapy versus monotherapy on KPC bacteraemic isolates, that the use of two or more drugs was superior to a single drug regime (21,22). However, these studies were done on KPC isolates, and further studies taking into consideration the carbapenemase epidemiology in our South African

setting need to be done. The INCREMENT study published in the Lancet Infectious Diseases in 2017, assessed the effect on mortality of combination versus monotherapy on bloodstream CPE infections utilizing mortality scores and also concluded that combination therapy was preferred in the setting of critically ill patients with high mortality scores (24).The use of monotherapy may still be considered in uncomplicated infections and its use has been particularly described in the treatment of uncomplicated urinary tract infections with aminoglycosides (18). Monotherapy can also be considered in less critically ill patients and should be considered in those with lower mortality risks (24). However, based on the available literature, further research into optimum drug management of CRE infection is warranted before any definitive conclusions are advocated.

The commonest carbapenem utilized in our setting in both monotherapy and combination therapy was imipenem. Possible reasons for the choice of carbapenem could be related to the susceptibility profile of imipenem in relation to the *bla*$_{\text{OXA-48}}$ genotype. It was noted that the majority of the *bla*$_{\text{OXA-48}}$ isolates were susceptible to imipenem (78.67%) while 25% of the *bla*$_{\text{NDM}}$ isolates were susceptible to imipenem. This was considered statistically significant ($p<0.001$). Since *bla*$_{\text{OXA-48}}$ is the commonest genotype in our setting, this may be a reasonable choice of a carbapenem. Colistin was only used as monotherapy in one case and as part of combination therapy in 12 cases. The limited use of colistin in our study was partly attributed to a shortage of colistin supplies during the study period and may not accurately reflect the antibiotic prescribing practices with regard to colistin in our setting. However, the use of colistin and its potential for toxicity has been highlighted in a review article by Doi, where its use as part of combination therapy as opposed to monotherapy is advocated (20). These antibiotics are currently considered our last line of defence, and concern about resistance to colistin has already been described in a South African setting (25).

Infection control practices were instituted immediately in the majority of cases. This could be due to the dedicated multidisciplinary IPC team at HJH consisting of nurses, microbiologists as well as infectious disease specialists. However, 25% of cases did not have these measures in place. Possible reasons for this could be a lack of formal IPC education programmes and hence a lack of understanding of the importance of these measures amongst all levels of staff. Furthermore, a lack of infrastructure in our current under resourced hospital environment such as sufficient isolation cubicles etc, further hampers the ability to implement these measures timeously and appropriately.

Half of the patients demised in our study sample. Possible reasons for the equivalent numbers of survivors versus deaths could be explained by taking cognizance of the fact that estimating the degree of morbidity and mortality directly attributed to infection can be particularly challenging. Multiple pre-existing patient factors and conditions, aside from infection, are important determinants and are contributory in predicting mortality outcomes. The findings in the Consortium on Resistance against Carbapenems in Klebsiella and other Enterobacteriaceae (CRACKLE) study illustrates this point and affirm that mortality should be assessed in totality and consideration should be given to both the infectious and non-infectious determinants when determining mortality (26).

A large proportion of patients that demised (75.5%) were of the *bla*$_{\text{OXA-48}}$ & variants genotype -this is most a likely a reflection of the high prevalence of this genotype in our setting. The paucity of other genotypes in our study hampered further analysis and definitive conclusions regarding its effect on mortality could not be drawn. Of those that survived, a negative culture post treatment was only recorded in 9.43%. This is a recognized limitation of this study as microbiological cure was only documented in the minority of cases.

Limitations

This study has several limitations. Firstly, and perhaps the most significant, is the retrospective nature of the study which was partly hampered by incomplete data in patient records. In addition, the accuracy of the data is dependent on adequate and meticulous record-keeping and given the retrospective nature of the study, limited control could be exercised over this process.

Secondly, our study was conducted in a single institution in Johannesburg, South Africa and this small sample size may not be representative of CRE infection/colonization in a broader South African context. Furthermore, the *bla*$_{\text{OXA-48}}$ & variants genotype predominated in our study and the lack of other genotypes hampers the ability to draw broader conclusions.

