THE EFFECT OF FIRST-LINE TB TREATMENT ON CARBAPENEM RESISTANCE IN FAECAL *ENTEROBACTERALES*

By
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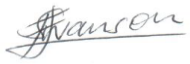
DECLARATION

I, Fay Liza Manuel (Swanson) declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed  Date: 25 August 2023

ABSTRACT

Carbapenems are used more regularly as empiric and targeted treatment options due to the emergence of resistant bacteria. There is an increased risk of mortality and treatment cost in cases where carbapenem resistant *Enterobacterales* (CRE) have been isolated. This is due to the fact that remaining therapeutic options are limited, potentially toxic and expensive. Antibiotic use is a risk factor for colonisation with resistant bacteria such as carbapenem resistant *Enterobacterales* (CRE) and carbapenemase-producing *Enterobacterales* (CPE). Tuberculosis treatment specifically has not been evaluated as a risk factor for CRE colonisation in the literature. This study aimed to determine whether patients receiving tuberculosis (TB) treatment are likely to be colonised with CRE two weeks after treatment is commenced by collecting a rectal swab before treatment commences and again two weeks after first line treatment starts. Each collected swab was screened using culture CARBA-R screening plate and the results evaluated. Although no CRE was isolated in these 18 patients, due to the small sample size, the possibility cannot be excluded. A future study sampling a larger patient group, with additional sampling time points later in the treatment regimen will be valuable to detect subsequent CPE / CRE colonisation in the population receiving TB treatment.

Keywords:

Tuberculosis treatment; *Enterobacterales*; carbapenem resistance

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Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the EDCTP.

DEDICATION

To the strongest women I know who now watch over me from above, Theresa May Manuel and Agatha Andrea Manuel.

LIST OF PRESENTATIONS

The following oral presentations were done during the course of this MSc study:

1. Presentation title: The Effect of first-line TB treatment on carbapenem resistance in faecal *Enterobacterales - project update*

    - Presenter - Fay Swanson
    - Event name - PanACEA Annual Meeting 2021
    - Date of presentation - 29 June 2021
    - Venue/Location – Virtual

2. Presentation title: The Effect of first-line TB treatment on carbapenem resistance in faecal *Enterobacterales - project update*

    - Presenter - Fay Swanson
    - Event name - TASK Consumption Meeting
    - Date of presentation - 27 June 2020
    - Venue/Location – Virtual

3. Presentation title: The Effect of first-line TB treatment on carbapenem resistance in faecal *Enterobacterales - project update*

    - Presenter - Fay Swanson
    - Event name - PanACEA Annual Meeting 2019
    - Date of presentation - 19 March 2019
    - Venue/Location - Maputo, Mozambique

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AMR	Antimicrobial resistance
AMS	Antimicrobial stewardship
ARG	Antibiotic resistance genes
AIDS	Acquired immunodeficiency syndrome
AST	Antimicrobial sensitivity testing
BSI	Blood stream infection
CAI	Community acquired infection
CDC	Centre for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CPE	Carbapenemase-producing *Enterobacterales*
CRE	Carbapenem-resistant *Enterobacterales*
CSE	Carbapenem-susceptible *Enterobacterales*
CTB	Centre for Tuberculosis
DOTS	Directly observed treatment short course
DPA	Dipicolinic acid
eHRB	Emerging highly-resistant bacteria
ESBL	Extended spectrum β-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GLASS	Global Antimicrobial Resistance and Use Surveillance System
GCP	Good Clinical Practice
HAI	Hospital acquired infection
HIV	Human immunodeficiency virus
HRZE	Isoniazid, rifampicin, pyrazinamide, ethambutol
ICF	Informed consent form
ICH GCP	International Conference on Harmonization for Good Clinical Practice
ICU	Intensive Care Unit
INH	Isoniazid

ISO	International Organisation for Standardisation
KPC	*Klebsiella pneumoniae* carbapenemase
LOS	Length of stay
LTCF	Long-term care facility
MDR	Multi-drug resistant
MDR - E	Multi-drug resistant *Enterobacterales*
MDRO	Multi-drug resistant organism
MHT	Modified Hodge test
MIC	Minimal inhibitory concentration
MTB	Mycobacterium tuberculosis
NICD	National Institute for Communicable Diseases
NHLS	National Health Laboratory Service
PBP	Penicillin-binding proteins
PTID	Participant Identification number
PZA	Pyrazinamide
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SANAS	South African National Accreditation System
TB	Tuberculosis
UN	United Nations
UTI	Urinary tract infection
WHO	World Health Organisation
XDR	Extensively drug resistant

CLARIFICATION OF TERMS

Antimicrobial agent: a substance that can kill, inhibit or slow the growth of microorganisms.

Bactericidal: an agent that can kill bacteria.

Bacteriostatic: an agent that inhibits the growth of bacteria but does not kill the bacteria

Carbapenems – a class of β lactam antibiotics that differ from penicillins and cephalosporins in their chemical structure and broader spectrum of activity

Carbapenemases - β-lactamases that have the ability to hydrolyse penicillins, cephalosporins, monobactams, and carbapenems thus rendering many β-lactams ineffective clinically.

Colonisation resistance - the ability of a diverse health population of microorganisms to prevent colonisation/invasion by other potentially pathogenic organisms.

Commensal - a microorganism that does not cause harm to the host.

Dysbiosis - (also called dysbacteriosis) a term for a microbial imbalance or maladaptation on or inside the body, such as an impaired microbiota.

Enterobacterales - a taxonomic order that contains many Gram-negative organisms such as *Escherichia coli, Klebsiella, Salmonella, Shigella, Serratia, Citrobacter and Proteus.*

ESKAPE pathogens - **E** *Enterococcus faecium*, **S** *Staphylococcus aureus*, **K** *Klebsiella pneumoniae*, **A** *Acinetobacter baumannii*, **P** for *Pseudomonas aeruginosa*, and **E** for *Enterobacter species*.

HRZE – Is an abbreviation for the four most common antimicrobial agents used in the treatment of tuberculosis

Microbiota - An "ecological community" of commensal, symbiotic and pathogenic microorganisms" found in and on all multicellular organisms studied to date from plants to animals. It includes bacteria, archaea, fungi and viruses.

Microbiome - Collective genomes of the microbes (composed of bacteria, bacteriophage, fungi, protozoa and viruses) that live inside and on the human body.

Nosocomial infection - infection or disease acquired whilst in a hospital where the patient was admitted for reasons other than the infection.

Prodrug – An inactive compound that is metabolised into a pharmacologically active drug.

Pseudomonas aeruginosa - Gram-negative rod-shaped bacteria that can cause a wide range of infections in individuals who are immunocompromised.

Resistome – the collection of all antibiotic resistance genes and their precursors in pathogenic and non-pathogenic bacteria.

Staphylococcus aureus - Gram-positive coccal bacteria that produce toxins and enzymes capable of causing a variety of infections.

Staphylococcus epidermidis - a Gram positive coagulase negative coccus that is normally a commensal but can cause infections in individuals who are immunocompromised.

Selection pressure - Any change in the environment that encourages particular mutations to succeed**.** For example, antibiotic use kills susceptible bacteria and allows microorganisms with resistant genes to survive and proliferate.

Subclinical TB – is a disease caused by viable tuberculosis bacteria that does not cause clinical TB-related symptoms. It does however cause other abnormalities that can be detected using existing radiologic or microbiologic laboratory assays.

Wound - an injury to a living tissue leading to the disruption of its normal structure and function.

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CHAPTER 1

1.1. INTRODUCTION

Mycobacterium tuberculosis (MTB) infections remain a major healthcare concern globally. According to the World Health Organisation (WHO), tuberculosis (TB) is one of the leading causes of death due to an infectious agent; this disease results in 1.5 million fatalities annually (Stephanie *et al*., 2021). The standard first line of TB treatment consists of a combination of antibiotics, rififour (rifampicin, isoniazid, pyrazinamide, ethambutol) taken over a six-month period. Compounding this, is the increased drug resistance classified as multi-drug resistant TB (MDR) and extensive drug-resistant TB (XDR) which is becoming increasingly challenging to treat due to cost, toxicity and duration of antibiotic therapy required. Treatment can last for up to two years with many serious side effects. South Africa is ranked as one of the 30 high TB burden countries with the incidences in the Western Cape being a huge public health concern.

Carbapenems are broad-spectrum antibiotics used more regularly as empiric and targeted treatment options due to the emergence of resistant bacteria. There is, however, an emerging increase in resistance to carbapenems leading to limited treatment options for patients with a poor prognosis. There is an increased risk of mortality and treatment cost in cases where carbapenem-resistant *Enterobacterales* (CRE) have been isolated. This is due to the fact that remaining therapeutic options are limited, potentially toxic and expensive. Patients requiring treatment are often seriously ill and in need of urgent medical care. There are a number of risk factors that can increase the risk of acquiring a resistant strain for example, being in a long-term care facility; admitted to hospital, especially intensive care or high care; comorbidities and invasive medical procedures. Antibiotic use is another important risk factor for colonisation with resistant bacteria such as carbapenem-resistant *Enterobacterales* (CRE) and carbapenemase-producing *Enterobacterales* (CPE). This may increase in regions where antimicrobial stewardship (AMS) is not an area of focus or adhered to when prescribing antibiotics. Areas of high TB incidence (e.g. South Africa) may also contribute to increased colonisation with CREs and CPEs.

Currently, there is very little information in the literature about TB treatment being a risk factor for CRE colonisation. The combination of TB treatment antibiotics and the prolonged period of exposure of six months could be a significant risk factor for colonisation with resistant

bacteria. This study therefore aimed to determine whether patients receiving tuberculosis (TB) treatment are more likely to be colonised with CRE two weeks after treatment has been commenced. A two-week period was selected as this coincides with the usual treatment period at TASK clinic where participants are admitted as in-patients and therefore easily accessible for sampling during this time.

1.2. AIM

TB treatment specifically has not been evaluated as a risk factor for CRE colonisation in the literature. This study aimed to determine whether patients receiving TB treatment are more likely to be colonised with CRE 14 days after treatment has commenced.

1.3. OBJECTIVES

i. Determine if there is an association between first-line TB treatment and gut CRE/CPE acquisition or colonisation after a 14 - day treatment period.

ii. Determine additional secondary effects of the first-line TB treatment

iii. To determine if the first-line TB treatment can be considered a risk factor for CRE/CPE colonisation

CHAPTER 2

LITERATURE REVIEW

2.1. Background to research problem

In South Africa, the TB epidemic poses a huge risk to the health sector as it may directly or indirectly lead to increased morbidity and mortality if not effectively managed (Dudley *et al*, 2018). South Africa is ranked as one of the 30 high TB burden countries globally by the WHO with the Western Cape Province reporting one of the highest TB incidences in the country (Dudley *et al*, 2018).

The standard TB regimen prescribed for the treatment of pulmonary tuberculosis is usually administered for at least six months, thus patients are exposed to antimicrobial effects for a prolonged period. This regimen contains a combination of agents: isoniazid, rifampicin, ethambutol and pyrazinamide that are taken daily.

Carbapenems are β-lactam antibiotics (Fig 1) belonging to three of the four Ambler classification classes for β-lactamases (Class A, Class B and Class D) and can be used to treat a variety of infections such as urinary tract infections, pneumonia, gynaecological, and soft tissue infections. The carbapenems are indicated for antimicrobial therapy in cases where resistance to first-line agents is expected since they have a broad spectrum of activity. The carbapenems are active against many gram-positive and anaerobic organisms but their main benefit has been a broader activity against resistant gram-negative bacteria especially ESBLs (Török *et al*, 2009).

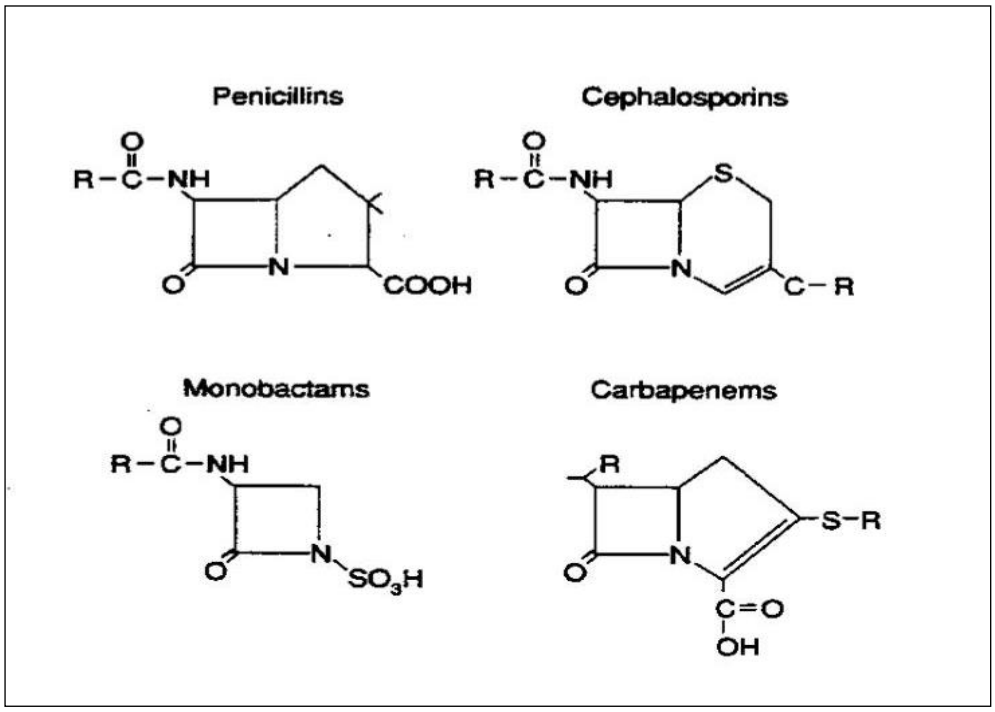


Figure 1. Structure of Beta-Lactam antibiotics

(Source: https://image.slideserve.com/604437/beta-lactam-structure-l.jpg)

The CRE organisms that are resistant to carbapenems due to the production of carbapenemases are known as CPE. The resistance to carbapenems in CPE is largely plasmid-mediated and is thus acquired. Carbapenemase production is the most common resistance mechanism (Teena *et al,* 2018). The incidence of carbapenemase-producing *Enterobacterales* (CPE) has been increasing in South Africa over recent years (Perovic *et al*, 2018).

Risk factors for acquiring CRE include exposure of patients to hospital high care settings, long-term care facilities (LTCF), invasive procedures or foreign devices and contact with a CRE patient. Patients who have co-morbidities and antibiotic exposure are also at risk and thus community-associated cases have been described (Van Duin *et al*, 2016 and Schwartz-Neiderman *et al*, 2016). This increased likelihood of exposure is due to numerous human medical advancements in surgery, neonatal care, organ and tissue transplants as well as haemato-oncology over the years. These advancements have led to increased length of hospitalisation and increases chance of colonisation. CRE occurs throughout the health care sector; the incidence of CRE colonisation is high in the health care sector globally. The occurrence in middle income countries where antibiotic access is not controlled (NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES (nicd.ac.za)).

The human microbiome is composed of bacteria, bacteriophages, fungi, protozoa and viruses that live in harmony inside and on the human body. Microbiomes exists in many sites of the body and are important. The gut microbiome of the intestinal tract is considered the most diverse with numerous species and is of major interest to researchers. The gut microbiome plays a role in healthy and disease states and has been of great interest over the recent years. (Bull and Plummer, 2014). Exposure of the gut to any agent that has antimicrobial effects, this includes but is not limited to antibiotics/antifungals/chemotherapeutic agents can have an effect on the composition of gastrointestinal bacteria and result in dysbiosis.

Treatment with any antibiotic class regardless of duration has been shown to result in dysbiosis (Namasivayam et al., 2017). As the microbiome composition is rapidly altered by exposure to antibiotics this makes the environment more conducive to colonisation with opportunistic pathogens or resistant organisms (Namasivayam et al., 2017). Antimicrobial therapy has an effect on the intestinal bacteria and can lead to horizontal transfer and selection of resistance due to selection pressure. Gut bacteria readily exchange antibiotic resistance genes with other bacteria in the gut. Studies indicate that the dysbiosis returns to their normal state but may not return to the same initial state after an insult (Willmann *et al*, 2015). An increase in risk of being colonised with a multi-drug resistant organism such as CRE results from exposure to prolonged antimicrobial therapy as well as other antimicrobial agents, which all may have an effect on the composition of the human gut flora (Hrabák *et al*, 2014).

A recent study investigated gut microbiota dysbiosis in individuals receiving TB treatment, individuals with active TB receiving treatment and those who have not yet started the treatment course. A dysbiosis was noted in bacterial and fungal gut populations of patients who have not yet started treatment. A severe dysbiosis was noted in the patients on anti-TB treatment with a decrease in microbial numbers noted for both bacterial and fungal populations. There are findings that the microbiome is rebuilt with higher proportions of fungi six months after treatment and therefore with a dysbiosis (Cao *et al*., 2021)

As TB regimens, such as the first-line TB medications are antimicrobials, they have also been shown to cause a dysbiosis of microbial flora noted when performing the gut microbiome studies (South African National TB Guidelines 2017 and Namasivayam et al., 2017). Microbial dysbiosis is in turn associated with an increased likelihood of the acquisition of organisms containing resistance genes. The mechanism by which this happens is due to the normal balance of the gut flora occurring. When the balance of microbial flora in an ecological niche is disrupted, there is less colonisation resistance. Colonisation resistance is a term that

describes the ability of a diverse health population of organisms to prevent colonisation or invasion by other potentially pathogenic organisms. This is because a niche is created when the normal flora is destroyed by an antimicrobial, allowing pathogenic organisms to invade and populate the area. Based on this evidence, there was an expectation to observe a change in carbapenem sensitivity patterns in *Enterobacterales* pre-treatment and after treatment was commenced due to the dysbiosis caused by the rifafour treatment itself. There was a likelihood that increasing carbapenem MIC values could be observed in participants during this project and thus increase the risk of CRE/CPE colonisation in the gut.

