MOLECULAR EPIDEMIOLOGY OF CARBAPENEM-RESISTANT *ENTEROBACTERALES* COLONIZATION IN AN INTENSIVE CARE UNIT

By

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A dissertation submitted in fulfilment of the requirements for the degree of

Master of Pharmacy in Pharmaceutics,

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A dissertation submitted to the School of Health Sciences, University of KwaZulu-Natal, Westville Campus, for the degree of Master of Pharmacy in Pharmaceutics.

This is a dissertation by manuscript with an overall introduction and final summary.

This is to certify that the content of this dissertation is the original research work of Mr. Osama Madni, carried out under our supervision at the Antimicrobial Research Unit (ARU), Discipline of Pharmaceutical Sciences, School of Health Sciences, Westville campus, University of KwaZulu-Natal (UKZN), Durban, South Africa:

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DECLARATION

I, Mr. **Osama Madni**, declare as follows:

1. That the work described in this dissertation has not been submitted to UKZN or any other tertiary institution for purposes of obtaining an academic qualification, whether by myself or any other party.

2. That my contribution to the project was as follows:
    a. The research reported in this dissertation, except where otherwise indicated, is my original work
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    a. Their words have been re-written, but the general information attributed to them has been referenced.
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Signed 

_____

Osama Madni

Student Number 220081240

Date: 16/04/2021

DEDICATION

“This thesis is dedicated to my beloved parents and family for their endless love, support and encouragement”.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABR	Antibiotic resistance
AmpC	Ampicillin cephalosporinase
ARGs	Antibiotic resistance genes
AST	Antimicrobial susceptibility testing
BLAST	Basic local alignment search tool
BLI	β-lactamase inhibitor
BREC	Biomedical Research Ethics Committee
CGE	Centre for Genomic Epidemiology
CPE	Carbapenemase-producing *Enterobacterales*
CPKP	Carbapenemase-producing *K. pneumoniae*
CRE	Carbapenem-resistant *Enterobacterales*
CRKP	Carbapenem-resistant *K. pneumoniae*
CTX-M	Cefotaximase-München
DABCO	Diazabicyclo-octane
DHP	Dehydropeptidase
DNA	Deoxy-ribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ERIC	Enterobacterial repetitive intergenic consensus
ESBL	Extended spectrum β-lactamase
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*.
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration
GES	Guiana extended spectrum
GIM	German imipenemase

HAI	Health care associated infection
HGT	Horizontal gene transfer
HIC	High income country
ICU	Intensive care unit
IDSA	Infectious Disease Society of America
IMEs	Integrative and mobilizable elements
IMI	Imipenem-hydrolysing β-lactamase
IMP	Imipenem-resistant pseudomonas type carbapenemase
IS	Insertion sequence
KPC	*Klebsiella pneumoniae* carbapenemase
LMIC	low- and middle-income country
LSBL	Limited spectrum β-lactamases
MBL	Metallo-β-lactamases
MDR	Multi-drug resistant
MGE	Mobile genetic element
MIC	Minimum inhibitory concentration
MLST	Multi-locus sequence type
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
NCBI	National Centre for Biotechnology Information
NDM	New Delhi metallo-β-lactamase
NHLS	National Health Laboratory Service
NICD	National Institute for Communicable Diseases
NMC-A	Not metallo-enzyme carbapenemase-A
NTC	No template control

OMP	Outer membrane protein
OXA	Oxacillinase
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
PDR	Pan-drug resistance
PMQR	Plasmid-mediated quinolone resistance
REP-PCR	Repetitive element palindromic-polymerase chain reaction
RMS	Restriction-modification system
RNA	Ribonucleic acid
RND	Resistance-nodulation-division
SIM	Seoul imipenemase
SME	*Serratia marcescens* enzyme
SNP	Single nucleotide polymorphism
SPM	Sao-Paolo metallo-β-lactamase
SHV-1	Sulfhydryl-variable
STs	Sequence types
TEM-1	Temoneira
UKZN	University of KwaZulu-Natal
UPGMA	Unweighted Pair-Group Method
VGT	Vertical gene transfer
VIM	Verona integron-encoded metallo- β-lactamase
WGS	Whole genome sequencing
WHO	World Health Organization
WSBL	Wider spectrum β-lactamases
XDR	Extremely drug resistant