Thirdly, limited conclusions could be drawn about the management of CRE infection/colonization at HJH. This was partly due to the retrospective nature of the study as well as well as unforeseen circumstances such as colistin shortages during the study period, which affected prescribing practices and may not accurately reflect true prescribing practices.

Lastly, this study was conducted in the year 2017 and the CLSI criteria at the time was used in the interpretation of the data. The EUCAST criteria was not considered as this is not the preferred method endorsed by the NHLS. This allowed for categorization of isolates into susceptible and resistant isolates categories only and the intermediate category was not considered in the interpretation of the data set. Subsequent changes in these definitions in later editions of the CLSI/EUCAST criteria will have to be considered in future studies.

Conclusions

This study provided a glimpse into the local CRE epidemiology in a single institution in Johannesburg, South Africa. From our study, the CRE prevalence was high with the three predominant organisms cultured in our setting being *Klebsiella pneumoniae* followed by *Enterobactercloacae* and *Escherichia coli*. Genotypically, *bla*$_{\text{OXA-48}}$ & variants predominates, while *bla*$_{\text{NDM}}$ represents the second commonest carbapenemase. This is in keeping with local South African epidemiology, however differs from the global picture of predominant *bla*$_{\text{KPC}}$ production. Significant differences in resistance patterns between *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM}}$ isolates to imipenem and meropenem was observed. Previous hospitalization in the last six months and current admission to an intensive care independently predicted mortality in our study. In addition, infection prevention and control measures are crucial in ensuring optimum care of CRE patients.

Recommendations

Potential exists in the future for conducting a prospective study evaluating CRE infection/colonization in multiple institutions over a prolonged period of time. This can be done in multiple provinces and incorporate both the public and private sector. This was will provide useful and comprehensive information about local CRE epidemiology.

Furthermore from our study it is clear that there is an urgent need for the development of local treatment guidelines and protocols to allow for standardization of treatment. While we are cognisant of the barriers that exist in the access to drugs and other resources, consistency in the management approach to CRE infections need to be exercised. This extends to both pharmacological management as well as the stringent implementation of infection control practices, in order to allow for the best possible outcome for all patients alike. The development of IPC Programmes with clearly defined goals and objectives, specifically targeted and under resourced institutions such as public

institutions in Southern Africa , will go a long way in ensuring optimum patient care and management. In addition, education and training programs directed at all levels of health care professionals as well as dedicated and appropriate surveillance programs will allow for improved management of CRE patients.

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LIST OF TABLES

TABLE 1: Descriptive statistics: Overall description of patients with culture positive CRE (N=106)				
	N	**%**	**Mean (SD)**	**Median (IQR)**
Demographics				
Gender				
Male	56	52.83		
Female	50	47.17		
Race				
African	68	64.15		
White	21	19.81		
Indian	9	8.49		
Coloured	8	7.55		
Age			52.63 (18.76)	
Admission details				
Ward				
Medical	38	35.85		
ICU	27	25.47		
Surgical	23	21.70		
Ward 12 HCU	10	9.43		
Orthopaedic	5	4.72		
ED	3	2.83		
Transfer in from another institution	10	9.43		
Duration of hospitalization prior to culture				12.5 (7 – 21)

(days)				
Previous hospital admission in the last 6 months	36	33.96		
Culture Details				
Specimen type/site of culture				
Urine	38	35.85		
Blood	28	26.42		
Pus	12	11.32		
Tracheal aspirate	11	10.38		
Catheter tip	9	8.49		
Fluid	6	5.66		
Tissue	2	1.89		
Organism cultured				
Klebsiella pneumoniae	94	88.68		
Enterobacter cloacae	7	6.60		
Escherichia coli	3	2.83		
Enterobacter aerogenes	1	0.94		
Serratia marcescens	1	0.94		
Clinical significance				
Colonization	42	39.62		
Infection	64	60.38		
Organism PCR				
bla$_{OXA-48}$& variants	75	70.75		
Not done	15	14.15		
bla$_{NDM}$	11	10.38		
Negative	4	3.77		
bla$_{NDM}$and *bla*$_{OXA-48}$& variants	1	0.94		