As the TB incidence in South Africa and the Western Cape are high in relation to the rest of the world, there is a significant proportion of the population on TB treatment, which in turn leads to a prolonged exposure of the gut microbiota to combination antibiotics. It was therefore worthwhile investigating a potential impact the TB regimen may have as a potential risk factor for acquiring CRE colonisation of the gut microbiota during this time.

2.2. *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is a species of pathogenic bacteria that belongs to the family *Mycobacteriacaea.* It is the causative agent of tuberculosis. It was first discovered by Robert Koch in 1882. *M. tuberculosis* has an unusual, waxy coating on its cell surface primarily due to the presence of mycolic acid, which makes the cells impervious to Gram staining. It is therefore more suitable to use acid-fast stains such as Ziehl-Neelsen (Fig 2) or fluorescent stains such as Auramine (Fig 3) to identify *M. tuberculosis* microscopically (https://en.wikipedia.org/wiki/Mycobacterium_tuberculosis)

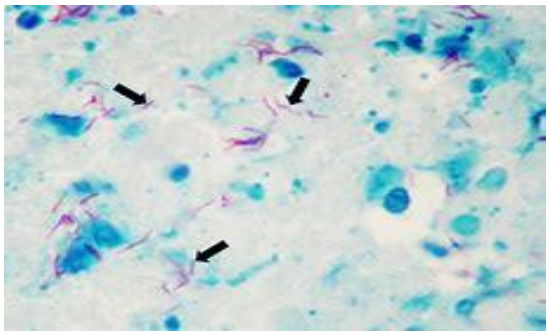


Figure 2. *Mycobacterium tuberculosis* – Ziehl Neelsen Stain: The acid-fast bacilli take up the red colour of the primary dye, carbol-fuschin, indicated by the arrows above. The non-acid-fast bacteria decolourize when acid-alcohol is added and take up the counterstain dye of methylene blue and appear blue.

(Source:*https://th.bing.com/th/id/R.9746d47c0b8466151cd81b48b647ce23?rik=h3yxfeOQppMIDw&pid=ImgRaw&r=0)*

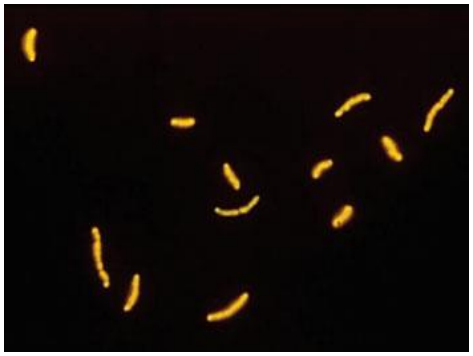


Figure 3. *Mycobacterium tuberculosis*, Auramine-O fluorescent stain: The presence of mycolic acids in the microbes' cell walls. Auramine (primary stain) binds cell-wall mycolic acids and resist decolourization by acid-alcohol. The counterstain, potassium permanganate is applied to quench fluorescence in the background.
(Source:https://globetechcdn.com/labmedica/images/stories/articles/article_images/2016-07-07/RLJ-244.jpg

2.3. *Mycobacterium tuberculosis* in the Western Cape

According to WHO statistics, approximately 66% of TB cases in the world are reported from eight countries, India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa (World health statistics 2021*)* (Fig 4).

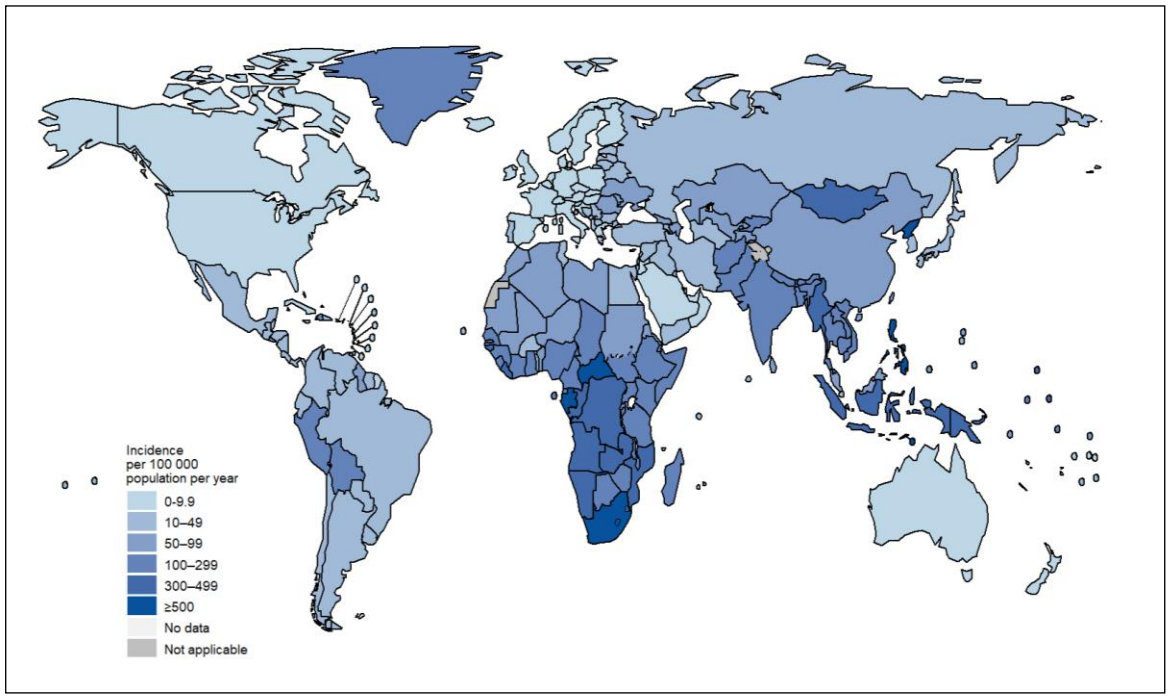


Figure 4. Estimated global TB incidence rates (2020)

The diagram demonstrates the global TB incidence rates where light blue is the lowest incidence rate and darker blue the highest rate. South Africa is indicated with the darkest blue and therefore one of the regions with the highest rate. Source: https://www.who.int/publications/digital/global-tuberculosis-report-2021/tb-disease-burden/incidence

Mycobacterium tuberculosis remains problematic in South Africa which is one of eight countries with the highest incidence rates in the world, with 781 cases reported per 100 000 populations (McIntosh *et al.*, 2018). Thirty of the high TB burden countries in the world accounted for 87% of the incidence globally; of this proportion, South Africa's incidence was 3% (Global Tuberculosis Report 2019). In the Western Cape a large proportion of the population are currently on treatment or have completed treatment relative to the global population. The TB incidence in the Western Cape (Fig 5) is higher than the rest of the country at 906 cases reported per 100 000 populations and has remained one of the highest reported numbers per province in the country (Dudley *et al.,* 2018).

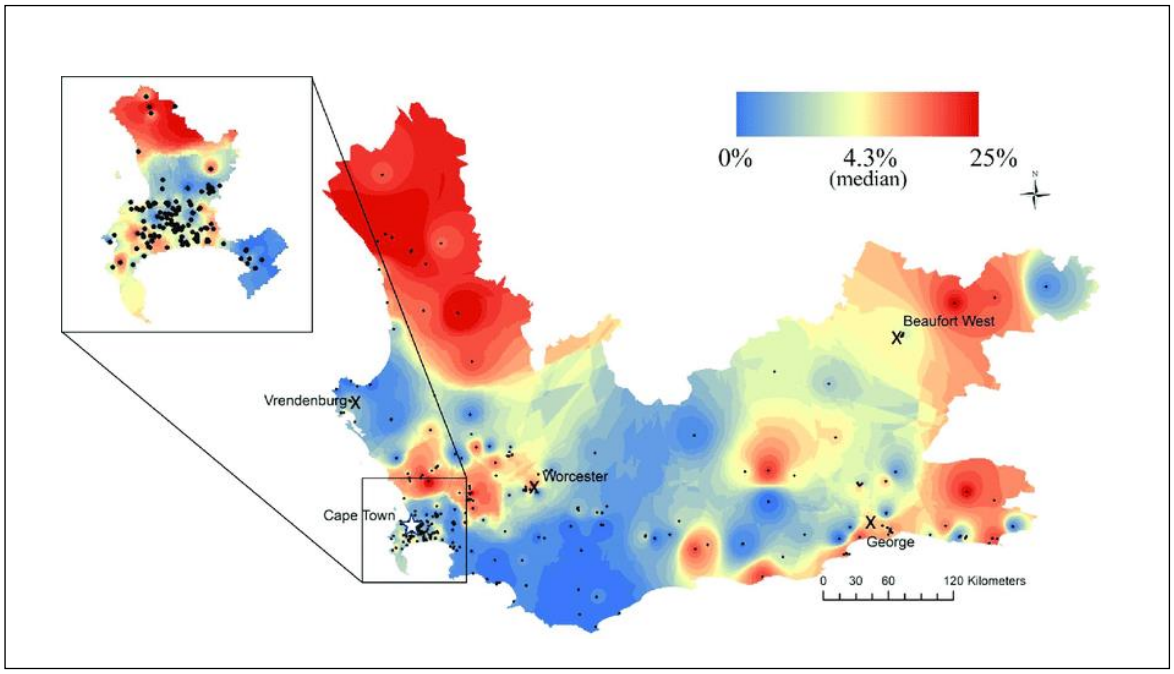


Figure 5. TB incidence in the Western Cape

The percentage of total clinic-diagnosed tuberculosis cases found to be rifampicin resistant in Western Cape Province between 2008 and 2013. The black dots indicate clinic locations.

(Source: https://www.researchgate.net/profile/Elizabeth-Ragan/publication/327149940/figure/fig2/AS:662262490595328@1534907090026/The-percentage-of-total-clinic-diagnosed-tuberculosis-cases-found-to-be-rifampicin.png)

The First National Tuberculosis Prevalence Survey was conducted in South Africa in 2018 as a collaborative effort between the South African Department of Health TB programme, WHO, South African Medical Research Council (SAMRC); the Human Sciences Research Council (HSRC); the National Institute for Communicable Diseases (NICD) to name a few.

The survey shows that currently, a high TB burden remains in South Africa with the number higher in males. A higher percentile of TB was detected in HIV-negative individuals who reported no symptoms opposed to the HIV-positive patients who had more symptoms and thus increasing the chances of detection. The HIV-negative patients were less likely to seek medical intervention due to a lack of symptoms, likely leading to the ongoing spread of the disease. The survey also states that TB is the leading cause of mortality due to infectious diseases in South Africa. It would be interesting to see an update to the survey taking the all the effects of the pandemic on the healthcare sector into account. (The First National TB Prevalence Survey | South Africa 2018).

Surveillance of communicable diseases in South Africa of both tuberculosis as well as carbapenemase resistance is conducted by the National Institute for Communicable Diseases (NICD). The Centre for Tuberculosis (CTB) is a part of the NICD, which conducts TB specific surveillance monitoring, epidemiological and drug resistance studies. A survey conducted by the NICD and the National department of health from 2012 to 2014 showed that MDR-TB numbers were stable compared to a similar survey conducted in 2001 and 2002 and that the MDR-TB numbers were lower compared to the rest of the world. (Health Systems Trust (hst.org.za). Although these numbers are promising, *Mycobacterium tuberculosis* remains a problem in South Africa and the Western Cape.

2.4. History of carbapenems

Looking at the history of carbapenems, understanding their use and the mechanisms by which organisms are resistant to these antibiotics as well as the prevalence of carbapenem resistance is necessary to understand the implications of this resistance in the public health setting.

Carbapenems (Fig 6) are ß-lactam antibiotics derived from thienamycin, which are produced by a Gram-positive bacterium, *Streptomyces cattleya*. Earlier studies have shown that the hydroxyethyl side chain of thienamycin structurally differs greatly from other ß-lactam antibiotics (penicillins and cephalosporins) and sets it apart in terms of microbial activity and spectrum (Papp-Wallace *et al.*, 2011). An increase in mainly cephalosporin resistant Gram-negative extended spectrum beta-lactamase (ESBL) pathogens highlighted the need for new antibiotics with ESBL activity.

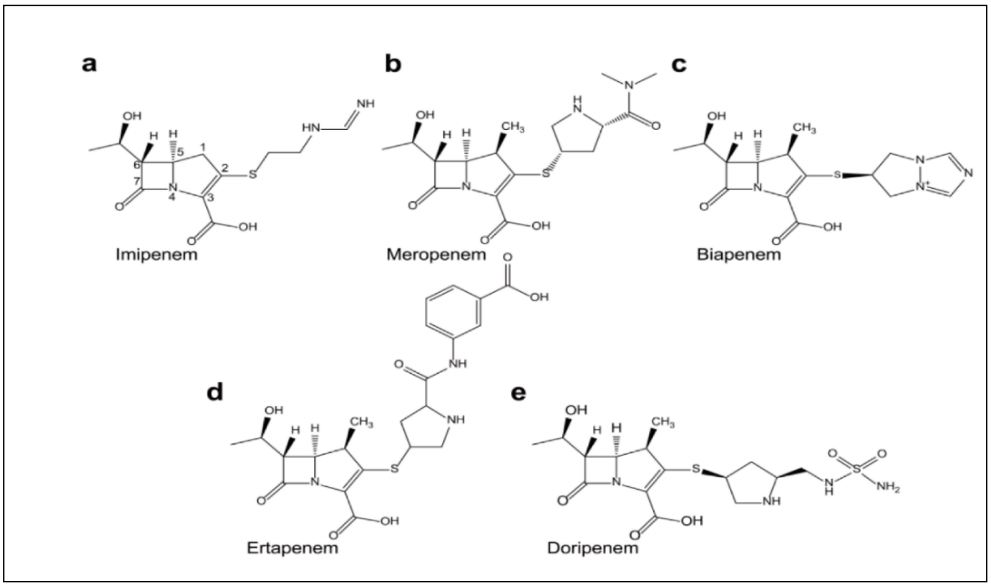


Figure 6. Structure of Carbapenems

(Source:https://www.mdpi.com/ijms/ijms-16-09654/article_deploy/html/images/ijms-16-09654-g001.png

Imipenem (Fig 6) was released in 1985 and later more-stable compounds, meropenem, ertapenem and doripenem all of which had a broader spectrum of coverage for resistant Gram-negative pathogens, were developed. These antimicrobials have an affinity for most high molecular weight penicillin binding proteins (PBPs) of Gram-positive and negative bacteria and can bind at multiple sites eventually resulting in cell autolysis. Carbapenemes, especially imipenem are also able to travel through the outer cell membrane of Gram-negative bacteria via membrane protein OprD. Penicillins and cephalosporins contain OprC and OprF membrane proteins.

Carbapenems are stable against many ß-lactamases, which also adds to the broad-spectrum coverage abilities (Török *et al.*, 2009). The carbapenem spectrum of antimicrobial activity includes Gram-negative and Gram-positive pathogens including anaerobes and they are frequently used as empiric and targeted treatment.

2.5. Antibiotic Resistance

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi and parasites undergo changes and over time render medicines used to treat these infections ineffective. This may lead to an increased risk of morbidity and mortality in infected individuals. Antimicrobials are used in humans, animals and the environmental sector and a lack of discretion while in use leads to an increase in AMR. The result is an increased risk of severe illness when conducting medical procedures, prolonged treatments and increased costs. The COVID19 pandemic has resulted in further concerns related to AMR.

Extended spectrum beta-lactamase (ESBL) production is the main mechanism of acquired resistance in Gram-negative bacteria. These ESBL producing organisms can be found in the hospital setting as well as, more recently, in the community (Díaz-Agero Pérez *et al,* 2019). The ESBLs are enzymes produced by organisms that contain antibiotic resistance genes carried on plasmids (BIOMÉRIEUX, 2018). These ESBL enzymes hydrolyse many cephalosporins making these antibiotics ineffective as treatment options for numerous infections caused by the ESBL producing organisms. An increase in resistance to existing Gram-negative agents such as cephalosporins is especially problematic as the broader-spectrum carbapenems are more likely to be prescribed (Papp-Wallace *et al*, 2011 and Van Duin *et al*, 2016).

There is an increased risk of mortality and treatment cost in cases where CRE have been isolated. This is because remaining therapeutic options are limited, potentially toxic and expensive (Richter *et al* 2016 and Van Duin *et al* 2016). Resistance to carbapenems can be intrinsic i.e., due to chromosomal carbapenemases, efflux pumps or reduction in outer membrane permeability due to porin loss, or plasmid-mediated, which is an acquired resistance mechanism. Resistance can also occur due to a combination of ESBL or AmpC ß-lactamase and a deficiency of porins in the outer membrane, namely ESBL and impermeability or AmpC and impermeability.

The Global antimicrobial resistance and use surveillance system (GLASS report 2021), noted a concerning trend of resistance to antibiotics used to manage multi-drug resistant bacterial infections (MDR), especially blood stream infections (BSIs) greatly limiting treatment options (GLASS report, 2021).