LIST OF APPENDICES

ABSTRACT

Background: Due to the high association with mortality and morbidity, carbapenem-resistant *Enterobacterales* (CRE) in general, and carbapenem-resistant *Klebsiella pneumoniae*, in particular, have been listed as high-priority pathogens by the World Health Organization (WHO) for the research and development of new antibiotics. Concomitant resistance to multiple antibiotics of different classes, impedes efficient clinical management of CRE infections. We characterized carbapenemase-producing *K. pneumoniae* (CPKP) isolates from sequential rectal screening of patients in a single intensive care unit (ICU) in a public hospital in the uMgungundlovu District of Kwazulu-Natal, South Africa, collected over one month. **Method:** Ninety-seven rectal swabs collected from consenting adult patients (n=31) on day 1, 3, 7 and weekly thereafter were screened for carbapenemase-production using Chrome-ID selective media. Fourteen CPKP were subjected to speciation and antibiotic susceptibility testing using the VITEK 2® automated system and their clonality was ascertained by ERIC/PCR. A sub-sample of eight isolates from five patients underwent whole genome sequencing (WGS) on the Illumina MiSeq platform followed by bioinformatics analysis to delineate the resistome, virulome, mobilome, clonality and phylogeography. **Results:** All isolates (100%) were resistant to ertapenem and meropenem and 71.4% (n=10) were resistant to imipenem. All isolates harbored the *bla*OXA-181 carbapenemase (100%, n=8) and also carried other β-lactamase genes such as OXA-1, CTX-M-15, TEM-1B and SHV-1. IncF, IncX3, and Col plasmid replicons groups and class I integrons (ln191 and ln27) were detected. All isolates belonged to the same sequence type ST307 and capsular serotypes (K102, O2v2) and several were associated with a single bed located in the ICU. All but one isolate carried the same plasmid multilocus sequence type [K7:A-:B-] and the same virulence repertoire was identified reflecting the epidemiological relationships between isolates. *Bla*OXA-181 were presumably located on a multi-replicon plasmid similar to that of *E. coli* p010_B-OXA181, and isolates were aligned with several South African and international clades, demonstrating horizontal and vertical transboundary distribution. **Conclusion:** OXA-181-producing *K. pneumoniae* belonging to ST307 was found to be potentially endemic in the hospital ICU environment of a public hospital in KwaZulu-Natal South Africa. The presence of a myriad of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in different permutations and combinations presents challenges to clinical management and infection prevention and control measures. This

necessitates a CRE screening programme and strict infection prevention and control measures to detect and eliminate this endemic clone

Molecular Epidemiology of Carbapenem-Resistant *Enterobacterales* Colonization in an Intensive Care Unit

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Agents that can eradicate or inhibit bacterial growth are known as antibiotics, with their life-saving advantages hailed after penicillin's first clinical use in the 1940s (Munita and Arias, 2016). The efficacy of antibiotics has dwindled because of the steady increase in bacterial resistance, a situation exacerbated by very few new antibiotics in the pipeline to replace the current antibiotics against which resistance has evolved (World Health Organization, 2014; Codjoe and Donkor, 2018).

Carbapenems are β-lactam antibiotics that are among the most effective antibiotics against Gram-positive and Gram-negative bacteria, providing a broad spectrum of antibacterial activity. Their molecular structure, along with β-lactam ring, gives added stability to most β-lactamases and extended-spectrum β-lactamases (ESBLs) (Meletis, 2016). Carbapenems are thus often considered "last-resort antibiotics" or "last-line agents" when critically ill patients are suspected of harbouring multi-drug resistant (MDR) bacteria (Ramsamy et al., 2020). The frequent use of carbapenems has allowed the evolution of carbapenem resistance, most commonly caused by carbapenemases which are β-lactamases capable of hydrolysing carbapenems (Pitout, Nordmann and Poirel, 2015). The Ambler classification method allows for the differentiation of carbapenemases into class A (e.g., KPC, SME, IMI, and GES), class B (e.g., NDM, VIM, and IMP), and class D (OXA-48-like). The carbapenemases of class A and class D are serine β-lactamases, and the carbapenemases of class B are metallo-β-lactamases (MBLs) (Cui, Zhang and Du, 2019).

Carbapenem-resistant *Enterobacterales* (CRE) are listed by the World Health Organization (WHO) as high priority for the research and development of new antibiotics (World Health Organization, 2017). Serious CRE infections have a higher risk of fatality than those caused by carbapenem-sensitive isolates (Martin et al., 2018). This threat is considerably higher when the carbapenem resistance in *Enterobacterales* is due to carbapenemase production (CPEs). Compared with the non-CPE infections (n=37,45%), CPE infections (n=46, 55%) were reported to cause four

times the risk of death within 14 days among hospitalized patients in an academic hospital in the USA between March 2013 and April 2016 (Tamma et al., 2017).

This study describes the molecular epidemiology of CPE colonization in patients from an intensive care unit (ICU) in a single public hospital in the KwaZulu-Natal area using whole-genome sequencings (WGS) and bioinformatics analysis. Delineating the molecular signatures of CPEs will direct containment strategies to improve patient outcomes.

1.2 Literature review

1.2.1 Antibiotics

Substances that interfere with the growth of micro-organisms (bacteria, viruses, fungi, and parasites) are called antimicrobial agents and act at concentrations that are tolerated by the hosts which they inhabit/infect (Balouiri, Sadiki and Ibnsouda, 2016). Antibiotics are a subdivision of antimicrobial agents that are produced wholly, or partially, by fermentation by micro-organisms and chemical synthesis to suppress the growth of or kill bacteria. Examples of antibiotics are penicillins and cephalosporins from moulds, polymyxins and bacitracin from bacteria, and aminoglycosides from actinomyces (Nemeth, Oesch and Kuster, 2015). Most of the antibiotics used in clinics today are from natural sources detected using variants of the Waksman platform strategy in the environmental material screens (Maarten et al., 2018). Synthetic antibiotics were traced back toward Paul Ehrlich, who identified different chemicals such as salvarsan involved in the syphilis treatment. Later, Domagk discovered the dye prontosil and its active metabolite sulfanilamide, which inhibited folic acid biosynthesis. This research has contributed to the development of sulfa drugs (Hutchings, Truman and Wilkinson, 2019). Medicinal chemistry and target-based compound library high-throughput screens replaced natural product studies in the mid-1990s and incorporated the idea of synthetic chemistry as a technique for the production of next-generation antibiotics. Strides in computational biology (system biology, bioinformatics, and metagenomics) and structural biological innovations (crystal protein target compositions, ligand screens by docking software, and the so-called designer drug strategy) drove synthetic biology as a third discovery strategy (Hoffman, 2020).