WCC at time of culture			11.47 (4.85)	
CRP at time of culture			151.70 (94.14)	
Co-morbidities				
HIV				
HIV positive	29	27.36		
HIV negative	59	55.66		
HIV status unknown	18	16.98		
ARV use	16	15.09		
CD4 cell count				113 (31 – 243)
HIV viral load				
Detectable	15			51.72
Undetectable	8			27.59
Not done	6			20.69
Renal failure	21	19.81		
Steroids/ Immunosuppressive therapy	19	17.92		
Diabetes	18	16.98		
Malignancy	15	14.15		

TABLE 2: Infection/Colonization per site and association with invasive devices (n=106)

Site	N	Infection	Colonization	Percentage of Infection associated with an invasive device	Percentage of Colonization associated with an invasive device	No device
Urine	38	9 (23.68%)	29 (76,31%)	18.42%	73.68%	7.89%
Blood	28	28 (100%)	0 (0%)	78.57%	0%	21.42 %
Pus	12	9 (75%)	3 (25%)	75%	25%	0%
Tracheal aspirate	11	7(63.33%)	4 (36.36%)	63.63%	36.36%	0%
Catheter tip	9	4 (44.44%)	5 (55.55%)	44.44%	55.55%	0%
Fluid	6	5 (83.33%)	1 (16,66%)	83.33%	16.6%	0%
Tissue	2	2 (100%)	0 (0%)	100%	0%	0%

TABLE 3: Carbapenemase gene versus MIC for PCR positive patients (n=87)				
Interpretation of imipenem MIC (CLSI Criteria)				
Carbapenemase gene testing	**Susceptible**	**Intermediate**	**Resistant**	**P Value**
bla$_{OXA-48}$ & variants	59 (78.67)	7 (9.33)	9 (12.00)	
bla$_{NDM}$	3 (25.00)	1 (8.33)	8 (66.67)	**<0.001**
Interpretation of meropenem MIC (CLSI criteria)				
Carbapenemase gene testing	**Susceptible**	**Intermediate**	**Resistant**	**P Value**
bla$_{OXA-48}$& variants	48 (64.00)	11 (14.67)	16 (21.33)	
bla$_{NDM}$	3 (25.00)	0 (0.00)	9 (75.00)	**0.001**
Interpretation of ertapenem MIC (CLSI criteria)				
Carbapenemase gene testing	**Susceptible**	**Intermediate**	**Resistant**	**P Value**
bla$_{OXA-48}$& variants	5 (6.67)	4 (5.33)	66 (88.00)	
bla$_{NDM}$	0 (0.00)	0 (0.00)	12 (100.00)	1.000

TABLE 4: Management and outcome	*N*	*(%)*	*Mean (SD)*
Infection Control			
Infection control measures instituted	80	(75.47)	
Contact screening			
No	33	(31.13)	
Yes	41	(38.68)	
No contacts	32	(30.19)	
Management			
Management			
Not treated	51	(48.11)	
*Treated appropriately	48	(45.28)	
**Treated inappropriately	7	(6.60)	
Antibiotic treatment			
Not treated	51	(48.11)	
Monotherapy	25	(23.58)	
Combination therapy	30	(28.30)	
Monotherapy			
Imipenem	14	(13.21)	
Ertapenem	5	(4.72)	
Meropenem	5	(4.72)	
Colistin	1	(0.94)	
Combination therapy			
Aminoglycoside+ Carbapenem	18	(16.98)	
Colistin+ Carbapenem	12	(11.32)	
Particular antibiotics used in combination therapy			
Amikacin + Imipenem	15	(14.15)	