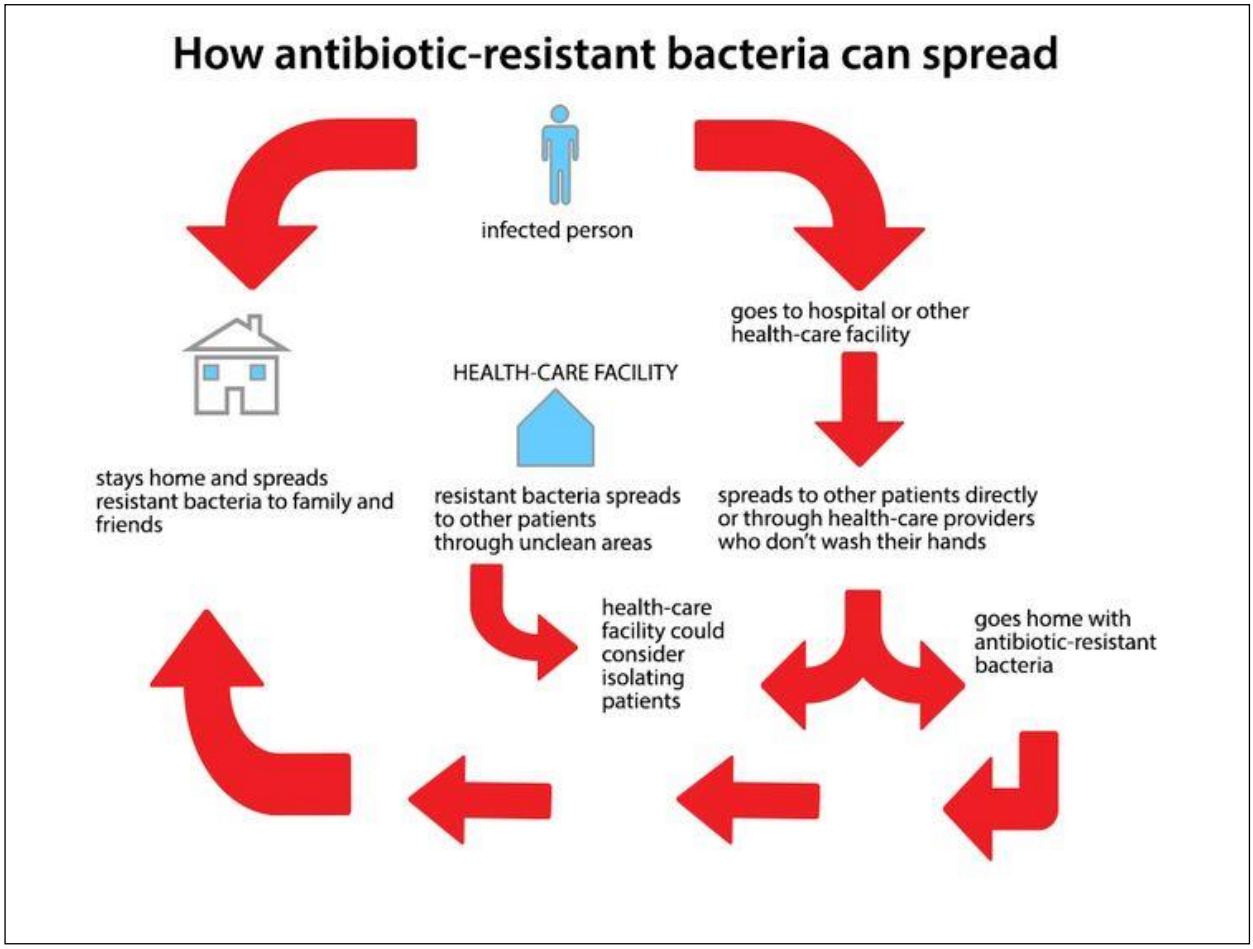


Figure 7. How easily antibiotic resistant bacteria can spread

(Source:https://i.pinimg.com/736x/d4/e6/c4/d4e6c45ec91627f5ce389fb816a5d342--alternative-health-medical-science.jpg)

2.6. Carbapenem resistance

CRE organisms have been defined by the Centre of Disease Control (CDC) as any *Enterobacterales* species confirmed resistant to any carbapenem (excluding Intermediate resistance) or any *Enterobacterales* confirmed to produce carbapenemases. Carbapenems were introduced as a last line of defence and infections caused by carbapenem-resistant organisms may thus be untreatable (Carbapenem Resistant *Enterobacteriaceae* (CRE) and others - NICD [accessed 14 March 2022]).

Organisms with intrinsic resistance, for example *Proteus mirabilis* should be confirmed to be resistant to a carbapenem other than imipenem due to the intrinsic resistance to this carbapenem (Van Duin *et al.*, 2016). CPE and CRE have been detected worldwide more

confirmed cases of infection/colonisation have been reported in recent years and is seen as a global healthcare threat. Initially the resistance was noted primarily in *Klebsiella pneumoniae* however, this is no longer the case. Carbapenemase resistance over the years have been increasingly isolated from *Enterobacterales* group and has therefore become known as CRE. CRE be due to widespread international travel, farming practices, especially with livestock, and even recreational water usage has facilitated the worldwide spread of resistance. There is a need for active surveillance across the globe to monitor and contain the spread of resistance and to ultimately feed into the best possible antibiotics prescribed as treatment.

Different carbapenemase genes have been found throughout the world such as *Klebsiella pneumoniae* carbapenemase (*bla*$_{KPC}$) found in outbreaks in America and Israel, and New Delhi metallo- β-lactamase (NDM) *bla*$_{NDM}$ have been isolated in Europe after being documented in India first. Verona integrin-encoded metallo- β-lactamase *bla*$_{VIM}$ has been problematic in Europe for some time, imipenemase gene (IMP) is commonly isolated in Asian countries and OXA-48 appears to be spreading in Europe (Cepheid 2018). In South Africa, it appears that most common genes detected were NDM and OXA-48 in hospitalised patients from 2015 to 2016 (Teena *et al*, 2018).

CREs are considered a major threat to the healthcare sector, the WHO, and CDC and is an urgent area of focus. *The* WHO policy guidance (WHO policy guidance on integrated antimicrobial stewardship activities) on integrated AMS activities is aimed at guiding policy makers within health departments and AMR national steering committees or its equivalent and all those responsible for formulating national action plans and policies. It covers areas of the healthcare sector as well as animal and plant or environmental sectors. *(*WHO policy guidance (WHO policy guidance on integrated antimicrobial stewardship activities) on integrated AMS activities). If these policies are adhered to across the globe, it will facilitate in curbing the alarming rate of AMR reported.

A retrospective study published in this year evaluated trends in CRE in a hospital in Malaysia where 168 isolates were noted over the 2014-2015 period. The predominant CRE species isolated over this period was *K. pneumoniae*. Of the 168 strains, the following carbapenemases were noted: *bla*OXA-48 (the most dominant), *bla*OXA-232, *bla*VIM and *bla*NDM. (Kong *et al.*, 2022).

An annual report released by the NICD shows that from 2015 to 2017 there has been an increase in CRE bacteraemia over many regions in South Africa. This includes, the Free State (lowest number of reported cases nationally), Gauteng (highest reported numbers), KwaZulu-

Natal (second highest) and the Western Cape (third most reported cases). *Klebsiella pneumoniae* was the most frequently isolated organism with the gene OXA-48 most dominant followed by the NDM gene (GERMS-SA-AR-2017-final.pdf (nicd.ac.za)).

The most recent update on the NICD report for 2019 NICD-Bulletin-Vol18-Iss3-December2020-GERMS-SA-ANNUAL-SURVEILLANCE-REPORT.pdf again gathered cumulative data from 2015 until 2019 examining bacteraemia infections for the following pathogens: *Candida* species and ESKAPE organisms (*Enterococcus, Staphylococcus aureus, Klebsiella, Acinetobacter, Pseudomonas* and *Enterobacteriaceae (Enterobacter* and *E. coli*) as well as CREs. An increase in CRE was noted during the 2019 period, an increase from the 2017 and 2018 surveillance years. This is concerning as an increase in isolating these resistant organisms would mean an increase in healthcare costs and poor prognosis for affected patients. (GERMS-Annual-Review-2019_.pdf (nicd.ac.za)).

The mechanisms of carbapenem and non-carbapenem resistance are highlighted in Figure 8:

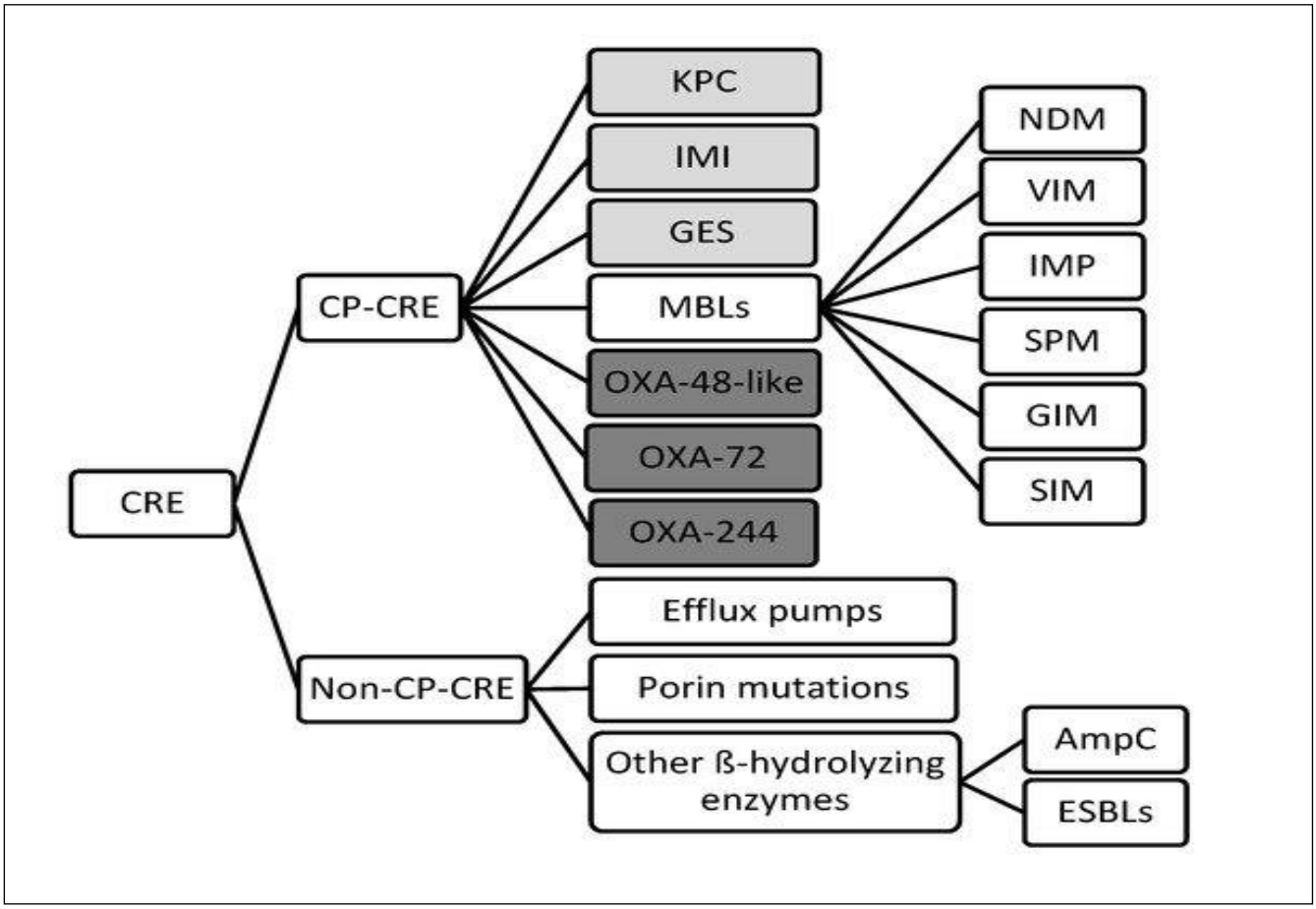


Figure 8. Mechanisms of carbapenem and non-carbapenem resistance

Source:https://www.mdpi.com/antibiotics/antibiotics-08-00122/article_deploy/html/images/antibiotics-08-00122-g001-550.jpg

2.7. CRE Colonisation

Risk factors for CPE/CRE colonisation/infection include in-hospital patients particularly in a high care, long term or ICU setting; previous exposure to antibiotics (not only carbapenems); mechanical ventilation and indwelling devices, i.e. urinary catheters. These organisms are mostly associated with hospitalisation i.e., hospital acquired infections (HAI). However, community acquired, or associated cases have been reported in the literature (Van Duin *et al.,* 2016). Colonisation does not necessarily lead to infection and can go undetected as in community-associated infections (CAI) (Kelly *et al.,* 2017). The aim of screening patients for CRE/CPE in a healthcare setting is to prevent transmission of these organisms from colonised and infected patients to staff and other patients (Richter *et al.*, 2016). A surveillance study in the South African public health sector in 2016 has shown an increase in CPE over a two-year period highlighting a need for better surveillance and enhancing infection control and AMS programmes (Perovic *et al.*, 2018).

A recent study on ESBL prevalence where patient's faecal swabs were screened for ESBL producing *Enterobacterales*, noted a high percentage of ESBL and carbapenemase producers (Díaz-Agero Pérez *et al.,* 2019). This is interesting, as all patients were screened soon after admission and did not have a prolonged hospital stay.

A study evaluating the time period patients are CRE carriers, examined patients from 2009 to 2010 where a CRE was detected during an in hospital. These patients were followed up with a rectal swab for analysis. Patients with previous hospitalisation exceeding one year, displayed a lower probability of CRE carriage and multiple hospitalisations extended the time period of carriers. (Zimmerman *et al.,* 2013). This risk factor was important for eliminating patients for the purposes of this project and patients were screened using a patient questionnaire.

In a study conducted in Peru, a 10-year observational study period on paediatric patients from January 2009 to 31 December 2018 found a changing infection pattern where increasing ambulatory care and community-acquired infections were noted. However, the article did not specify the type of ambulatory care received. There is a need to evaluate CRE and CPE in hospital as well as in patients receiving ambulatory care (Ubillus Arriola *et al.*, 2021).

A retrospective observational study conducted from January 2019 to June 2020 at the teaching hospital of Terni in Italy where ASP and infection control measures were successfully implemented, noted an increase in CRE isolated during the COVID-19 pandemic. From March

2020 the ICU was used for patients with COVID and infection control measures, additional staff training and an increase in ICU staff were adapted to accommodate for the virus. Despite these measures a marked increase from 6.7% in 2019 to 50% for March and April 2020 in CRE was detected. It is believed that increased staff members to the ward increased time spent on patient care since approximately two thirds of the CRE-positive patients moved to the prone position likely contributed to the sharp increase in CRE isolates and colonisation noted during this time. (Tiri *et al*, 2020)

It is known that there are many risk factors that predispose a patient to acquiring a CPE / CRE, and antibiotic therapy is one of them (Schwartz-Niederman *et al*, 2016). There are many effects of antibiotic exposure depending on the drug however, studies have shown that dysbiosis occurs commonly (Hrabák *et al.*, 2014). Dysbiosis affects colonisation resistance. The TB regimen consists of a prolonged regimen of a number of antimicrobials thus resulting in prolonged dysbiosis which lasts after treatment has been completed (Namasivayam et al., 2017).

The differences between CP-CRE and non-CP-CRE cultures in a population of US veterans were evaluated over a five-year period from 2013 to 2018. Participants who received a diagnosis of chronic heart failure or gastroesophageal reflux disease (GERD) in 2017 or 2018 were all associated with an increased risk of CP-CRE. More than 90 days of proton pump inhibitor (PPI) exposure was more frequent among those without previous antibiotic exposure and where CP-CRE was isolated. Interestingly, there is the diagnosis and treatment for GERD, with PPI as a risk factor. There was also a link between race, particularly belonging to the African American race, which indicated the need for even further studies (Wilson *et al*, 2021). An article looking at disparities in racial and ethnic antimicrobial use showed that during a survey from 2014 to 2015, white people reported twice as many antibiotic prescriptions filled than other ethnic groups. Olesen *et al*, 2018. Another study showed there are gender inequalities in antibiotic prescribing as well where woman where prescribed more antibiotics than men (Schröder *et al.*, 2016).

After searching through the literature, no association was found between the TB treatment regimens and the potential effect on faecal CPE colonisation or resistance. This was therefore a worthwhile field to explore, especially in the Western Cape where tuberculosis remains a major problem.

There is a possibility that TB patients on current treatment or completed treatment can be hospitalised for a different reason and require carbapenem treatment for ailments other than

TB the Western Cape and South Africa. It is crucial for medical practitioners to be aware of any potential secondary effect that the TB treatment may cause.

The aim of this project was to screen a population of patients that is not ordinarily screened for CPE / CRE colonisation. Screening is normally performed for patients on admission to a hospital where patients are deemed to be at high risk of being colonised (i.e., recent hospitalisation, transfer from a high CRE prevalence setting etc.) as well as regular screening of patients in high care/ ICU/haemato-oncology patients who are at risk of CRE colonisation and infection. This is to ensure that infection control measures can be instituted correctly if need be and that high-risk patients that are known to be colonised are monitored closely for infection.