Antibiotics can be divided into two classes; bacteriostatic, and bactericidal, in which the former inhibit or stop the growth and multiplication of bacteria but do not kill them like the latter, and killing taking place by the immune system (Nemeth, Oesch and Kuster, 2015).

In general, major groups of antibiotics i.e., β-lactams, aminoglycosides, tetracyclines, macrolides, and glycopeptides can be categorised in terms of their mechanisms of action, that is, according to targets of their action. Two commonly used antibiotic classes, β-lactams and glycopeptides, interact with extracellular cross-linking steps and weaken the peptidoglycan layer's mechanical strength interfering with cell wall biosynthesis, however, the former group because of their relative efficacy and safety are the most clinically significant group (Sekyere, Govinden and Essack, 2016; Walsh and Wencewicz, 2016). Another class of antibiotics, such as the lipopeptides daptomycin and lanthionine-containing peptides disrupt the peptidoglycan assembly process which is important to maintain the integrity of the cell membrane, and acts as a barrier to organize the passage of ions and small molecules across the cell membrane (Walsh and Wencewicz, 2016).

Unlike the last two classes, there are other classes that block macromolecular metabolism either by altering the synthesis of bacterial proteins that perform many different functions of life, growth, and bacterial cell division. These acts either on small subunits of ribosomes (tetracyclines and aminoglycosides) or large subunits (erythromycins, streptograms and lincosamides). Yet another affects DNA replication or block transcription of DNA into RNA, e.g., processes performed with DNA gyrase enzyme and bacterial RNA polymerase enzymes that are inhibited by fluoroquinolones and rifamycin respectively (Davies and Davies, 2010; Walsh and Wencewicz, 2016). Sulfonamide antibiotics, considered to be the fifth group, act as competitive inhibitors of para-amino benzoate in the maturation of folate in which DNA biosynthesis is also affected (Walsh and Wencewicz, 2016).

1.2.2 Antibiotic resistance

The life-saving benefits of antibiotics had been recognized since penicillin was first introduced in the 1940s (Munita and Arias, 2016). Alexander Fleming who discovered penicillin was the first one to emphasize that resistance would occur if antibiotics were excessively and inappropriately used during his 1945 Nobel Prize elocution (World Health Organization, 2014). Antibiotic resistance (ABR) happens when bacteria can circumvent the mechanisms that antibiotics use against them or develop this ability with time. Infections caused by bacteria resistant to antibiotics are generally more challenging to manage, can cause relapses and significant mortality rates (Christaki, Marcou and Tofarides, 2020). According to studies conducted worldwide, particularly during the last two decades, the world is moving towards the "post-antibiotic era" largely attributed

to the inappropriate use of antibiotics. Clinical as well as financial medical outcomes are adversely affected by ABR in terms of higher morbidity and mortality, extended hospital stays, increased healthcare costs, and expensive or higher toxic alternative therapies (Butler, Blaskovich and Cooper, 2017). Infectious diseases remain a dominant cause of death globally, since the antibiotics used to cure them are being exhausted with rising ABR. Modern medicine that is highly dependent on antibiotics for bone marrow and organ transplants, surgeries, treating immune-compromised patients and cancer patients, and normal hip and knee replacement (Cassini et al., 2019) is compromised because of ABR.

Antibiotic resistance is a growing global health challenge caused by the consumption of antibiotics. Results from the analysis of the relationship between antibiotic use and resistance over time concluded from a study carried out in 76 countries in health systems around the world between 2000 and 2015, reflected a global health crisis, especially in low- and middle-income countries (LMICs), that showed 2.5 times the antibiotic consumption of high-income countries (HICs). It underlines the need for global monitoring of antibiotic usage to inform policies to minimize antibiotic use and resistance while maintaining access to those life-saving drugs (Klein et al., 2018).

ABR develops in bacteria through many mechanisms that could be intrinsic, acquired, or adaptive. Intrinsic resistance is due to the bacterium's inherent characteristics. Examples include the resistance to glycopeptides shown by Gram-negative bacteria due to outer membrane impermeability (Christaki, Marcou and Tofarides, 2020). Some bacteria are known to have a natural resistance to the antibiotics synthesized by them and to those with similar mechanisms of action (Davies and Davies, 2010; Munita and Arias, 2016). Acquired resistance occurs when an earlier susceptible bacterium acquires a resistance mechanism by either a mutation or the attainment of the new genetic material of exogenous origin by horizontal gene transfer (HGT) (Christaki, Marcou and Tofarides, 2020). The innate ability of bacteria to divide in a few minutes into millions of cells can lead to daughter cells with genomes that differ from parent cells. These mutational changes may confer antibiotic resistance when the new genome expresses proteins that can protect the antibiotic binding site from being altered or broken down by an antibiotic. (Davies and Davies, 2010; Munita and Arias, 2016). Adaptive resistance is described as resistance toward individual or more antibiotics caused by involvement of a particular environmental factor (e.g.,

pH, stress, nutrient conditions, ion concentrations, antibiotic sub-inhibitory levels). Unlike intrinsic or acquired resistance, adaptive resistance is temporary which makes bacteria respond more rapidly to antibiotics' challenge. The adaptation reverts typically to the previous state once the inducing signal is removed (Lee, 2019). Modulation, particularly of the expression of efflux pumps and porins, is involved in developing adaptive resistance (Motta, Cluzel and Aldana, 2015).