Amikacin + Meropenem	3 (2.83)	
Colistin + Imipenem	8 (7.55)	
Colistin + Meropenem	4 (3.77)	
Duration of antibiotic treatment (days)		7.85 (3.46)
Time from culture to antibiotic treatment (days)		6.25 (2.13)
Outcome		
Discharged	53 (50.00)	
Demised	53 (50.00)	
Cure		
Clinical	22 (20.75)	
Culture	10 (9.43)	

***Treated with an appropriate choice of antibiotics where clinically indicated(ie: infection)**

****Treated where not clinically indicated (ie: colonisation)**

TABLE 5: Logistic regression models of clinical features and mortality				
Clinical Feature	**Univariable model**		**Multivariable model**	
	OR (95% CI)	**P value**	**OR (95% CI)**	**P value**
Ward				
Surgical/Medical/Orthopaedic/ED	Reference		Reference	
ICU/ Ward 12 HC	3.05 (1.32 – 7.06)	**0.009**	4.67 (1.40 – 15.51)	**0.012**
Clinical significance				
Colonization	Reference		Reference	
Infection	1.61 (0.73 – 3.53)	0.235	2.64 (0.66 – 10.51)	0.168
Specimen culture site				
Non-bacteraemic	Reference		Reference	
Bacteraemic	1.80 (0.75 – 4.34)	0.189	1.46 (0.38 – 5.66)	0.580
Number of co-morbidities				
None	Reference		Reference	
One	1.77 (0.72 – 4.38)		3.30 (0.97 – 11.24)	0.057
Two or more	1.22 (0.46 – 3.25)		2.74 (0.70 – 10.77)	0.149
Antibiotic treatment				
Not treated	Reference		Reference	
Monotherapy	0.60 (0.23 – 1.56)	0.293	0.46 (0.12 – 1.74)	0.254
Combination therapy	0.58 (0.23 – 1.44)	0.241	0.25 (0.061 – 1.03)	0.055
Susceptible carbapenems				
None	Reference		Reference	
One only	0.51 (0.16 – 1.67)	0.267	0.21 (0.04 – 1.21)	0.081
Two or more	0.55 (0.21 – 1.40)	0.208	0.35 (0.10 – 1.27)	0.111

Carbapenemase gene testing				
Bla$_{OXA-48}$	Reference		Reference	
Bla$_{NDM}$	1.22 (0.36 – 4.21)	0.747	0.87 (0.19 – 3.98)	0.860
Previous Hospital admission				
Previous hospital admission in the last 6 months	2.36 (1.03 – 5.40)	**0.042**	3.37 (1.19 – 9.53)	**0.022**

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FIGURE 1: Description of organism subtype