2.8. Sample/ Specimen type

Although the stool sample remains the gold standard when sampling from the gastro-intestinal tract, there are some factors to consider when screening patients for colonisation. A rectal swab is preferred when screening for organisms colonising the gastro-intestinal tract because the collection is faster i.e., can be collected by the patient and there is no need to wait until the patient can produce a stool sample. There are articles and studies from forty years ago stating that rectal swabs are preferred to stool samples for the isolation of *Shigella* species, *Vibrio cholera* as well as *S. aureus* and can be linked to the organisms potentially concentrating to the intestinal mucosa (Crossley *et al.*, 1980).

A comparative study relating to infection control screening for resistant *E. coli* isolated from the gut was also revised. A stool sample, rectal swab and perirectal swab were collected from each screened participant and the swab results were compared to the stool sample as the gold standard. It was reported that compared to stool samples, a sensitivity of 90% and specificity of 100% for both rectal and perirectal swabs was obtained (Lautenbach *et al.*, 2005).

Gut microbiota studies also suggest that the microbiota detection in stool and rectal swabs were highly similar with rectal swab collection being more convenient and easier to collect than stool and in healthier patients can be self-collected at the patient's convenience. In a study where 30 stool and rectal swab samples were collected from each participant, a complete bacterial analysis was obtained in 29 of the 30 swabs when compared to the stool samples (Bassis *et al.*, 2017). Studies have shown links between human intestinal microbiota

and an increasing number of diseases. When the microbiota is in the normal healthy state, it has a positive effect on general health and well-being, while a dysbiotic state affects general health (Jovel *et al.*, 2018).

Screening for CRE does not require large volumes of stool however, a limitation is the sample amount, which is less with a swab than a stool sample. By specifying that the swab should be visibly soiled with stool increases the sample volume obtained with the rectal swab and therefore the sensitivity of the assay is dependent on the amount of stool soiling the swab.

2.9. Sample analysis

. There are currently a number of techniques, algorithms and media used for the screening process. The usual methods include screening for carbapenem resistant *Enterobacterales*/carbapenemase producers directly on the sample or a two-step approach using a culture-based screen for CRE and then if a CRE screen is positive, using molecular methods to confirm CPE isolates. The various laboratory methods to detect CRE/CPE include phenotypic testing methods and/or molecular genetic techniques (BIOMÉRIEUX, 2018).

Two characteristics are important when evaluating test assays for use in the laboratory, namely the sensitivity and specificity.

2.9.1. Phenotypic tests

The modified Hodge test (Fig 10) is simple, cost effective and able to detect certain carbapenemases more effectively than others namely KPC-producing *Enterobacterales.* A limitation however is that it often gives a false positive and false negative result with a sensitivity of around 58 % and specificity of around 93% (Hrabák *et al.*, 2014).

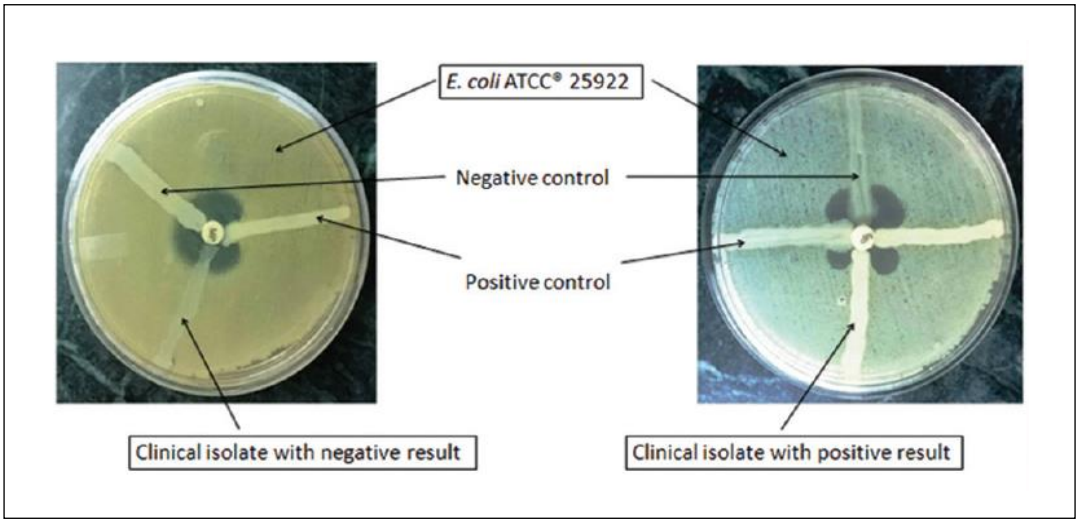


Figure 9. Modified Hodge test

If the test organism produces any carbapenemase enzyme, it will hydrolyze the diffused antibiotic in the vicinity of the streaked lines. This will enhance the growth of *E. coli*, which will otherwise be inhibited by the antibiotic, in the vicinity of the streak. Following the incubation, observe for the enhanced, indented growth of the *E. coli* and formation of clover leaf-like indentation at the intersection of the streak of the test organism (and controls, if used) and the zone of inhibition of the *E. coli*.

(Source:https://th.bing.com/th/id/R.28de02a7432b12559a19f71a25191c95?rik=F3n3iLlaFm1Wqg&riu=http%3a%2f%2fwww.mjdrdypu.org%2farticles%2f2017%2f10%2f4%2fimages%2fMedJDYPatilUniv_2017_10_4_365_213930_f1.jpg&ehk=VKH0BYi3D7NfDtcV4cSJejAvRXTWgmwjvAdIgyjJFxE%3d&risl=&pid=ImgRaw&r=0)

Other phenotypic methods include the detection of b) metallo-ß lactamases due to inhibition of chelating agents such as Ethylene diamine tetra-acetic acid (EDTA) and dipicolinic acid (DPA) performs well for screening for carbapenemase producing *K. pneumoniae* and *E. coli.* However, more data are needed for detection of carbapenemase production for other *Enterobacterales.* The sensitivity and specificity are low and both the above tests can be reported after 24 hours only, which is time consuming (Hrabák *et al.*, 2014 and Teena *et al*, 2018).

Detection of KPCs based on boronic acid inhibition and its derivatives are limited to KPCs only and is thus not ideal in the clinical setting.

2.9.2. Colorimetric assays

Colorimetric assays are based on changes in colour after the carbapenem within the assay is hydrolysed by a carbapenemase enzyme contained by the organism. The test example RAPIDEC® Carba NP (Fig 11) is simple, cheap and can be interpreted within 3 to 5 hours and is considered reliable for Ambler Classes A and B. Limitations however are, that pure growth is needed before inoculation, ability to detect OXA-48 is unclear and as it is a screening test, positive results will have to be confirmed with PCR (Hrabák *et al.*, 2014). This test may be better used in a clinical setting; however, it will not add much value to this project with a sensitivity of 72.5 % and specificity of 100% (Hrabák *et al.*, 2014).

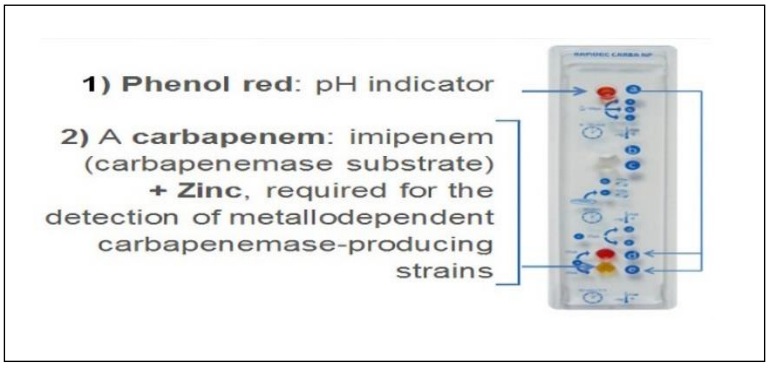


Figure 10. RAPIDEC® Carba NP

The RAPIDEC® CARBA NP test detects Carbapenem hydrolysis by Carbapenemase-producing bacteria: *Enterobacterale, Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Hydrolysis acidifies the medium which results in the change in color of the pH indicator – indicating specifically the presence of transmissible Carbapenem resistance.

(Source:https://th.bing.com/th/id/R.5c6cbb84905f49b59cc9a658836511a7?rik=erRfTpi6aZi2hw&riu=http%3a%2f%2fwww.biomerieux-diagnostics.com%2fsites%2fclinic%2ffiles%2fready-to-use.jpg&ehk=nwJPJjF2tN4XHCSE3YB0q2f%2fpSV1OpWRILxDZjJHefY%3d&risl=&pid=ImgRaw&r=0&sres=1&sresct=1)

2.9.3. Spectrophotometric testing

Spectrophotometric testing for carbapenemase activity has been suggested as a reference method, however it is labour intensive and unavailable in routine diagnostic laboratories. It has a reported 100% sensitivity and 98.5% specificity respectively (Hrabák *et al.*, 2014). It was not used for this study, as it was not available for use.

2.9.4. Screening using cultivation media

A variety of screening options do exist, these include the use of various media as well as various antibiotics i.e., MacConkey agar plates containing imipenem or using the MacConkey plate with imipenem, meropenem and/or ertapenem discs. Commercially prepared selective media specifically for screening purposes have also been used e.g., CHROMagar-KPC (Hy-labs) (Fig 12). The different methods and agar plates have varying performance, but the overall sensitivity and specificity is acceptable as a screening method; a limitation of many agar plates is the inability to detect all OXA-48 producers due the low level of carbapenem resistance in some of these strains (Hrabák *et al.*, 2014). A plate from BIOMÉRIEUX, ChromID® CARBA SMART agar (CARBA/OXA) (Fig 13) has a good sensitivity (95.9%) and specificity (96.6%) and the ability to detect many OXA-48 strains. The manufacturer recommended specimen is either a stool or rectal swab inoculated directly onto the culture medium. A limitation of the CARBA SMART agar, as with many other media is that multi-resistant organisms other than CPE could potentially grow and organisms with low carbapenemase activity may not grow on the media (BIOMÉRIEUX 2017).

Figure 11. CHROMagar-KPC

CHROMagar™ KPC is a selective and differential chromogenic culture medium, intended for use in the qualitative direct detection of gastrointestinal colonization with carbapenem-resistant *Enterobacteria* (CRE). The test is performed with rectal swab and stools from patients to screen for CRE colonization. Results can be interpreted after 18-24 h of aerobic incubation at 35-37 °C where carbapenem-resistant *E. coli* is indicated with a dark pink to red colour change and *Klebsiella, Enterobacter* and *Citrobacter* with a metallic blue colour.

(Source:https://www.chromagar.com/images_multimedia/000177-500x500.jpg?PHPSESSID=ef53906bda972c60f3812ca9768edb90)

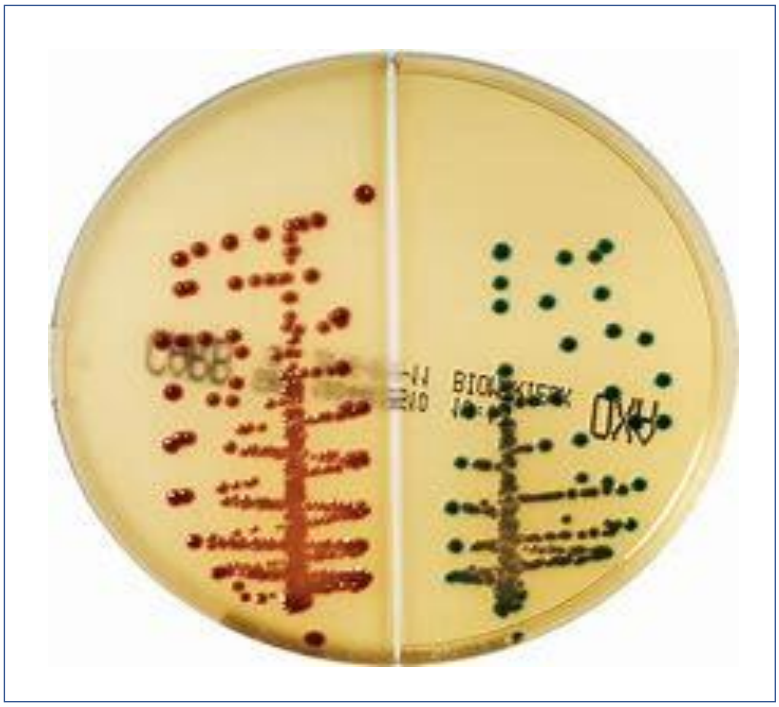


Figure 12. CARBA SMART agar

(Left): *Escherichia coli* pink to burgundy colonies; (right) - *Klebsiella, Enterobacter, Serratia, Citrobacter* (KESC), bluish-green to bluish-grey colonies.

(Source:https://th.bing.com/th/id/OIP.L1Xc7jaZk5ZQyBsbyuki8wHaHa?w=218&h=218&c=7&r=0&o=5&pid=1.7)

Various antibiotics have been used either in commercially prepared CRE screening agar plates or as discs placed on media for CRE screening. Susceptibility patterns of various CPE organisms may differ. Certain CPE organisms can show MICs lower than the Clinical European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints and even a small reduction in minimum inhibitory concentration (MIC) should be further examined for CPE (high index of suspicion in any non-susceptible isolates. Meropenem was suggested as the screening antibiotic due to it having the better sensitivity and specificity compared with other carbapenems, However, for bacteria producing OXA-48 ß-lactamase, temocillin was suggested to be used instead of meropenem (GERMS-SA-AR-2017-final.pdf (nicd.ac.za)).

Literature suggests that the OXA-48 ß-lactamase is prevalent in many areas, including South Africa and that the EUCAST guidelines were unable to identify many OXA-48 ß-lactamase producers. (BIOMÉRIEUX 2017 and GERMS-Annual-Review-2019 .pdf (nicd.ac.za)

It is suggested that using temocillin disks (30 µg) and piperacillin-tazobactam disks (100/10 µg) with revised cut-off values increases the sensitivity of screening methods. The use of susceptibility patterns as a screening method is useful despite the absence of an acceptable screening antibiotic that is sufficiently sensitive and specific. Once the screen is positive for a CRE or a suspicious organism with reduced susceptibility to one or more of the carbapenems molecular methods for gene detection should be performed to confirm a CPE (Hrabák *et al*, 2014).

Both the VITEK® 2 and Xpert® Carba-R (Fig 13) systems are internationally approved and validated methods used in many diagnostic laboratories for identification and susceptibility testing (BIOMÉRIEUX, 2018). The Vitek 2 is used to perform identification and susceptibility on the suspected CRE organisms isolated on the screening media. This will then confirm the identification of the organism as a member of the *Enterobacterales* and the carbapenem MIC's will also be elucidated. Molecular techniques are important to identify the presence of a gene responsible for carbapenemase production and thus reduced susceptibility to carbapenems.

Figure 13. Xpert®Carba-R system cartridge

X-pert®Carba-R system consists of a single-use disposable cartridge that containing the PCR reagents, the entire PCR process occurs within system. The cartridges are self-contained, reducing the risk cross-contamination between samples.

(Source:https://th.bing.com/th/id/OIP.LX7I7kaG6xxOLSUM3R1KSgHaFB?w=249&h=180&c=7&r=0&o=5&pid=1.7)

2.9.5. Molecular techniques

Molecular techniques yield a rapid and a more precise result (due to their improved sensitivity and specificity) compared to the other methods. These tests can however be costly and may require skilled personnel to perform and interpret the test results (Hrabák, *et al.,* 2014). A variety of options are available which include in-house assays or commercially prepared kits with sensitivity and specificity in the order of about 100% and 99% respectively. Testing can also be performed directly on the sample for example a stool or rectal swab sample or from pure bacterial cultures (Cepheid®2018). A limitation of using these methods directly on stool is that the test will only detect the targeted gene (Hrabák *et al.*, 2014) and will not detect *Enterobacterales* that are resistant to carbapenems by another resistance mechanism. Criteria for selecting a molecular test to have a rapid, easy to use and interpret, cost effective and cover as many target genes as possible.

The Xpert® Carba-R system is used by many diagnostic laboratories due to its ease of use. It detects the five most common circulating genes namely: *Klebsiella pneumoniae* carbapenemase (*bla*$_{KPC}$), New Delhi metallo- β-lactamase (NDM) *bla*$_{NDM}$, Verona integrin-encoded metallo- β-lactamase *bla*$_{VIM}$, Oxacillinase-48-type *bla*$_{OXA-48,}$ Imipenemase - *bla*$_{IMP}$ (Cepheid®2018). A limitation is that other less common genes like OXA-23, GES or PER are not catered for in the panel and thus it is important to be aware of local epidemiology and the limitations of this test. The Xpert® Carba-R system performance characteristics has a sensitivity (from pure bacterial cultures) of 100% and specificity (from pure bacterial cultures) of 98.1%. When tested directly from stool sample (rectal swabs), the sensitivity is 94.7% and specificity of 97.8% (Cepheid®2018). For the purposes of this screening study, the Xpert® Carba-R system will be used from pure cultures only due to the improved sensitivity and specificity of the assay as compared to the direct sample.

Eazyplex® SuperBug is a molecular diagnostic test used with the Genie II platform to detect carbapenemase producing organisms (CPOs) as well as certain ESBL genes directly from rectal swab samples. This test has the ability to detect and identify the major types of carbapenemases via the bacterial DNA within 30 minutes. This testing platform can detect the following genes: KPC, NDM, OXA-48 & OXA-181, VIM, CTX-M-1 7 CTX-M-9 group with an overall resulting sensitivity of 100% and specificity of 99.4%(Eazyplex® SuperBug).