Resistance is can be disseminated by vertical gene transferral (VGT) to daughter cells from mother cells and is maintained through this form of expression in generations of bacteria. For cases where bacteria are exposed to sub-lethal antibiotic doses, resistance genes to these agents may grow over time (Wright, 2010).

HGT can take place by three mechanisms. Transformation is genetic recombination where free bacterial DNA elements enter the recipient bacteria and are inserted into its chromosome. Transduction requires a bacteriophage transfer of genetic material (Holmes et al., 2016; Christaki, Marcou and Tofarides, 2020; Munita and Arias, 2016). Resistance genes also are typically harboured on conjugative plasmids that are mobile genetic elements (MGEs) capable of initiating conjugation between two bacterial cells of the same or different species by means of sex pili expression. During conjugation, these plasmids are transferred to other bacteria. HGT has been described as being an important factor in the spread of antibiotic resistance (Partridge et al., 2018).

Bacteria express various mechanisms of antibiotic resistance, including enzymatic action, by which bacteria can modify the chemical composition of antibiotics by means of hydrolysis (β-lactam antibiotics), acetylation (aminoglycosides and tetracyclines), methylation, etc., making the antibiotic ineffective (Munita and Arias, 2016). In other cases, bacteria express target modifications (target replacement, target site enzymatic alterations, target position mutations, target position protection, excess target production, or target bypass) and decreased antibiotic accumulation due to lower permeability and/or enhanced efflux (Christaki, Marcou and Tofarides, 2020).

1.2.3 β-Lactam antibiotics

β-lactams are well tolerated, effective and the most widely used antibiotics in clinical medicine. They are flexible in that they can be structurally modified to give rise to numerous active forms, a malleability not possessed by other antibiotic classes. β-lactams are antibiotics of choice for

treating bacterial infections (Bush and Bradford, 2016; Sekyere, Govinden and Essack, 2016; Klein et al., 2018). All have an integral four-member lactam ring that can be fused in order to form bicyclic ring structures or exist as isolated rings. β-Lactams, similar to other classes of antibiotics, have experienced continuous improvement following their initial discovery with respect to features such as potency, range of activity, pharmacokinetics, safety profiles, and resistance development. The types of substitutions added to the basic nucleus determine the activity of a particular β-lactam compound, e.g., substitutions in the 7-α region of cephalosporins confer enhanced stability against β-lactamases, while may also decrease the antibiotic activity against certain species. Replacing sulphur in the nucleus with oxygen can increase biological activity. The β-lactam compound's chemical composition is therefore a balance between biological activity, stability of β-lactamase, and toxicity (Davies and Davies, 2010).

The antibacterial activity of β-lactams is derived from their similarity to the terminal D-Ala–D-Ala subunit of the peptidoglycan stem pentapeptide, with both β-lactam amide and neighbouring carboxylate (or sulfonic acid in monobactams) groups resembling terminal carboxylate of D-Ala–D-Ala and the peptide bond. The interaction of the β-lactam ring with nucleophilic serine of target penicillin-binding proteins (PBPs) prevents the formation of cross-linking peptidoglycan transpeptide (Tooke et al., 2019). This includes inactivating the transpeptidases needed for the bacterial cell wall synthesis terminal step. At the enzymatic active site, the PBPs, which comprise these transpeptidases, all have a serine that is acylated by the β-lactams in a reaction which is essentially irreversible within a bacterial cell. PBPs undergo fast acylation followed by gradual deacylation to restore active enzyme and an inactive ring-opened β-lactam, which is also hydrolysed (Bush and Bradford, 2019). Peptidoglycan is an integral constituent of the cell wall of the bacteria. The PBP structure suggests high variation among species, but it is well preserved in a specific bacterial genus. Alterations can enhance bacterial strength and virulence, offering protection from environmental stresses, natural hydrolytic enzymes, and antimicrobials. It is crucial to the growth and division of cells, with its net-like structure, consisting of peptide cross-linked saccharide chains (Pazos and Peters, 2019).

Several additional penicillin derivatives and related β-lactam groups have been discovered since the benzyl penicillin discovery in the 1920's. Each new class of β-lactam has been established either to extend its spectrum to cover additional bacterial species or to tackle varying mechanisms

of resistance that have emerged in the target population of bacteria, e.g. the evolution of β-lactamases has driven the production of new β-lactams (De Angelis et al., 2020).

Four main classes of β-lactam antibiotics are currently in clinical use. They contain three types of bicyclic structure: the penicillins in which the four-membered β-lactam ring fused to a thiazolidine ring, the cephalosporins in which the coupling component is a six-membered dihydrothiazine, and the carbapenems in which a five-membered pyrroline completes the bicyclic system. The monobactams are a fourth monocyclic β-lactam (Tooke et al., 2019).

1.2.3.1 Penicillins

The term 'penicillin' is commonly used to refer to various compounds with a structure based on β-lactam ring and the same antibacterial activity as the original molecule, benzylpenicillin (penicillin G). For example, penicillin G is more active against Gram-positive bacteria, particularly cocci, several streptococci but less effective against Gram-negative bacteria (Lobanovska and Pilla, 2017). The quest for more potent penicillins against staphylococcal β-lactamases came shortly after the introduction of penicillin G as the first β-lactam to be used clinically in 1940. Penicillin G was followed by another natural penicillin, phenoxymethyl penicillin (Penicillin V)that could be orally administered (Sekyere, Govinden and Essack, 2016). The classification of penicillins is based on chemical substitutions for residues attached to the β-lactam ring. Thereafter the second generation of semi-synthetic penicillins were developed to counteract penicillinase-producing Gram-positive strains that emerged due to excessive use of the first generation, e.g., oxacillin, dicloxacillin, and methicillin. The relatively limited range of these antibiotics' action with the need for broader coverage of Gram-negative bacteria expanded the second generation. The third generation of broad-spectrum penicillins, also referred to as aminopenicillins, were introduced in the 1960s. Examples of this group are amoxicillin and ampicillin and are more successful against a larger group of Gram-negative bacteria. The next generation of penicillins comprising carboxypenicillins and ureidopenicillins further broadened the penicillin coverage spectrum against Gram-negative bacteria and showed good activity against *Pseudomonas aeruginosa* (Lobanovska and Pilla, 2017).