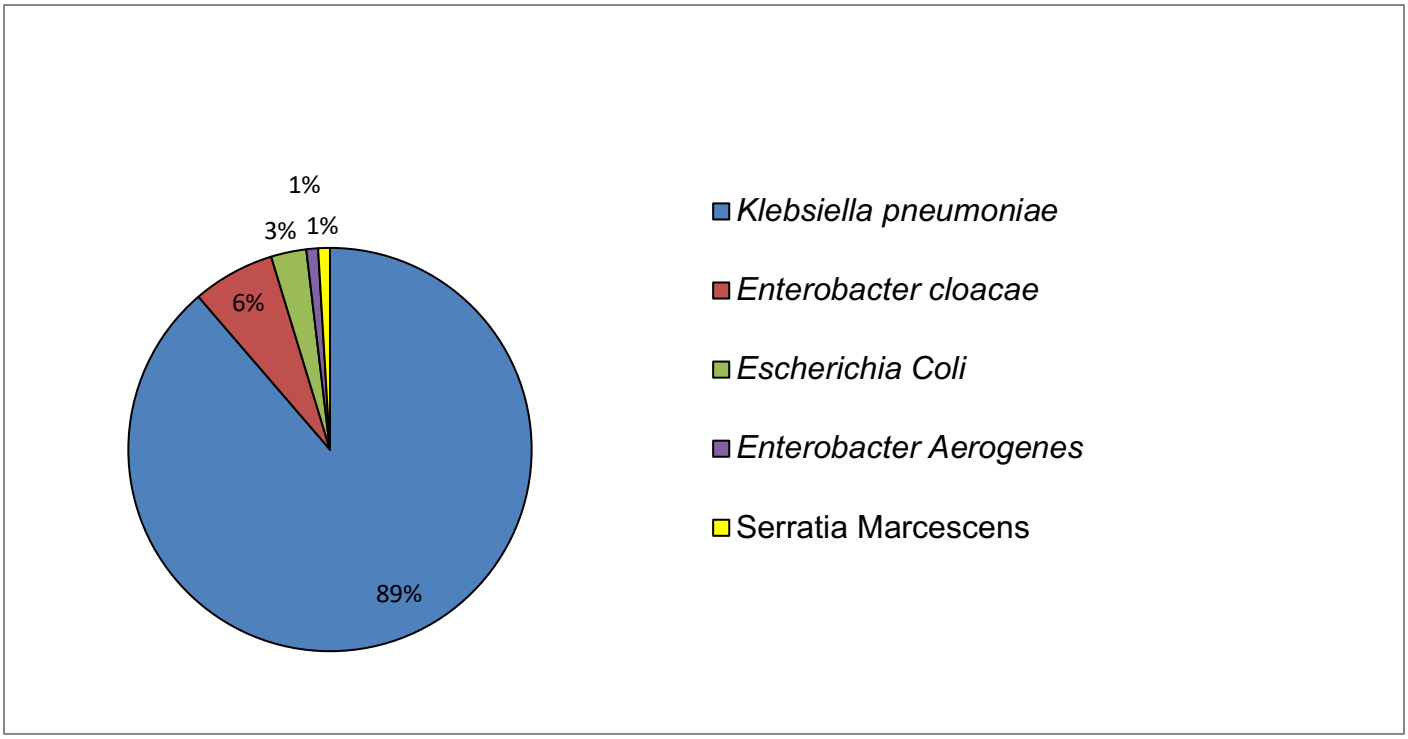
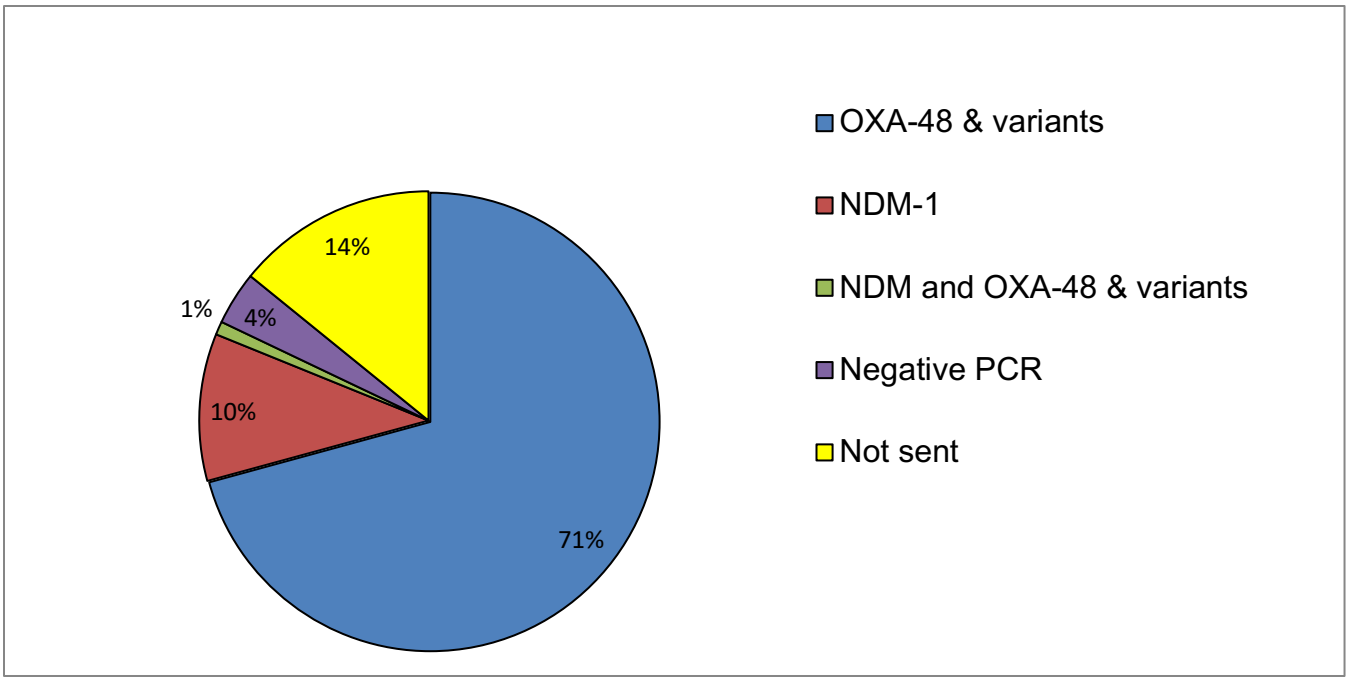