A comparative study between real-time PCR Xpert Carba-R and Eazyplex® SuperBug to detect carbapenemase and/or ESBL genes in *Enterobacterales* was conducted directly from bronchoalveolar lavage fluid samples. BAL samples were spiked with CPO-*Enterobacterales* at varying concentrations*.* Both testing assays had the same preparation times of 15 minutes however for the Xpert Carba R results for the 5 genes detected was available in 50 minutes and showed 100% correlation between the PCR and sequencing method opposed to the 30 minutes for the Eazyplex® SuperBug CRE for the 7 genes detected. However, decreased

correlation was noted with the sequencing at the lower concentrations of spiked organism (Vergara *et al,* 2020).

When comparing the performance of the BIOMÉRIEUX, ChromID® CARBA SMART agar to the performance of the Xpert® Carba-R system, directly from samples we can see that the culture medium is just over 1% more sensitive however, the Xpert direct method is more specific than ChromID culture medium by a little over 1% (Hrabák *et al*, 2014).

Figure 14. Cepheid GeneXpert instrument and semi-loaded cartridge

(Source:https://www.bioworld.com/ext/resources/BMT-source/2020/3-23-Cepheid-GeneXpert-SARS-CoV-2.png?height=635&t=1585000100&width=1200)

CHAPTER 3

RESEARCH DESIGN AND METHODOLOGY

3.1. Research Design

3.1.1. Study design: prospective cohort study

3.1.2. Study participants: The aim was to collect swabs from 25 (50 swabs) adult participants (male/female participants who were enrolled and sampled from 01 February 2020 to July 2021). Participants were already part of various existing research projects meeting the inclusion criteria at the TASK clinical site.

Participants were enrolled if they met the following inclusion criteria:

- Have not yet been started on first-line TB treatment or any components of the first-line TB treatment for another indication.
- Consent has been given (Potential participants will be consented at a TASK clinical site)
- Have been diagnosed with rifampicin susceptible *M. tuberculosis* pulmonary infection. (Diagnosis made at state primary health-care clinics other than TASK clinical sites using the GeneXpert MTB/RIF Ultra assay)
- Were older than 18 years of age
- Completed the study questionnaire

3.1.3. Ethics approval and informed consent

Ethics permission was obtained from the CPUT Health and Wellness Sciences Research Ethics Committee (HWS-REC) [Registration Number NHREC: REC- 230408-01]; approval reference number: CPUT/HW-REC 2019/H31.
Participants were consented by the staff at the TASK clinical site who were ICH-GCP trained and compliant with the requirements of GCP. Once consented, the rectal swabs were self-collected at the site. The Investigator, myself (Fay Swanson) and other researchers had no participant contact during the course of this project. The TASK clinical site staff obtained informed consent from patients who were willing to participate in this study. The patients were only allowed to sign consent and partake if they met both the inclusion and exclusion criteria.

Informed consent was obtained for each participant in accordance with South African Good Clinical Practice Guidelines. Participant confidentiality was maintained by including the age, gender and initials on the request forms only.
The investigator, myself, only had contact with samples, the laboratory request form and consent forms once completed.

For this study, due to ease of collection as well as for comparable sensitivity, rectal swabs were collected from the required patients after signed consent was obtained.

Ethics approval had to be renewed as only 15 participants were sampled by 06 December 2020 which fell short of the 25 patient target.
The ethics renewal was granted until 10 November 2021. At this point, three additional patients were sampled bring the total participant number to 18 sampled.

3.1.4. Unique PTID format

Below is an example of the unique participant identification used on swabs and request forms:

Example 1		**Example 2**
First participant with two initials only		Final participant with three initials
FS – 01 - Sample 1 FS – 01 - Sample 2	OR	FLS – 25 – Sample 1 FLS – 25 – Sample 2

3.2. Methodology

For this study, a stepwise diagnostic approach was performed making use of culture media to screen for CRE. If the screening test was confirmed as positive, the organism identification and susceptibility was performed on the VITEK® 2 automated system to confirm the presence of a CRE. Molecular gene testing then followed to confirm whether the organism was a CPE. Both the pre-treatment base-line sample and the sample at 14 days were screened and identified using the same laboratory methods. Any changes in organism susceptibility between the two time points noted were noted and analysed. This sample analysis was conducted in the microbiology laboratory and the most effective and economical screening procedure required elucidation.

3.3. Sample collection

The sample of choice for the screening methods was a rectal swab. This was chosen for logistical reasons as the patient sample can be collected at any time instead of waiting until the participant can produce a stool sample (Hrabák *et al*, 2014; Lautenbach *et al*, 2005; Jazmati *et al*, 2018). All sampled rectal swabs were visibly soiled with stool. All relevant isolates were stored long term for further investigation if needed.

Standard gel swabs containing Stuart Transport medium (Fig. 15.B) were used. The swabs were stored at room temperature before use and were used within the expiry date.

Figure 15.A. Amies transport swab medium

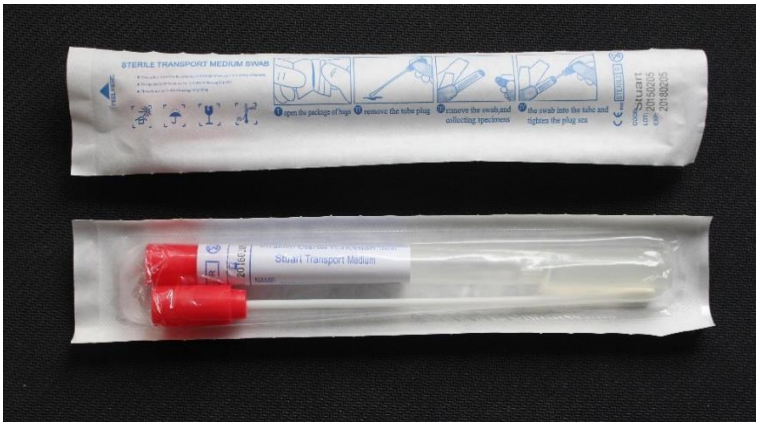


Figure 15.B. Stuart transport medium

Figure 15. Transport media swabs

Patient swabs were collected from the TASK clinical site in Bellville, Cape Town from 01 February 2020 to 30 July 2021. All swabs were provided by and analysed at Lancet Laboratories in Century City, Cape Town, according to the flow diagram below (Fig 16).

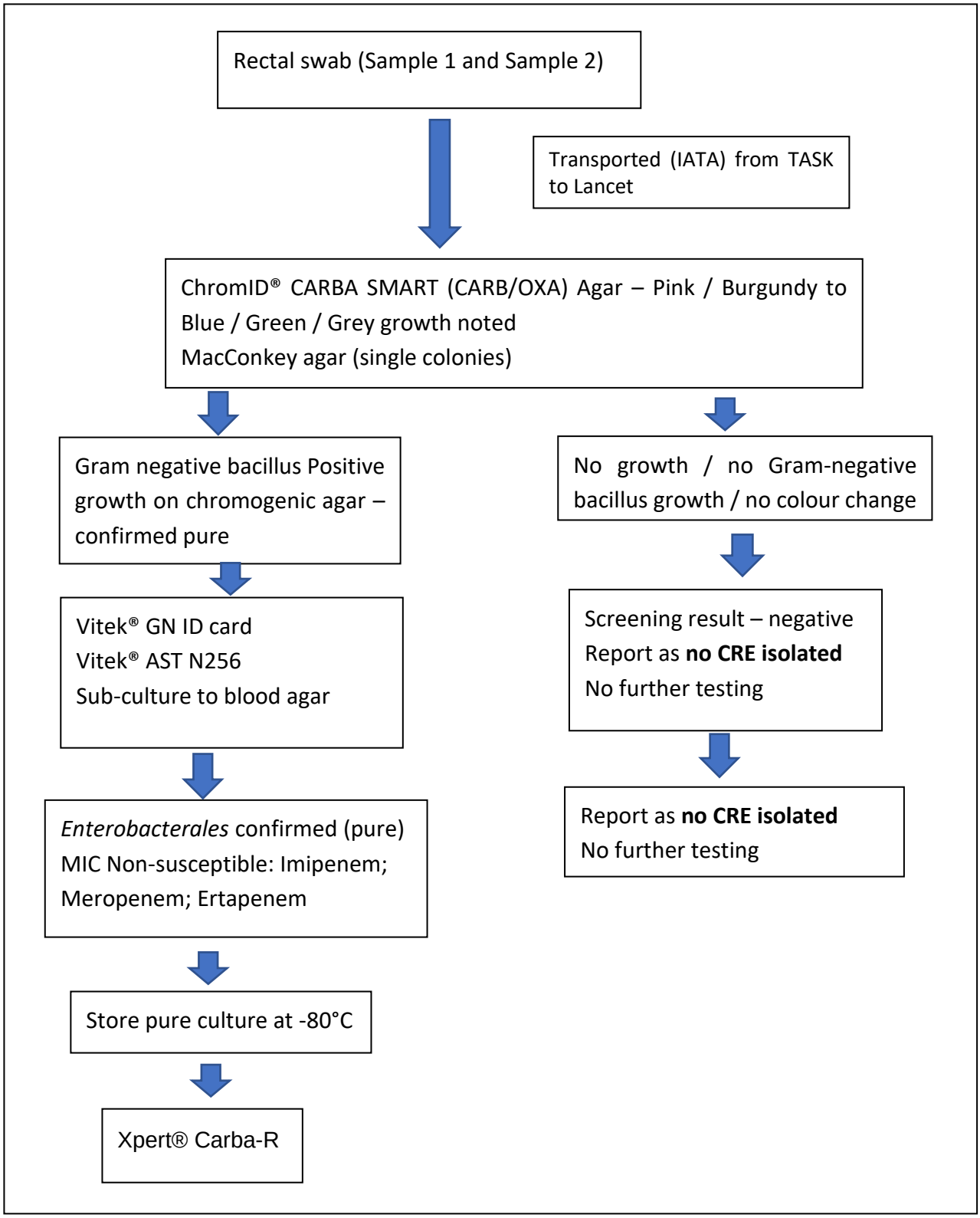


Figure 16. Lancet Process Flow diagram: screening, identification and sensitivities

Sampling commenced before first-line tuberculous treatment was initiated (day 1) and again after 14 days of standard first-line TB treatment (day 14). There was also a questionnaire asked of the participant by TASK site staff after the informed consenting process at the sampling stage.

The swabs and the questionnaire were labelled with participant initials, participant number, collection date and sample number. The participant unique number had either the two or three-letter prefix PID- and two digits e.g., 01.
Once collected, the swabs were kept in the refrigerator at 2-8 ° C at the TASK site for maximum of 72 hours until ready to be transported in accordance with the IATA guidelines in a specimen transport box labelled as biohazardous.

The TASK site SOP for specimen transport was followed for this process. The questionnaire was incorporated into the request form (Appendix C); it contained the specimen collection details (date and time) and participant initials as above which ensured that the traceability of the samples from the TASK site to Lancet Laboratory, Microbiology Lab, Century City, Cape Town, South Africa SANAS accredited laboratory in ISO15189 where microbiological testing occurred.

On receipt at the laboratory, swabs were assessed and documented to ensure that the sample was visibly soiled. Both swabs, baseline and day 14 samples, were screened for carbapenemase resistance using a commercial chromogenic agar plate. Innoculate the specimen directly onto the ChromID® CARBA SMART (CARBA/OXA) plates which has reached room temperature. Biomériuex recommends inoculating the CARBA portion first, then the OXA portion. Incubate aerobically at 35ºC +/- 2ºC by inverting the plates and evaluate after 18-24 hours. If CRE was noted, further testing was performed to determine if the carbapenem resistance was due to the presence of one of the most common carbapenemase genes.

Quality control and maintenance of laboratory assays, media, reagents, and equipment were in accordance with the relevant supplier and manufacturer instructions also complying with ISO15189, Lancet, Century City Facility Accreditation Number M0627. This accreditation number means that the laboratory has demonstrated technical competency for the scope of tests as well as an operational quality management system (Appendix E). The current accreditation certificate has an effective date of 02 August 2018 and the certificate expired on: 01 August 2022 which covered the period of sampling and participant swab screening for the purposes of this project.

The specimen request form was stored with the Lancet generated final report according to ICH-GCP and GCLP guidelines.

3.4. Screening of Samples

Screening processes followed the procedure summarised below (BIOMÉRIEUX 2017, BIOMÉRIEUX 2018, Cepheid®2018):

1. Each swab was inoculated onto two agar plates. a) BIOMÉRIEUX ChromID® CARBA SMART (CARB/OXA) Agar plate, reference number 414685, b) MacConkey medium plated for single colonies to assess for purity of culture.
2. Agar plates were incubated aerobically at 37°C for 24-48 hours.
3. For this study, and based on the current media options, BIOMÉRIEUX ChromID® CARBA SMART, reference number 414685, was the better option based on performance, however, it could not be used on its own.
4. The BIOMÉRIEUX ChromID® CARBA SMART (CARBA/OXA), reference number 414685, agar was assessed for the presence of relevant colonies as per package insert. Pink / Burgundy and Blue / Green / Grey colonies were sub-cultured onto MacConkey agar. Colonies from this medium were used for the identification and susceptibility testing using the BIOMÉRIEUX Vitek®2 system. A 0.5 to 0.63 McFarland Standard was utilised.
5. Using sterile cotton swabs, the isolates to be tested were selected.
6. A suspension was prepared by transferring several pure isolated colonies from the agar plates to 4 mL of sterile saline. Using a calibrated V2C DensiChek Meter, the suspension was adjusted to the 0.5-0.63 McFarland standard range (Fig 17).
7. The prepared suspensions were then placed in the cassette (Fig 18). [The suspension could not be older than 30 minutes before inoculating the cards].
8. For sample traceability, cards were scanned and barcodes isolated.

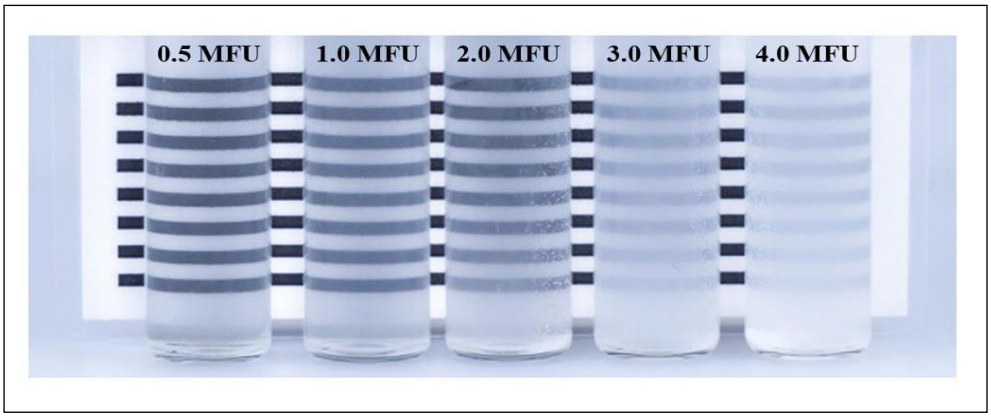


Figure 17: Mcfarland standards showing the concentration of the organism suspension

https://microbenotes.com/wp-content/uploads/2020/05/McFarland-Standards-Principle-Preparation-Uses-Limitations.jpg

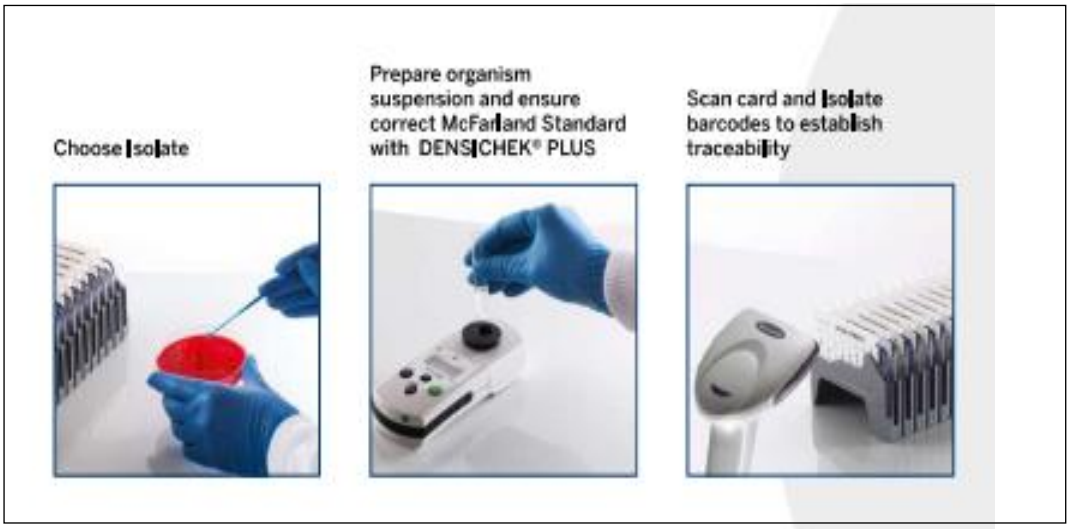


Figure 18: Loading the VITEK® 2 system with a bacterial suspension

1. The BIOMÉRIEUX VITEK® 2 Gram-Negative identification card (GN), reference number 21341, was used for identification and Vitek® 2 AST N256 card, reference number 421982, for susceptibility testing.

2. Vitek® AST N256 card Susceptibility test card MIC results were interpreted for sensitivity, intermediate or resistance by using the latest CLSI 2019 M100, 29th ed. Document programmed in the Vitek® AST software. (Figure 17).