1.2.3.2 Cephalosporins

In the 1950s, a new path for the production of the cephalosporins was opened with the discovery of naturally occurring cephalosporin C (Sudo et al., 2014). First-generation cephalosporins include

cephalothin, cefazolin, cephapirin, cephradine, cephalexin, and cefadroxil. They have effective coverage of most Gram-positive cocci, such as streptococci and staphylococci. In contrast, they have limited coverage of Gram-negative bacteria such as *Neisseria meningitidis*, *Haemophilus. influenza*. *Klebsiella pneumoniae*, *Escherichia. coli*, and *Proteus mirabilis* are Gram-negative bacteria that remain more sensitive to first-generation cephalosporins. Cephalosporins of the second generation possess less activity toward Gram-positive cocci compared with first generation cephalosporins but have enhanced activity against Gram-negative bacteria. Cefuroxime and cefprozil are members of second generation cephalosporins with antibacterial activity able to withstand the Temoneira (TEM-1) β-lactamase occurring on transmissible plasmids in *N. gonorrhoeae* and *H. influenzae*, in addition to the ampicillin cephalosporinase (AmpC) for the latter as well as sulfhydryl-variable (SHV-1). (Sekyere, Govinden and Essack, 2016). Third generation cephalosporins have additional coverage of Gram-negative bacteria and are frequently used to treat infections caused by bacteria resistance to first- and second-generation cephalosporins or different β-lactam resistant infections caused by drug-resistant *N. meningitidis, H. influenza*, or *Streptococcus pneumonia*, and more importantly, *Pseudomonas aeruginosa*. Ceftriaxone, cefotaxime, ceftazidime, cefpodoxime, cefdinir, and cefixime belong to the third generation. In general, the ability to withstand hydrolysis by β-lactamases increases as we move from the first generation to the fifth. Third generation cephalosporins confer added stability to β-lactamases than first or second generations, particularly those produced by *Klebsiella*. spp, *E. coli*, and *Haemophilus influenza*. Fourth generation cephalosporins include cefepime and cefpirome which have the same spectrum of activity as the third generation in addition to much more noteworthy coverage of β-lactamase-producing Gram-negative bacteria. Cephalosporins of the fifth generation include ceftaroline. It differs from the rest of the cephalosporins by its indication for methicillin-resistant *Staphylococcus aureus* (MRSA). While *Enterococcus faecalis* and *Listeria monocytogenes* are also covered with ceftaroline, it does not embrace *Pseudomonas aeruginosa* (Bui and Preuss, 2020).

1.2.3.3 Cephamycins

Cephamycins were regarded as a possible answer to the emerging threat of bacteria resistant to cephalosporins. The pharmacology of this class of compounds, derived from a bacterial natural product, cephamycin C, is similar to that of cephalosporins, and these compounds (including cefoxitin, moxalactam, and cefotetan) are also known as second-generation cephalosporins. The

characteristic 7-methoxy side chain in the cephamycin cefoxitin gives stability against β-lactamases of TEM type, including ESBLs. It has beneficial antibacterial activity against methicillin-susceptible *Staphylococcus aureus* (MSSA) and also enteric bacteria, that do not contain high levels of AmpC (Bush and Bradford, 2016). With an antimicrobial activity comparable to that of cephalosporins of the 3rd and 4th generation, the novel oxacephem moxalactam, or latamoxef, has excellent hydrolysis stability against β-lactamases (Bush and Bradford, 2016).

1.2.3.4 Carbapenems

In the 1980s, a new β-lactam group of antibiotics stable to hydrolysis by all β-lactamases including ESBLs was introduced. They are bactericidal antibiotics with proven effectiveness against bacteria producing ESBLs (Hawkey and Livermore, 2012). Carbapenems are noteworthy for their stability, except for the evolving carbapenemases mainly found in Gram-negative bacteria (Kiratisin et al., 2012). The mechanism of action of carbapenems occurs after penetration into the bacterial cell wall and attachment to particular PBPs that include 1a, 1b, 2, and 3 that leads to the killing of bacteria. Current knowledge describes the inhibition of transpeptidase as the predominant target enzyme of carbapenem. A covalent bond is established through transpeptidation by PBPs, specifically the carboxypeptidase and transpeptidase enzymes that inhibit their peptide cross-linking actions during the biosynthesis of peptidoglycan resulting in cell death by autolytic action (Codjoe and Donkor, 2018). There is a lot of focus on the substantial nature of the glycan backbone. When PBPs are repressed, the strength of the cell wall is compromised, and because of autolysis, the glycan backbone fails and, ultimately, osmotic pressure in Gram-negative bacteria destroys the cell (Papp-Wallace et al., 2011).