FIGURE 2: Description of Organism PCR



CHAPTER 3: APPENDICES

3.1 DATA COLLECTION SHEET

DATA COLLECTION SHEET: CARBAPENEM RESISTANTENTEROBACTERALES AT HELEN JOSEPH HOSPITAL (Circle or Fill in)		
1. PATIENT DETAILS		
Study number		
Gender	Male/Female	
Ward	Medical/ Surgical/Orthopaedics/Psychiatry/Other/ICU/Ward 12 HC	
Admission Date		
Transfer in from another institution	Yes/No	
Reason for admission		
Duration of hospitalization prior to culture		
Total length of hospital admission		
2. DETAILS ABOUT CULTURE		
Specimen type/site of culture	Blood/Urine/Pus/Sputum/Tracheal aspirate/Tissue/Rectal swab/Other	
Organism cultured	*Klebsiella pneumoniae/E.coli/E.cloacae*/Other	
Colonization versus Infection	Colonization/ Infection	
Date cultured		
Organism PCR	Class A: KPC/GES Class B: VIM/IMP/NDM Class D: OXA-48	
WCC/CRP at time of culture	WCC:	CRP:
3. PATIENT PROFILE: RISK FACTORS FOR CRE ACQUISITION		
COMORBIDITIES		

HIV Status: Positive/Negative	CD4:	VL:	ARV naïve	On ARVS's
DM	Yes/No			
Malignancy	Yes/No			
Prior exposure to immunosuppressives	Yes/No			
Other co-morbidities				
ANTIBIOTIC HISTORY(preceding 12 months)				
Prior exposure to penicillin antibiotics	Yes/No			
Prior exposure to cephalosporin antibiotics	Yes/No			
Prior exposure to carbapenem antibiotics	Yes/No			
Prior exposure to fluoroquinolone antibiotics	Yes/No			
Prior exposure to aminoglycoside antibiotics	Yes/No			
Prior exposure to macrolide antibiotics	Yes/No			
Prior exposure to lincosamide antibiotics	Yes/No			
Prior exposure to nitromidazole derivatives	Yes/No			
INVASIVE DEVICES				
Urinary catheter	Yes/No			
Central line	Yes/No			
Mechanical ventilation	Yes/No			
Quinton line	Yes/No			
Other drains (pigtail/ICD etc)	Yes/No			
Surgical procedures	Yes/No			
4. MANAGEMENT PLAN				
Management plan	Treated/ Not treated			
Date treatment initiated				
Time from culture to treatment				

Antibiotic used	<u>Polymixin:</u> Colistin	<u>Aminoglycoside:</u> Amikacin	<u>Carbapenem:</u> Imipenem Meropenem Ertapenem	<u>Other</u> Fosfomycin Rifampicin Tigecycline	
Eradication of the organism: Negative culture or clinical parameters of recovery/ failure	Yes	No	Not done		
Treatment duration					
Patient Outcome	Discharged	Demised	Other		
5. INFECTION CONTROL MEASURES					
Seen by IPC team	Yes/No				
Patient isolated already on visit by IPC	Yes/No				
Date Isolated					
Time from culture to isolation					
Isolation for duration of admission versus de-isolation					
IPC precautions adhered to	Yes/No				
Active surveillance of contacts via rectal swabbing	Yes/No				