For this study, participants were screened using the ChromID culture media, the organisms identified as *Enterobacteriaceae* using the VITEK® 2 method. Lolecular testing using the Xpert® Carba-R system from pure cultures would then follow. Both the Xpert® Carba-R system and Eazyplex® SuperBug are able to detect both of the most common genes in South Africa, gene OXA-48 most dominant followed by the NDM gene. Both assays are comparable although the Eazyplex® SuperBug was slightly less sensitive than the Xpert® Carba-R for the purposes of this project. The Xpert® Carba-R was also the validated assay in use at the time of sample analysis at the laboratory of choice, Lancet Laboratory, Century City, Cape Town.

MIC values for ertapenem, imipenem and meropenem were documented. As latest CLSI criteria were used for MIC breakpoints (CLSI 2019 M100, 29th ed.), MIC values that fell in the non-susceptible range (intermediate/resistant) were tested for carbapenemase production using Cepheid Xpert® CARBA-R, reference number GXCARBAR-10.

As imipenem MIC for *Proteus spp*, *Providencia spp* and *Morganella morganii* tend to be higher than meropenem MIC's due to intrinsic mechanisms of resistance other than production of carbapenemases, these isolates were documented but not part of the analysis.

Cepheid Xpert® Carba-R, product code: GXCARBAR-10 is an assay performed on the GeneXpert instrument for the detection and differentiation of the gene sequences below by utilising real-time polymerase chain reaction (PCR):

Klebsiella pneumoniae carbapenemase *bla*$_{\mathbf{KPC}}$,
New Delhi metallo- β-lactamase (NDM) *bla*$_{\mathbf{NDM}}$,
Verona integrin-encoded metallo- β-lactamase *bla*$_{\mathbf{VIM}}$,
Oxacillinase-48-type *bla*$_{\mathbf{OXA\text{-}48}}$,
Imipenemase - *bla*$_{\mathbf{IMP}}$

Table 1. Interpretation summary of Xpert® Carba-R results

Instrument results	Interpretation	Reported result
IMP Detected VIM, NDM, KPC, OXA-48 Not detected	IMP DNA sequence Detected VIM, NDM, KPC, OXA-48 Not Detected All probe check results pass	Imipenemase - *bla*$_{\text{IMP}}$ Detected
VIM Detected IMP, NDM, KPC, OXA-48 Not Detected	VIM DNA sequence Detected IMP, NDM, KPC, OXA-48 Not Detected All probe check results pass	Verona integrin-encoded metallo- β-lactamase *bla*$_{\text{VIM}}$ Detected
NDM Detected IMP, VIM, KPC, OXA-48 Not Detected	NDM DNA sequence Detected IMP, NDM, KPC, OXA-48 Not Detected All probe check results pass	New Delhi metallo- β-lactamase *bla*$_{\text{NDM}}$, Detected
KPC Detected IMP, NDM, VIM, OXA-48 Not Detected	KPC DNA sequence Detected IMP, NDM, VIM, OXA-48 Not Detected All probe check results pass	*Klebsiella pneumoniae* carbapenemase *bla*$_{\text{KPC}}$ Detected
OXA-48 Detected IMP, NDM, VIM, KPC Not Detected	OXA-48 DNA sequence Detected IMP, NDM, VIM, KPC Not Detected All probe check results pass	Oxacillinase-48-type *bla*$_{\text{OXA-48}}$, Detected
IMP, NDM Detected VIM, KPC, OXA-48 Not Detected	IMP, NDM DNA sequences Detected VIM, KPC, OXA-48 Not Detected All probe check results pass	Imipenemase - *bla*$_{\text{IMP}}$ Detected New Delhi metallo- β-lactamase *bla*$_{\text{NDM}}$, Detected
INVALID	Presence or absence of IMP, VIM, NDM, KPC, OXA-48 DNA Sequences cannot be determined. Repeat test. All probe check results pass	Report repeated result

ERROR	Presence or absence of IMP, VIM, NDM, KPC, OXA-48 DNA Sequences cannot be determined. Repeat test. One or more probe check results FAILED.	Report repeated result
No Result	Presence or absence of IMP, VIM, NDM, KPC, OXA-48 DNA Sequences cannot be determined. Repeat test. Probe check results Not Applicable ? Power failure or test stopped	Report repeated result

For the purposes of this study, only pure *Enterobacterales* isolates were to be tested according to the Cepheid Xpert®Carba-R package insert and added below:

For this study, bacterial isolates were to be used only, however rectal or perirectal swabs can be used for direct analysis as well. Prepare a 0.5 McFarland pure suspension of the bacterial isolate using saline or broth and vortex the 0.5 McFarland suspension. To prepare, remove a Xpert Carba-R Assay cartridge, a sample reagent vial and a transfer pipette from the kit. Open the vial of the sample reagent.

Using a loop, transfer 10 µL of the 0.5 McFarland suspension to a 5 mL vial of sample reagent. Rotate the loop at least three times in the sample reagent. As a precaution, ensure that the sample transferred in the loop does not burst before adding to the sample reagent The leftover sample in the sample reagent vial can be retained at 2–28 °C for up to five days if a retest is required.

Cap the sample reagent vial tightly and vortex at high speed for 10 seconds.

Open the CARBA-R cartridge lid and the sample reagent cap. Using the transfer pipette provided, aspirate the prepared sample up to the mark on the pipette (approximately 1.7 mL) and transfer the material into the sample chamber large opening of the Xpert Carba-R Assay cartridge.

Close the cartridge lid and place the cartridge into the GeneXpert instrument within 30 minutes of adding the sample to the cartridge. Program the instrument with the sample details, open the instrument module door and load the cartridges
Close the door to start the test, a green light is visible on the door. When the test is finished, the light turns off and releases the door system lock.
The used cartridges should be disposed in the appropriate specimen waste container.

The system interprets the results from measured fluorescent signals and embedded calculation algorithms (Cepheid®2018).

CHAPTER 4

RESULTS

4.1. Study Participants

Potential participants were consented by the TASK study nurse who was GCP trained using the ICF form (Appendix A) before any project related investigations were performed. In total, 18 patients were sampled out of the 25 as we originally planned. This was due to the COVID-19 pandemic and the various governmental restrictions imposed, resulting in an immediate decrease in participants seen at the site. Potential participants were reluctant to undergo additional study investigations at a very uncertain time.

During this study it was important to assess the risk of CRE colonisation of the participants, other than TB treatment as the participants could have been exposed to other risk factors for CRE colonisation during the 14-day clinic stay at the TASK site. A questionnaire was completed by each participant to assess their exposure to other risk factors (Appendix C).

Prior to the South African COVID-19 lockdown, nine patients were sampled rapidly in a six-week period from February 2020 to March 2020 without any monetary compensation. On 26 March 2020, South Africa was placed under Lockdown level 5 and as a result, in the next four months there were no participants to sample due to various reasons related to the restrictions. Many patients did not attend their routine clinic visits or chose not to seek medical assistance due to the lockdown restrictions imposed at the time. In the later weeks, when there were eligible patients, three potential patients declined consent as there was no monetary compensation and they did not wish to be included in the project as a result.

As there were no sample testing screening positive, the CLSI guidelines were not used for this study. The potential breakpoints were as follows; according to CLSI guidelines, the MIC breakpoints of imipenem and meropenem for susceptible, intermediate, and resistant are ≤1, 2, ≥4 µg/mL respectively. Whilst, according to EUCAST, the MIC breakpoints of colistin for susceptible and resistant are ≤2 and >2 µg/mL respectively. The antibiotic susceptibility of ertapenem was obtained from Medical Microbiology Diagnostic Laboratory (MMDL) of UMMC where the disc diffusion test was carried out.

4.2. Patient Questionnaire

In total, 36 swabs were collected from 18 patients. At collection it was noted that the swabs were visibly soiled with faecal matter and were therefore good quality specimens for laboratory investigations.

There were no positive results screened for either the baseline or the 14-day swabs, therefore no CRE was isolated from the 18 sampled patients for this project.

From the questionnaires obtained from the 18 patients, two were exposed to risk factors prior to receiving first line TB treatment and being sampled. The questionnaire was included in the lab request form and addressed the same set of questions at baseline sampling and again at 14-days (appendix C). From the completed patient questionnaires, PTID 02 was hospitalised for three days in November 2019 within four months of being sampled for this project and was on antibiotic treatment for a chest infection six days before the official TB diagnosis and sampling for this project. PTID 06 was treated with antibiotics for pyrexia within one year of being sampled for this project.

The outcome for the patient questionnaires is summarised in Table 2.

Table 2. Patient Questionnaire Responses

Patient Number	Hospitalised in last year?	Have you been hospitalised in a high care / ICU ward?	Any contact with ICU/LTCF discharged person?	Any operations/ procedures/ catheters inserted in last 2 years?	Any antibiotics in last year, e.g., TB Rx?	Any chronic illness: Diabetes, Asthma, etc.?
1	No	No	No	No	No	No
2	Yes*	No	No	Yes*	Yes*	No
3	No	No	No	No	No	No
4	No	No	No	No	No	No
5	No	No	No	No	No	No
6	No	No	No	No	Yes*	No
7	No	No	No	No	No	No
8	No	No	No	No	No	No
9	No	No	No	No	No	No
10	No	No	No	No	No	No
11	No	No	No	No	No	No
12	No	No	No	No	No	No
13	No	No	No	No	No	No
14	No	No	No	No	No	No
15	No	No	No	No	No	No
16	No	No	No	No	No	No
17	No	No	No	No	No	No
18	No	No	No	No	No	No

i. **PTID 02** was hospitalised for three days in November 2019 within four months of being sampled for this project and was on antibiotic treatment for a chest infection six days before the official TB diagnosis and sampling for this project

   PTID 06 received antibiotic treatment for pyrexia within one year of sampling and had an ESBL producing *E. coli* isolated from the baseline swab which was not isolated at 14 days.

Patient 2 confirmed during the participant questionnaire process to visiting the local clinic for a chest infection and leaving with antibiotics in tablet form. It is unclear what antibiotic was prescribed, if the course was completed and if the duration of the course. This was

approximately one week prior to consenting to participate in this study. This is significant as this recent antibiotic treatment could have led to dysbiosis regardless of the antibiotic prescribed or duration of treatment (Namasivayam et al., 2017). The same patient was hospitalised for three days at Tygerburg hospital in November 2019 although it is unclear if there was any exposure to the high care or ICU settings however, the patient did experience either an operation or medical procedure during this time, which is an added risk factor. Patient 2 was therefore exposed to at least two risk factors before partaking in this study.

Patient 6 confirmed antibiotic usage within the last year to treat pyrexia and although the actual antibiotic prescribed, duration of use or whether the course has been completed is unclear. Patient 6 has been exposed to at least one risk factor which likely led to dysbiosis (Namasivayam et al., 2017). The remaining 16 patients did not indicate a positive response to risk factor exposure.

4.3. Laboratory Data

Participants were consented using the ICF (Appendix A) and after recruiting and sampling, participants slowed down during the COVID-19 pandemic. Out of the 18 participants swabbed and processed for this study, no CRE was detected during screening at either baseline or after two weeks or 14 days.

The laboratory results are summarized in Table 3.

Table 3. Carbapenem-resistant *Enterobacterales* screening results

Patient #	Swab	CRE screening result	Swab	CRE screening result
1	B	CRE-	D14	CRE-
2	B	CRE-	D14	CRE-
3	B	CRE-	D14	CRE-
4	B	CRE-	D14	CRE-
5	B	CRE-	D14	CRE-
6	B	CRE-	D14	CRE-
7	B	CRE-	D14	CRE-
8	B	CRE-	D14	CRE-
9	B	CRE-	D14	CRE-
10	B	CRE-	D14	CRE-
11	B	CRE-	D14	CRE-
12	B	CRE-	D14	CRE-
13	B	CRE-	D14	CRE-
14	B	CRE-	D14	CRE-
15	B	CRE-	D14	CRE-
16	B	CRE-	D14	CRE-
17	B	CRE-	D14	CRE-
18	B	CRE-	D14	CRE-

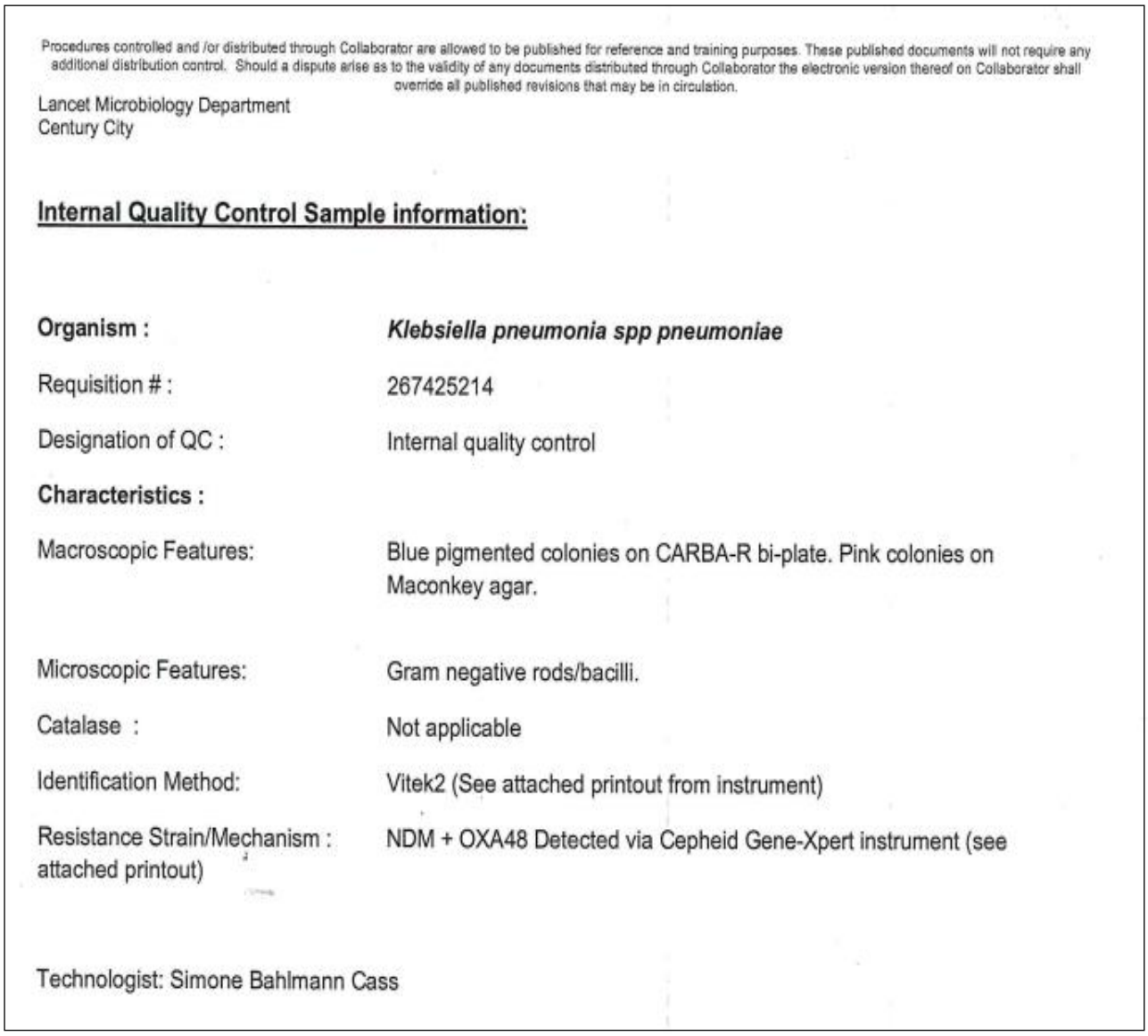
B	Baseline
CRE-	CRE NOT isolated
D14	After 14 days

All collected swabs for the 18 patients sampled were negative with a final result of CRE Not isolated, see Appendix F for laboratory report issued by Lancet laboratory.

The supplier provides a certificate of analysis for the ChromID® CARBA SMART media available on the Biomérieux website and downloaded for record purposes with each shipment arrival to verify the quality of the media received.

Lancet laboratory performs internal quality checks on all reagents and media upon receipt for new lot numbers and new shipment batches. A known, previously identified positive and negative control is used as a quality check for the ChromID® CARBA SMART screening media. A previously identified CRE-resistant *Klebsiella pneumoniae spp pneumoniae* strain, NDM and OXA-48 positive using the VITEK® 2 ID and sensitivity methods and Xpert Carba-R Assay cartridge was used as the positive control for the screening plates used during this study as well as a previously identified CRE-sensitive negative control (see figure 19 and figure 20).

If the quality control checks fail at any of the above mentioned steps, the test will be repeated and if still unsuccessful, the media and reagents will not be used for any patient samples. Reagents, media and testing kits will only be placed into circulation for routine testing if all of the above quality checks passed.