Several examples, including meropenem, imipenem, doripenem, panipenem, ertapenem, and biapenem, are used worldwide due to the growing resistance of the *Enterobacterales* to cephalosporine antibiotics (Patel and Bonomo, 2013). However, in general, due to their concentration-independent killing effect for bacteria, carbapenems are prioritized to treat infectious or life-threatening infections over other types of antibiotics (Watkins and Bonomo, 2013).

Imipenem was used as the first therapeutic agent for non-carbapenemase-producing enteric bacteria after failure of chemically unstable thienamycin. Its spectrum of activity covers non-

fermentative and anaerobic Gram-positive and Gram-negative bacteria. Thereafter, more agents such as meropenem, ertapenem, and doripenem, were developed which vary slightly in their antibacterial activity, i.e., meropenem and doripenem have higher activity compared to imipenem against *Enterobacterales* and *P. aeruginosa*. Biapenem and tebipenem are two carbapenems licensed for Japan with the same range of activity as earlier ones, in addition to providing greater hydrolytic stability compared to imipenem or meropenem for MBLs (see section 1.2.5.1.2) (Bush and Bradford, 2016).

Comparatively, doripenem is reasonably stable against hydrolysis by most β-lactamases among the carbapenems. Evidence has shown that doripenem also is less sensitive and much slower in carbapenemase hydrolysis (from 2 to 150 fold) than imipenem (Chahine, Ferrill and Poulakos, 2010; Queenan et al., 2010). Ertapenem, meropenem, and imipenem are poorly orally absorbed, and require parenteral administration (Watkins and Bonomo, 2013).

Imipenem, doripenem, and meropenem have an in vivo half-life of almost one hour, whereas ertapenem has an estimated half-life of four hours, making it suitable for once-daily administration. Imipenem is known, in contrast to the other carbapenems, for its dose-dependent gastrointestinal side-effects. Many carbapenems are degraded via the dehydropeptidase-1 (DHP-1) enzyme found within the renal tubules and need co-administration with a DHP-1 inhibitor like cilastatin. The latter types of carbapenems, including doripenem and ertapenem, require no DHP-1 inhibitor since they are more stable. These compounds differ in their linking to PBP, thereby providing distinct behaviour variations against different bacterial species (Codjoe and Donkor, 2018). For example, a study evaluated the selectivity of 22 commercially available β-lactams for PBPs in live *E. coli* DC2. *E. coli* possesses three class A (PBP1a, PBP1b, and 26 PBP1c) and two class B (PBP2 and PBP3) high molecular weight PBPs, also has seven low molecular weight (class C) (PBP4, PBP5, PBP6, PBP6b, PBP7, PBP4b, and AmpH). Meropenem among these β-lactams inhibited PBP2 and PBP4. PBP2, PBP4, PBP7, and PBP8 were inhibited by doripenem and faropenem and are therefore identified as not selective (Kocaoglu and Carlson, 2015).

1.2.3.5 Monocyclic β-lactams

.These are β-lactams containing a monobactam nucleus with substitution in their monocyclic ring, i.e. sulfonic acid substituent (aztreonam) and group substitution of O-sulfates (BAL30072, novel monosulfactam), with the former being the only one authorized to be used clinically in 2016 (Bush

and Bradford, 2016). They are resistant to β-lactamases and effective against Gram-negative rods, though not on bacteria or anaerobes that are Gram-positive (Fernandes, Amador and Prudêncio, 2013). Aztreonam was effectively used for multidrug-resistant β-lactamase-producing species before the advent of serine carbapenemase and ESBLs, but BAL30072 demonstrated synergistic activity in combination with β-lactamase inhibitors and greater stability to serine carbapenemase against hydrolysis compared to aztreonam (Mushtaq et al., 2013; Bush and Bradford, 2016).

Strains expressing MBLs confer resistance to all β-lactam antibiotics in Gram-negative bacteria, except for monobactams, which are inherently stable against MBLs. However, established first-generation monobactam drugs such as aztreonam have limited clinical effectiveness against strains that express MBL because they are frequently co-expressed with other serine β-lactamases in clinical isolates. Studies had been carried out to optimize novel monobactams by increasing their stability to serine β-lactamases, while preserving intrinsic stability against MBL. This led to the discovery of LYS2288 (compound31). In the presence of all classes of β-lactamases, LYS228 is potent and displays potent activity against CRE (Reck et al., 2018).

1.2.3.6 β-lactam/β-lactamase inhibitor combinations

Clavulanic acid was the first β-lactamase inhibitor identified in 1972, followed by sulbactam in 1978 and tazobactam in 1984, named the classical β-lactamase inhibitors (Toussaint and Gallagher, 2015). Clavulanic acid has a novel clavam structure and is an inhibitor for staphylococcal penicillinases and most of the plasmid-encoded penicillinases present in *Enterobacterales*. Clavulanic acid's inhibition spectrum spans most class A β-lactamases, including ESBLs and, to a lower extent, serine carbapenemases. It firstly acylates the β-lactamase active site serine with temporary inhibition that includes hydrolysis of clavulanic acid before the enzyme completes the inactivation. Clavulanic acid acts against β-lactamase-producing enteric bacteria synergistically with penicillins and cephalosporins to inhibit particular β-lactamases, enabling the β-lactam counterpart to kill the bacteria. A variety of penicillanic acid sulfones are synthesized by medicinal chemists with a similar activity spectrum and perform the same general inhibitory/inactivation mechanism as clavulanic acids, such as sulbactam and tazobactam. In general, none of the inhibitors have substantial antibacterial efficacy in monotherapy, although there are several notable exceptions (Bush and Bradford, 2016). Sulbactam shows less activity against β-lactamases of class A than clavulanic acid or tazobactam; however, it is more active

against β-lactamases of class C and demonstrates antimicrobial activity against *A. baumannii*. Tazobactam shows better activity than the others against cefotaximase-München (CTX-M) type enzymes (class A group) and can inhibit particular Class C and D β-lactamases (insignificantly) (Toussaint and Gallagher, 2015).