3.2 ETHICAL CLEARANCE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Romana Jassat

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M180120

NAME: (Principal Investigator)	Dr Romana Jassat
DEPARTMENT:	Internal Medicine Helen Joseph Hospital
PROJECT TITLE:	Carbapenem Resistant Enterobacteriaceae (CRE) at Helen Joseph Hospital
DATE CONSIDERED:	26/01/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof P. Ive, Dr J. Nel and Dr R. Chomba
APPROVED BY:	CB Penny Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	31/01/2018

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in January and will therefore be due in the month of January each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

3.3 RESEARCH COMMITTEE PERMISSION LETTER

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Gauteng Department of Health
Helen Joseph Hospital
Enquiries: Dr. M. Mukansi
Research Committee: Chairperson
Tel :(011) 489-0306/1087
Fax :(011) 489 1038
E mail:murimisi.mukansi@wits.ac.za

22 November 2017

To whom it may concern

Subject: HELEN JOSEPH HOSPITAL RESEARCH COMMITTEE APPLICATION

PROTOCOL TITLE: Carbapenem Resistant Enterbacteriacea (CRE) at Helen Joseph Hospital.

Protocol Ref No: Dr. Romana Jassat
Ethic Clearance: Pending
Principal investigator: Dr. Romana Jassat

Department: Internal Medicine

Committee Recommendations
Conditional access approval is given while waiting the final ethical clearance certificate from the University of Witwatersrand HREC.

As this is all independent research project it remains the responsibility of the researcher to recruit participants from the relevant department within the hospital and acquire their individual voluntary consent to participate in your study.

Dr. Murimisi Mukansi
Chairperson of the HJH Ethic and Research Committee

3.4 TURNITIN ORIGINALITY REPORT: Student Number: 305431

ORIGINALITY REPORT

8%	4%	4%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Posters", Clinical Microbiology and Infection, 2009 Publication	1%
2	Submitted to University of Limpopo Student Paper	1%
3	www.samj.org.za Internet Source	1%
4	Marta C. Nunes, Anne von Gottberg, Linda de Gouveia, Cheryl Cohen et al. "Persistent High Burden of Invasive Pneumococcal Disease in South African HIV-Infected Adults in the Era of an Antiretroviral Treatment Program", PLoS ONE, 2011 Publication	<1%
5	Vincent van Almsick, Beniam Ghebremedhin, Niels Pfennigwerth, Parviz Ahmad-Nejad. "Rapid detection of carbapenemase-producing Acinetobacter baumannii and carbapenem-resistant Enterobacteriaceae using a bioluminescence-based phenotypic method",	<1%

	Journal of Microbiological Methods, 2018 Publication	
6	worldwidescience.org Internet Source	<1%
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8	Submitted to Rutgers University, New Brunswick Student Paper	<1%
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10	Gopi Patel, Robert A Bonomo. "Status report on carbapenemases: challenges and prospects", Expert Review of Anti-infective Therapy, 2014 Publication	<1%
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16	"Posters", Clinical Microbiology and Infection, 05/2009 Publication	<1%
17	Virginia M. Pierce, Patricia J. Simner, David R. Lonsway, Darcie E. Roe-Carpenter et al. "Modified Carbapenem Inactivation Method for Phenotypic Detection of Carbapenemase Production among Enterobacteriaceae", Journal of Clinical Microbiology, 2017 Publication	<1%
18	Suay-García, Pérez-Gracia. "Present and Future of Carbapenem-resistant Enterobacteriaceae (CRE) Infections", Antibiotics, 2019 Publication	<1%
19	Submitted to University of KwaZulu-Natal Student Paper	<1%