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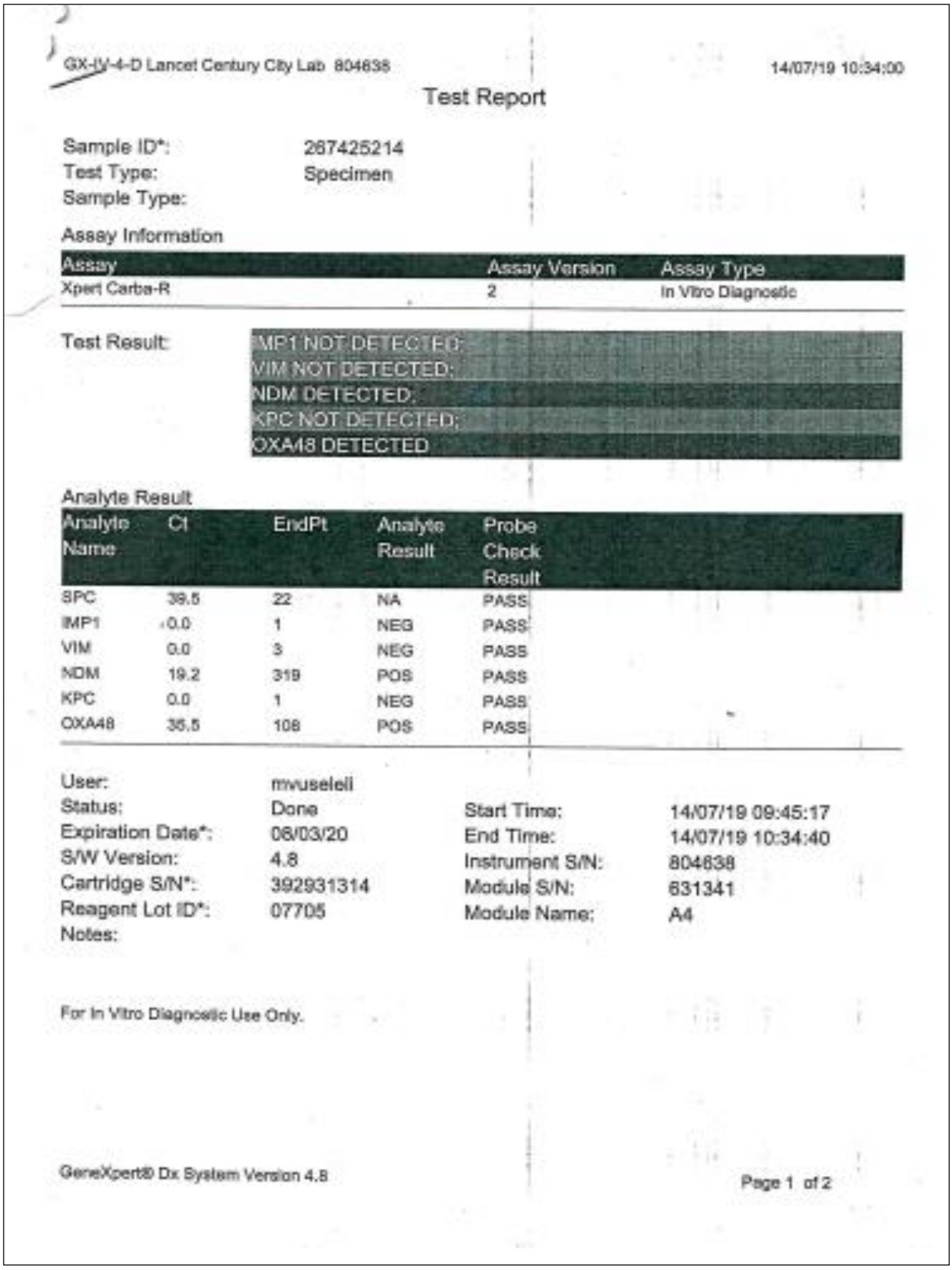
Lancet Microbiology Department
Century City

Internal Quality Control Sample information:

Organism :	***Klebsiella pneumonia spp pneumoniae***
Requisition # :	267425214
Designation of QC :	Internal quality control
Characteristics :	
Macroscopic Features:	Blue pigmented colonies on CARBA-R bi-plate. Pink colonies on Maconkey agar.
Microscopic Features:	Gram negative rods/bacilli.
Catalase :	Not applicable
Identification Method:	Vitek2 (See attached printout from instrument)
Resistance Strain/Mechanism : attached printout)	NDM + OXA48 Detected via Cepheid Gene-Xpert instrument (see

Technologist: Simone Bahlmann Cass

Figure 19: Internal quality control information used for the verify the functionality of the ChromID® CARBA SMART screening media used for this study



GX-IV-4-D Lancet Century City Lab 804638 14/07/19 10:34:00

Test Report

Sample ID*: 287425214
Test Type: Specimen
Sample Type:

Assay Information

Assay	Assay Version	Assay Type
Xpert Carba-R	2	In Vitro Diagnostic

Test Result:

IMP1 NOT DETECTED;
VIM NOT DETECTED;
NDM DETECTED;
KPC NOT DETECTED;
OXA48 DETECTED

Analyte Result

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
SPC	39.5	22	NA	PASS
IMP1	0.0	1	NEG	PASS
VIM	0.0	3	NEG	PASS
NDM	19.2	319	POS	PASS
KPC	0.0	1	NEG	PASS
OXA48	35.5	108	POS	PASS

User: mvuselelI
Status: Done
Expiration Date*: 06/03/20
S/W Version: 4.8
Cartridge S/N*: 392931314
Reagent Lot ID*: 07705
Notes:

Start Time: 14/07/19 09:45:17
End Time: 14/07/19 10:34:40
Instrument S/N: 804638
Module S/N: 631341
Module Name: A4

For In Vitro Diagnostic Use Only.

GeneXpert® Dx System Version 4.8 Page 1 of 2

Figure 20: The printout obtained from the GeneXpert system, the Xpert Carba-R cartridges were used to confirm the CRE-resistant strain for the *Klebsiella pneumoniae spp pneumoniae* strain

4.4. Data Analysis

At the proposal stage of this project, the data were to be expressed as mean, standard deviation and 95% confidence interval. The paired *t* test was to be used to assess for a difference in the carbapenem susceptibility and resistance patterns between baseline and after 14 days of HRZE treatment. The relative risk was to be assessed to determine if 14 days of exposure to HRZE increases the probability for development of carbapenem resistance.
For all tests, a *P*-value <0.05 was to be considered significant.

The COVID-19 pandemic caused significant delays in the data compilation for this project, and as a result, the proposed total sample number goal of 25 participants was not reached; only 18 participants were sampled.

From the data obtained, no patients had positive CRE results. The 95% confidence interval for the proportion observed, in this case 0%, is 0 to 19%. This is due to the low number of patients. It could be that the occurrence of CRE conversions is very rare and one would therefore have to take a large number of observations to get any reliable results.

CHAPTER 5

DISCUSSION

5.1. Discussion of Results

A detailed search of the literature, indicated no previous study that explored first line TB treatment as a risk factor for CRE / CPE. From the 18 patients sampled in this study, there were no carbapenem-resistant *Enterobacterales* isolated; all patients' screening tests were negative and there was therefore no need for molecular investigations since no significant organisms were isolated.

Although the sampled patients were a small group, the results could indicate that the sampled patients did not develop CPE/CRE during the 14-day period as the swabs appeared soiled with faecal matter and the screening media used, ChromID® CARBA SMART was verified using the quality checks in figures 19 and 20.

A study conducted in mice (Le Guern *et al*, 2019), indicated that the timing of exposure to CPE as well as antibiotic usage may be a key component when considering colonisation. In this study, mice were exposed to CPE and a single dose of clindamycin, an antimicrobial which *Enterbacterales spp*. are resistant to. A single clindamycin injection within a week before or after CPE exposure induced gut colonisation for at least 100 days.

Since the first line TB treatment regimen is a six-month course, our study may indicate that patient screening should extend to at least the end of the treatment and antibiotic exposure. The CRE questionnaires completed before each swab collection did however indicate that patient 2 was exposed to a hospital environment within one year of being sampled with antibiotic treatment and patient 6 had received antibiotic treatment other than the TB treatment within the last year. According to the questionnaires, the remaining patients were not exposed to any additional risk factors however. All patients were exposed to the TASK clinical site during the 14-day period which is an added risk factor.

Participants were easily accessible at the TASK clinical site during the 14-day period as they were partaking in a separate study in addition to this study which greatly facilitated the collection of the baseline and 14-day samples.
Based on the results obtained and discussed in chapter 4, a second project with additional time points to cover pre, during and post treatment should be considered.

This project however, had been granted limited funding which had to be considered when selecting time points

A study by (Namasivayam et al., 2017) showed that combination TB treatment does have a significant effect on the microbiome, and profound dysbiosis was noted 1 – 2 years after the combination therapy.

The selected methods for the project are sound and aligned with the routine detection methods. A study by Knight *et al.*, 2018 demonstrated that the use of culture and PCR provides the optimal balance of cost and risk of days averted. As limited funds were available for our study, it was important to balance cost and sensitivity of results. As there were no positive screening results for the 18 patients sampled, only the screening media were used to analyse the swabs. According to the Lancet process flow in figure 16, after a negative screening result, the final report is negative, see final patient result report in appendix F.

By using the patient questionnaire obtained at both time points, we were able to exclude other risk factors.

From the first line combination therapy, rifampicin is a broad-spectrum antibiotic which inhibits bacterial RNA polymerase, is a broad-spectrum antimicrobial that is used for non-mycobacterial infections and has a broad spectrum of activity including possible effect on other organisms in the gut microbiota including some Gram-positive and Gram-negative bacteria in combination or alone. CRE may be generally uncommon in the population on TB treatment however, there are too few patients sampled on this study.

The first line treatment prodrugs are Pyrazinamide (PZA) and Isoniazid (INH) which are activated only within the *Mycobacterium*. Pyrazinamine is activated to pyrazinoic acid in the bacilli where it interferes with fatty acid synthase and interferes with the organism's ability to synthesize new fatty acids, required for growth and replication.

INH is activated by bacterial catalase, and once activated, INH inhibits the synthesis of mycolic acids, an essential component of the bacterial cell wall. These prodrug properties may have reduced the anticipated effect of the combination therapy on the microbiome and subsequent dysbiosis as the drug is only active once inside the mycobacterium. These mechanisms could influence the dysbiosis properties of these microbial agents.
Literature supports significant dysbiosis (Namasivayam et al., 2017). However, this project suggests that within the small group of 18 patients sampled, 14 days of treatment may not

have been sufficient time to detect the resultant CRE and CPE. The population sampled were community-based before admission to the TASK clinic and diagnosed with pulmonary TB but were otherwise well.

TB remains a problem worldwide according to the WHO, 2020. The global reported TB cases in the WHO regions are as follows: South-East Asia (44%), Africa (25%) and the Western Pacific (18%), with smaller shares in the Eastern Mediterranean (8.2%), the Americas (2.9%) and Europe (2.5%).
Eight countries account for two thirds of the global total: India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%) and South Africa (3.6%). According to the GERMS-Annual-Review, 2019, the number of CRE bacteraemia cases are steadily increasing annually from 2015 to 2019. The literature shows that both TB and CRE remains a concern. To date, it is not clear from the literature whether there is an association and if TB medication is a separate risk factor for the colonisation by CPE / CRE. It is therefore difficult to determine the risk of CRE carriage, CRE carriage in the general population in TB endemic areas in the world, and the duration of time on treatment required to be colonised with CRE.

The 95% confidence interval for the proportion observed, in this case 0%, is 0 to 19%. This is due to the low number of patients. It could be that the occurrence of CRE conversions is very rare, and one would therefore have to take a larger number of patient investigations to get more representative results.

5.2. Limitations of the Study

A significant limitation with this study is that 18 patients is a small sample number of patients and since there has been no CRE noted at screening after 14-days treatment, it cannot be ruled out that it can occur. The sampled patient group is not large enough to determine this. Furthermore, the 14-day period may also not be sufficient to detect changes to the *Enterbacterales spp*. in the gut microbiome using the screening methods, and it could take longer for patients to be colonised with CRE while on TB treatment. For this study, a 14-day period was selected as the patients were already admitted to the TASK clinic for this period thus facilitating the sample collection process for both time points.

The detection of CRE using culture methods may take a longer period of exposure to first-line treatment to be detected. Of the 18 patients sampled, CRE was not isolated in any patient. For future studies, a larger proportion of the population will have to be sampled and over a treatment period exceeding the 14-days. Potentially the six-month period for the first line treatment regimen as the increased time period covers the duration of the treatment regime and the dysbiosis likely increases during this time (Namasivayam et al., 2017).

5.3. Sampling complications due to the COVID-19 pandemic

Sampling commenced as from 07 February 2020 and within a six-week period, nine participants were fully sampled, participant no. 09 producing the second swab by 17 March 2020. Thereafter, there was a four-month period where no participants were sampled due to the strict lockdown restrictions limiting movement and encouraging social distancing during the COVID19 pandemic. During the four month-period where no patients were sampled, the study nurses received feedback that the patients were not willing to consent to be sampled if they were not offered any money and some were just fearful of too many medical interventions at an uncertain time.

In the month of May 2020, three potential participants declined to participate as they were not offered any money while participating in the project. Lockdown restrictions directly affected many people in the community, some with reduced employment or losing their jobs therefore potential participants were in need of money.

Many people experienced a shift in the stability of their jobs and were forced to work reduced hours with less pay or even losing their employment entirely and therefore their source of income almost overnight. People were in dire need of money; more than was usual for the South African population. An ethics amendment was therefore requested and obtained to compensate participants R100 after the second swab was collected. Due to this compensation of participants as a study amendment, the ICF was amended first and used for the consenting process by July 2020 (Appendix B). Thereafter, nine additional patients were swabbed over a ten-month period.

An application for a protocol deviation was applied for and was approved by the CPUT HWC-REC on 17 July 2020; participants could now receive payment of R100,00 after the second swab (14 day) was collected. The patient no. 10 was sampled on 20 July 2020, soon after the deviation approval.

Due to the COVID19 pandemic, sampling of the 25 participants was not completed by the December 2020 cut off and an ethics extension was applied for and granted on 09 November 2020 until 10 November 2021. Participant sampling however slowly continued during this time period until a total of 18 were sampled by November 2021.

Further limitations include:

- Swabbing process – if Rectal swabs are not soiled with faecal matter – insufficient sample obtained may influence the isolate recovery and growth and thus affecting sensitivity of detection resulting in false negative results. This limitation is however unlikely for this study as each swab collected was visibly soiled with sufficient faecal matter.

- the Xpert® Carba-R panel detects five of the most common gene sequences responsible for carbapenemase production Additional genes such as OXA-23, GES and PER may not be detected by the Xpert® Carba-R system. For this study, no swabs were screening test positive and thus no Xpert® Carba-R testing performed.

- Sensitivity of culture may be less than the sensitivity (sensitivity 95.9% and specificity 96.6%) of performing PCR directly on samples (sensitivity and specificity in the order of about 100% and 99% respectively)

- If patients have other risk factors for CRE/CPE colonisation that they are exposed to at the same time as their TB treatment that they have not disclosed in the questionnaire, it may affect the results. This may also be true for unknown risk factors for CPE/CRE acquisition not covered by the questionnaire.

- The effects of the COVID19 pandemic resulted in a smaller participant sample size than planned which has have affected the outcome of this project.

5.4. Recommendations for Future Studies

A future study sampling a larger patient group, with additional sampling time points later in the 6-month treatment regimen will be valuable to detect subsequent CPE / CRE colonisation in the population receiving TB treatment as well as secondary side effects.

Tuberculosis remains a problem worldwide according to the WHO which means there will be a substantial proportion of the population on treatment particularly in endemic areas. Even though there was no resistance detected in the participants that were sampled in this project, it is still worthwhile to further explore the hypothesis by sampling a larger sample size, over a longer time period, using an alternate detection method.

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APPENDICES

Appendix A. Original ICF

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM
(CPE/CRE TASK /LANCET study)

TITLE OF RESEARCH PROJECT:
The effect of first-line TB treatment on carbapenem resistance in faecal Enterobacterales.

REFERENCE NUMBER:

……

STUDY CO0ORDINATOR:
Silvia Nunes (Site manager TCRC)

ADDRESS:
Department of Biomedical Sciences, Faculty of Health & Wellness Sciences
Cape Peninsula University of Technology, Bellville, 7535, Cape Town, South Africa

CONTACT NUMBER:
021 917 1044 (TASK Applied Science: TCRC)

We would like to invite you to participate in a research study looking at the bacteria in your stool samples. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research involves. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

What does this research study involve?
Tuberculosis (TB) is a curable infectious disease that is spread by coughing. TB is caused by a "bad" bacterium called *Mycobacterium tuberculosis*. TB mainly affects the lungs and is diagnosed from sputum (coughed up spit). TB treatment or medication must be taken every day for six months at least.

When people take antibiotics like the medication used to treat TB, it can affect the good bacteria. It is also more likely that other "bad" bacteria that are more resistant to killing by other antibiotics can enter the body or become more abundant in the body. We are interested in finding out how these bacteria are effected by TB treatment. Specifically, we will look at some of the organisms in the stool to see if they have now developed resistance to an antibiotic group called "carbapenems". Carbapenems are antibiotics that are used regularly to treat serious infections in hospital. If organisms become "resistant" to this antibiotic group, this means the antibiotic will no longer work. It is thus important to see what affects these bacteria and if TB treatment is one of the reasons why this could happen.

We will collect one rectal swab (from inside your anal area) before you start your treatment (at baseline) and one rectal swab (from inside your anal area) after 14 days of treatment. These swabs will be sent to TASK Laboratory and Lancet laboratories where we will grow bacteria from both swabs.

Why have you been invited to participate?
You have been invited to participate in this study because you are diagnosed with TB and about to be treated for it. You also meet our eligibility criteria and are a participant at a drug study at TASK.

What procedures will be involved in this research at each time-point?
Collection of one rectal swab by either a TASK nurse or yourself.
Answering a questionnaire about your recent medical history

Are there any risks involved?
You will receive the same care whether or not you decide to take part in the study. We will take care that your information is protected. All participants and their samples will be assigned specimen numbers, respectively, to ensure confidentiality. This de-identified information will be stored in a password protected database. Only the Investigator will have access to the code.