None of these inhibitors are effective in inhibiting MBLs and their limited activity toward serine carbapenemases does not translate into clinical susceptibility due to the carriage of multiple β-lactamases in the bearing organisms (Bush, 2015). Even with the substantial inhibitory activity observed in clavulanic acid and tazobactam action against single ESBLs, it is not sufficient to protect large amounts of their accompanying penicillins and/or co-expression of many β-lactamases (Jones-Dias et al., 2014).

The advent of inhibitor-resistant bacterial strains and inadequate inhibitory action of conventional β-lactam inhibitors against carbapenemases resulted in the development of the 'second generation' of β-lactamase inhibitors (BLIs) that include avibactam, vaborbactam, relebactam, zidebactam, and nacubactam as highly active inhibitors of Ambler class A and C β-lactamases (Docquier and Mangani, 2018).

A new class of non-β-lactam β-lactamase inhibitors has developed, based on a novel joined diazabicyclo-octane (DABCO) structure. One of the first inhibitors, avibactam, has a wider spectrum of activity covering class C cephalosporinases, and some class D oxacillinases (Ehmann et al., 2013). Avibactam has a unique inhibition mechanism; this does not have a β-lactam core but retains the potential to target β-lactamase carbamylateits covalently through the opening of the avibactam ring. In contrast to 'older' βlactamase inhibitors, the reaction is reversible, with deacylation leading to regeneration of the preserved avibactam capable of rebinding rather than hydrolysing (Ehmann et al., 2012). This mechanistic distinction from the older inhibitors helps make avibactam particularly effective in protecting the β-lactam companion against hydrolysis by chromosomal and plasmid-mediated β-lactamases (Lahiri et al., 2014). Avibactam has been combined with ceftazidime for therapeutic use and is being developed in combination with ceftaroline or aztreonam (Livermore et al., 2015). Other DABCOs involve RG6080 and relebactam in combination with imipenem. Both exhibit a comparable spectrum of activity to avibactam, however, relebactam is less active against class-D β-lactamases, such as OXA-48 (Hecker et al., 2015).

Zidebactam combination with cefepime for the prevention of Gram-negative infections demonstrated potent activities against *Entrobacterales* and *P. aeruginosa* producing β-lactamases of clinical significance (Sader et al., 2017). Enmetazobactam (formally AAI101) is a penicillanic acid sulfone identified as an ESBL inhibitor with action against selected enzymes of class C and class D carbapenemases, and also some class A carbapenemases produced by *Enterobacterales* (Tselepis et al., 2020). The combination of cefepime/enmetazobactam yielded better results than recently approved combinations, including ceftazidime/avibactam, ceftolozane/tazobactam, and imipenem/relebactam against MDR ESBLs-producing *Enterobacterales* strains (Morrissey et al., 2019). Nacubactam, also has demonstrated efficacy against class A and class C β-lactamases in combination with meropenem, and improved antibacterial killing of ceftazidime-avibactam-resistant isolates has been observed in vitro (Monogue et al., 2018).

New structures mainly oriented towards impairing MBLs have been discovered in the last few years, with the compound ANT2681 (Antabio) possibly being one of the most important examples. This novel thiazole-carboxylate inhibitor is the product of a pyridine-2-carboxylic acid hit-to-lead program in medicinal chemistry and is a preclinical candidate with clinical potential for development as a particular inhibitor of MBLs (Davies et al., 2020). By interaction with the dinuclear zinc ion cluster present in the active site of these enzymes, ANT2681 inhibits the activity of MBLs (Everett et al., 2018). The inhibitor shows high NDM-1 affinity, lower VIM-1 affinity, and very slight IMP-1 affinity. It has shown effectiveness in a mouse thigh model with an NDM-1-producing clinical isolate of *K. pneumoniae*. ANT2681 is therefore undergoing preclinical development to combine it with meropenem as a potential therapy for MBL-producing CRE-induced serious infections (Vázquez-Ucha et al., 2020).

The boronic acid inhibitor RPX70099 is another novel class of synthetic non-β-lactam β-lactamase inhibitors (vaborbactam). This is developed in combination with meropenem against serine-based carbapenemase-producing pathogens, and has been known to be an effective serine β-lactamase inhibitors for several years (Lapuebla et al., 2015). This combination was approved because of its potent activity against Ambler class A carbapenemases, such as KPC, by the Food and Drug Administration (FDA) in October 2017 (Ackley et al., 2020).

In acknowledging the need for viable solutions to the small new antibiotic pipeline, the Infectious Diseases Society of America (IDSA) initiated the '10×'20 campaign' in 2010, aiming for the

production and regulatory approval of 10 novel, effective and safe systemically administered antibiotics by 2020 (Boucher et al., 2013). There was significant interest in combinations of β-lactam/β-lactamase inhibitors, promising in vitro action against MDR, XDR, and even PDR Gram-negative bacteria, as novel agents to counter β-lactam antibiotic resistance (Magiorakos et al., 2012). Over the last decade, the FDA has licensed six β-lactam containing agents: ceftaroline, ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam and cefiderocol. four of which are β-lactam antibiotics paired with β-lactamase-inhibitors (Bush and Bradford, 2019).