Are there any benefits to your taking part in this study and will you get told your results?
You will not directly benefit from this study however the results of the research will help us to better understand the effects of TB treatment on the bacteria already in the body.

How long will your rectal swab be stored and where will it be stored?

Your rectal swab will be stored at Lancet Laboratories and destroyed 7 days after processing and will not have your name on it. Certain resistant bacteria, bacterial DNA and RNA will be stored at TASK Laboratory, Glenlily, Parow for an indefinite amount of time, until they are

used up. The resistant bacteria, bacterial DNA and RNA will not be destroyed unless you so wish. The samples will not have your name attached to them only a specimen number.

How will your confidentiality be protected?
All information that is collected during this study, including your test results, personal data and research data will be held in strictest confidence. Data that may be reported in scientific journals will not include any information that identifies you.

Will you or the researchers benefit financially from this research?

You will not be paid to take part in this study case report forms.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties

Declaration by participant

By signing below, I ……. agree to take part in the study entitled *"The effect of first-line TB treatment on carbapenem resistance in faecal Enterobacteriaceae."*

Please read, tick off each of the boxes and sign the form if you agree to take part in this study.

I understand what this study is about and know how to contact the investigators if I want to.

I understand that rectal swabs will be collected from me/the participant and that tests will be done. I will also be required to answer a questionnaire regarding my recent medical history.

I understand that all the information given to the investigators and all test results will be kept private and confidential.

I understand that I will not benefit financially from this study.

I understand that I am free to withdraw myself/the participant from this study if I want to.

I understand that my/the participant's care will not be affected if I refuse to take part in this study.

Tick the option you choose:

I agree that bacteria grown from my rectal swab samples can be stored indefinitely after the project is completed and that researchers may then use it for additional/future research in the field of tuberculosis and antimicrobial studies. The original rectal swab will be destroyed after processing for this study is completed. Any future research on my samples will have to be approved by an accredited South African Research Ethics Committee and I retain the right to withdraw my samples at any time, without penalty.

Signed at (*place*) on (*date*)

Signature of participant Signature of witness

Declaration by research personnel

I *(name)* …… declare that:

I explained the information in this document to ……..
I encouraged him/her to ask questions and took adequate time to answer them.
I am satisfied that he/she adequately understands all aspects of the research as discussed above.
I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ..... on (*date*) ……..

Signature of research personnel Signature of witness

Declaration by Interpreter

I *(name)* …… declare that:

I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) ……. using the language medium of Afrikaans/Xhosa.
We encouraged him/her to ask questions and took adequate time to answer them.
I conveyed a factually correct version of what was related to me.
I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ..... on (*date*) ……..……..

Signature of interpreter

Appendix B. Amended ICF

PARTICIPANT INFORMATION AND INFORMED CONSENT FORM (CPE/CRE TASK /LANCET study)

TITLE OF RESEARCH PROJECT:
The effect of first-line TB treatment on carbapenem resistance in faecal Enterobacteriaceae.

REFERENCE NUMBER:

……

INVESTIGATOR:
Fay Swanson

ADDRESS:

Department of Biomedical Sciences, Faculty of Health & Wellness Sciences
Cape Peninsula University of Technology, Bellville, 7535, Cape Town, South Africa

CONTACT NUMBER:
021 100 3606 (TASK Applied Science)

We would like to invite you to participate in a research study looking at the bacteria in your stool samples. Please take some time to read the information presented here which will explain the details of this project. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research involves. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part initially.

What does this research study involve?
Tuberculosis (TB) is a curable infectious disease that is spread by coughing. TB is caused by a "bad" bacterium called *Mycobacterium tuberculosis*. TB mainly affects the lungs and is diagnosed from sputum (coughed up spit). TB treatment or medication must be taken every day for six months at least.

When people take antibiotics like the medication used to treat TB, it can affect the good bacteria. It is also more likely that other "bad" bacteria that are more resistant to killing by other antibiotics can enter the body or become more abundant in the body. We are interested in finding out how these bacteria are effected by TB treatment. Specifically, we will look at some of the organisms in the stool to see if they have now developed resistance to an antibiotic group called "carbapenems". Carbapenems are antibiotics that are used regularly to treat serious infections in hospital. If organisms become "resistant" to this antibiotic group, this means the antibiotic will no longer work. It is thus important to see what affects these bacteria and if TB treatment is one of the reasons why this could happen.

We will collect one rectal swab (from inside your anal area) before you start your treatment (at baseline) and one rectal swab (from inside your anal area) after 14 days of treatment. These swabs will be sent to TASK Laboratory and Lancet laboratories where we will grow bacteria from both swabs.

Why have you been invited to participate?
You have been invited to participate in this study because you are diagnosed with TB and about to be treated for it. You also meet our eligibility criteria and are a participant at a drug study at TASK.

What procedures will be involved in this research at each time-point?
Collection of one rectal swab by either a TASK nurse or yourself.
Answering a questionnaire about your recent medical history

Are there any risks involved?
You will receive the same care whether or not you decide to take part in the study. We will take care that your information is protected. All participants and their samples will be assigned specimen numbers, respectively, to ensure confidentiality. This de-identified information will be stored in a password protected database. Only the Investigator will have access to the code.

Are there any benefits to your taking part in this study and will you get told your results?
You will not directly benefit from this study however the results of the research will help us to better understand the effects of TB treatment on the bacteria already in the body.

How long will your rectal swab be stored and where will it be stored?

Your rectal swab will be stored at Lancet Laboratories and destroyed 7 days after processing and will not have your name on it. Certain resistant bacteria, bacterial DNA and RNA will be stored at TASK Laboratory, Glenlily, Parow for an indefinite amount of time, until they are

used up. The resistant bacteria, bacterial DNA and RNA will not be destroyed unless you so wish. The samples will not have your name attached to them only a specimen number.

How will your confidentiality be protected?
All information that is collected during this study, including your test results, personal data and research data will be held in strictest confidence. Data that may be reported in scientific journals will not include any information that identifies you.

Will you or the researchers benefit financially from this research?
You will not be paid to take part in this study case report forms, however, R100 will be given to you once the second swab has been collected the end of the study to compensate you for your participation.

Important information: In the unlikely event that this research leads to the development of a commercial application or patent, you or your family will not receive any profits or royalties

Declaration by participant

By signing below, I ……. agree to take part in the study entitled *"The effect of first-line TB treatment on carbapenem resistance in faecal Enterobacteriaceae."*

Please read, tick off each of the boxes and sign the form if you agree to take part in this study.

I understand what this study is about and know how to contact the investigators if I want to.

I understand that rectal swabs will be collected from me/the participant and that tests will be done. I will also be required to answer a questionnaire regarding my recent medical history.

I understand that all the information given to the investigators and all test results will be kept private and confidential.

I understand that I will not benefit financially from this study.

I understand that I am free to withdraw myself/the participant from this study if I want to.

I understand that my/the participant's care will not be affected if I refuse to take part in this study.

Tick the option you choose:

I agree that bacteria grown from my rectal swab samples can be stored indefinitely after the project is completed and that researchers may then use it for additional/future research in the field of tuberculosis and antimicrobial studies. The original rectal swab will be destroyed after processing for this study is completed. Any future research on my samples will have to be approved by an accredited South African Research Ethics Committee and I retain the right to withdraw my samples at any time, without penalty.

Signed at (*place*) on (*date*)

Signature of participant Signature of witness

Appendix C. Study Laboratory Request Form

Site information:		
RECTAL SWAB (day 1) ☐	COLLECTION DATE: (DD/MMM/YYYY)	COLLECTION TIME: (HH:MM)
RECTAL SWAB (day 14) ☐	COLLECTION DATE: (DD/MMM/YYYY)	COLLECTION TIME: (HH:MM)

SUBJECT ID:	SUBJECT INITIALS:
TIME POINT (Tick where applicable)	☑
Day 1 (Sample 1)	☐
Day 14 (Sample 14)	☐

PARTICIPANT QUESTIONNAIRE		
Have you been hospitalised in the last year?	YES ☐	NO ☐
If yes, so when and how long?		
Have you been hospitalised in a high care or ICU ward?	YES ☐	NO ☐
Have you had any contact with a person discharged from ICU or high care or LTCF?	YES ☐	NO ☐
Have you had any operations, catheters inserted in the last two years?	YES ☐	NO ☐
Have you been on antibiotics in the last year?	YES ☐	NO ☐
If yes what for, what antibiotic and duration?		
Do you have any chronic illnesses e.g. Diabetes, Asthma, etc.	YES ☐	NO ☐
If yes, what chronic medication do you take?		

Laboratory information:		
RECEIVED BY:	SIGNATURE:	DATE & TIME STAMP:

SWAB QUALITY:	SOILED ☐	NOT SOILED ☐
COMMENTS:		

Appendix D: Study Participant Visit Log

Protocol: "The effect of first-line TB treatment on carbapenem resistance in faecal *Enterobacteriaceae*"

Investigator: F Swanson Site: Task Clinical Research Centre

PARTICIPANT VISIT LOG

No.	Participant Initials	Participant Number	Swab Sample 1 (date)	Swab sample 2 (date)
e.g. 1	FS	FS - 01	N/A	N/A
e.g. 2	FLS	FLS - 25		
1	RUT	01	7 FEB 2020*	20 FEB 2020
2	B-M	02	10 FEB 2020	24 FEB 2020
3	DLW	03	10 FEB 2020*	25 FEB 2020*
4	ALD	04	12 FEB 2020*	28 FEB 2020
5	EPS	05	14 FEB 2020*	28 FEB 2020
6	BMK	06	14 FEB 2020*	02 MAR 2020
7	B-M	07	14 FEB 2020*	28 FEB 2020
8	MLB	08	19 FEB 2020	02 MAR 2020
9	K-J	09	02 MAR 2020*	17 MAR 2020
10	L-B	10	20 JUL 2020	5 AUG 2020
11	RJ (RMJ)	11	22 JUL 2020	6 AUG 2020
12	ATS	12	29 JUL 2020	12 AUG 2020
13	SJH	13	08 OCT 2020	22 OCT 2020
14	L-K	14	15 OCT 2020	29 OCT 2020

Protocol: "The effect of first-line TB treatment on carbapenem resistance in faecal *Enterobacteriaceae*"

Investigator: F Swanson Site: Task Clinical Research Centre

PARTICIPANT VISIT LOG

No.	Participant Initials	Participant Number	Swab Sample 1 (date)	Swab sample 2 (date)
e.g. 1	FS	FS - 01	N/A	N/A
e.g. 2	FLS	FLS - 25		
15	T - N	15	26 OCT'20	04 NOV 2020
16	S - P	16	30 MAR 2021.	13 Apr 2020
17	S - M	17.	07 APR 2021.	21 April 2021 (2nd attempt)
18	L C-H	18	13 MAY 2021	27 MAY 2021.
19				
20				
21				
22				
23				
24				
25				

Appendix E: Accreditation Certificate - Lancet laboratory

CERTIFICATE OF ACCREDITATION

In terms of section 22(2) (b) of the Accreditation for Conformity Assessment, Calibration and Good Laboratory Practice Act, 2006 (Act 19 of 2006), read with sections 23(1), (2) and (3) of the said Act, I hereby certify that:-

LANCET LABORATORIES (PTY) LTD

Co. Reg. No.: 1996/006950/07
Practice No.: 5101055

CENTURY CITY LABORATORY

Facility Accreditation Number: **M0627**

is a South African National Accreditation System accredited facility
provided that all conditions and requirements are complied with

This certificate is valid as per the scope as stated in the accompanying schedule of accreditation, Annexure "A", bearing the above accreditation number for

MEDICAL TESTING LABORATORY
CHEMISTRY, ENDOCRINOLOGY, HAEMATOLOGY,
MICROBIOLOGY, PCR AND SEROLOGY

The facility is accredited in accordance with the recognised International Standard

ISO 15189:2012

The accreditation demonstrates technical competency for a defined scope and the operation of a quality management system

While this certificate remains valid, the accredited facility named above is authorised to use the relevant accreditation symbol to issue facility reports and/or certificates

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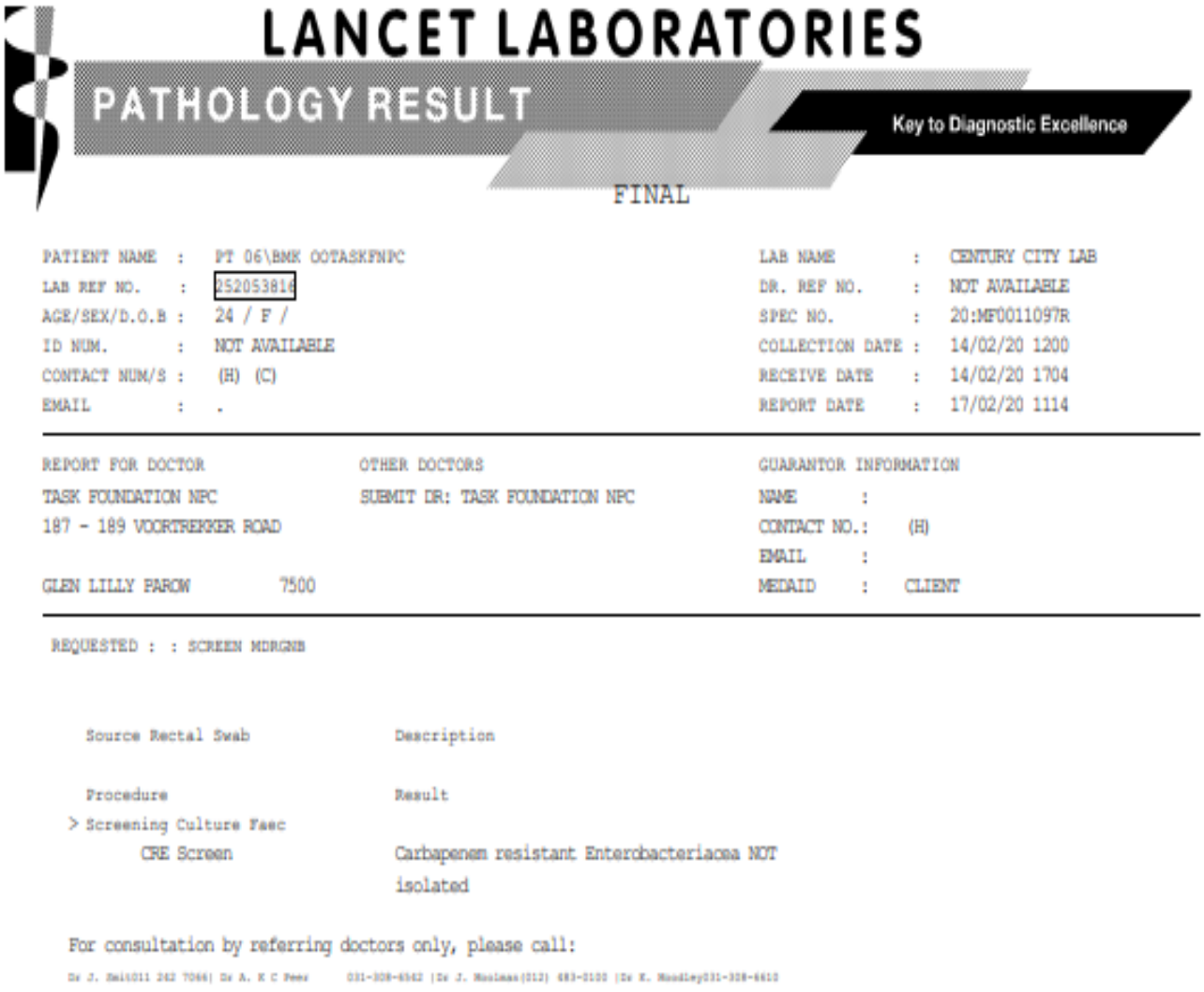
Mr R Josias
Chief Executive Officer

Effective Date: 02 August 2018
Certificate Expires: 01 August 2022

Appendix F: Example of Patient Laboratory Report



LANCET LABORATORIES

PATHOLOGY RESULT

Key to Diagnostic Excellence

FINAL

PATIENT NAME :	PT 06\BMK OOTASKFNPC	LAB NAME :	CENTURY CITY LAB
LAB REF NO. :	252053816	DR. REF NO. :	NOT AVAILABLE
AGE/SEX/D.O.B :	24 / F /	SPEC NO. :	20:MF0011097R
ID NUM. :	NOT AVAILABLE	COLLECTION DATE :	14/02/20 1200
CONTACT NUM/S :	(H) (C)	RECEIVE DATE :	14/02/20 1704
EMAIL :	.	REPORT DATE :	17/02/20 1114

REPORT FOR DOCTOR	OTHER DOCTORS	GUARANTOR INFORMATION
TASK FOUNDATION NPC	SUBMIT DR: TASK FOUNDATION NPC	NAME :
187 - 189 VOORTREKKER ROAD		CONTACT NO.: (H)
		EMAIL :
GLEN LILLY PAROW 7500		MEDAID : CLIENT

REQUESTED : : SCREEN MDRGNB

Source Rectal Swab — Description

Procedure	Result
> Screening Culture Faec	
CRE Screen	Carbapenem resistant Enterobacteriacea NOT isolated

For consultation by referring doctors only, please call:

Dr J. Smith011 262 7066| Dr A. K C Peer 031-328-6562 |Dr J. Moolman(012) 683-0100 |Dr K. Moodley031-328-6610