1.2.4 β-Lactam resistance mechanisms

It is acknowledged that β-lactams remain both the most prescribed class of antibiotics and the most significant in terms of sales confirming their continuing central role in bacterial infection management (Klein et al., 2018). The efficacy of β-lactam antibiotics depends on the accessibility of its targets, their susceptibility to β-lactamases enzymatic inactivation, and the ability of β-lactam to suppress target PBPs. Changing one or a combination of these parameters can contribute to resistance (Kong, Schneper and Mathee, 2010), and like any other antibacterial drug used within the clinical setting, their use is sooner or later accompanied by the development of resistant strains, which can spread quickly on a global scale after the acquisition of resistance determinants (Khan, Maryam and Zarrilli, 2017). β-lactam resistance may be intrinsically expressed by chromosomal genes or acquired through plasmids. Plasmid-mediated resistance due to HGT is a more worrisome form of resistance because of the ease of spread. Multiple resistance mechanisms to β-lactams, including target modification, efflux pumps, and hydrolysing β-lactamases enzymes, have been identified (Docquier and Mangani, 2018).

The biggest single cause of resistance to β-lactam antibiotics is antibiotic-inactivating enzymes, the β-lactamases, that are versatile enzymes present in a number of bacterial species with a restricted range of molecular structures. A commonality is the ability to hydrolyse β-lactam ring-containing chemical compounds (Kong, Schneper and Mathee, 2010; Bush, 2018). β-lactamases often contain a reactive serine residue at their active site (serine β-lactamases) or at least one divalent zinc atom (MBLs) that acts catalytically. Serine β-lactamases are varied in structure and hydrolytic pattern; however, all of these enzymes hydrolyse β-lactams at their catalytic site in a multi-step reaction via a serine residue (Bush and Bradford, 2019).

Decreased permeability of the lipopolysaccharide structure of the Gram-negative bacterial cell wall, and alteration or loss of trans-membranous hydrophilic protein channels, the porins, function as another resistance mechanism that hinders β-lactams from accessing their PBP targets. This mechanism only results in minimal resistance but may function synergistically with the expression of β-lactamases or active efflux to impose high resistance levels. Efflux systems consist of cytoplasmic membrane proteins which use the proton-motive force to extrude drugs. Also, bacteria seek to alter PBP-target affinity for β-lactam antibiotics, acquire resistant PBPs or increase PBP-target amounts in the absence of β-lactamases as other mechanisms of resistance (Fisher and Mobashery, 2016; Bush, 2018).

The production and proliferation of inactivating enzymes were identified early after the antibiotic class β-lactam was discovered and clinically administered. The β-lactam-hydrolysing enzymes have increased their spectrum of activity over time, starting with penicillinases, followed by cephalosporinases, then to ESBLs, and more recently MBLs and other carbapenemases. The MBLs also had a significant effect on the efficacy of carbapenems used to treat multi-resistant Gram-negative bacilli (Garcia, 2013).

Two main physiologically important enzyme properties, i.e., structure and function, are the basis of the nomenclature for β-lactamases. In 1980, Ambler first described structural classes that used four amino acid β-lactamase sequences to define class A enzymes including serine at the active site and class B MBLs. Since then, based upon the molecular size and unique homologous motifs, class C and class D serine-based β-lactamases have been identified (Bush and Bradford, 2020). Philippon et al., have recently proposed modifications to the Ambler's class A on the basis for a structure-based and phylogenetic classification. Two subclasses (A1, A2), six major clusters (e.g., wider spectrum β-lactamases (WSBL), naturally limited spectrum β-lactamases (LSBL), and several additional clusters were defined based on conserved (>90%) and unique patterns. Residues such as V231GDKTG and S70TFKAL were recognized for subclass A1, V231AHKTG and S70VFKFH for subclass A2 (Philippon et al., 2019).

Functional classification schemes have been established for over 50 years based on biochemical properties, such as substrate compositions, relative hydrolysis rates compared to the conventional β-lactams, and interactions with inhibitors. Bush et al., suggested revised functional classification based on a combination of biochemical properties and structural class assignments in 1995, with

Bush and Jacoby adding additional subgroups in 2010. To identify β-lactamases that include more than a single group or class, other functional names are frequently used. For instance, carbapenemases, β-lactamases efficient in hydrolysing carbapenems at a clinically significant rate, may correlate to structural class A, B, or D, or may relate to functional group 2df or 2f, or to group 3. ESBLs may contain Class A, C or D enzymes or may be classified as groups 1e, 2be, 2de, and 2e (Bush and Bradford, 2020).

Ambler class A serine β-lactamases are the largest number of enzymes with a comprehensive activity spectrum. Penicillinases related also to functional group 2b, are broad-spectrum enzymes, as they hydrolysed the first approved for medicinal use penicillins and cephalosporins. In this group also belong two frequently occurring β-lactamases identified in *Enterobacterales* designated as TEM-1 and SHV-1 most often conferring resistance in *E. coli* and *K. pneumoniae,* respectively (Beceiro et al., 2011). Other enzymes belong to the same group, and 2be subgroup are ESBLs. The first evolving ESBLs were derivatives of TEM and SHV that had amino acid substitutions resulting in an 'expanded' profile of the substrates, and they hydrolyse penicillins and cephalosporins that are both narrow and broad in their microbiological spectrum. For the first decade of ESBL discovery, they were ubiquitous, but in recent years, due to the worldwide spread of CTX-M-producing isolates, their importance has diminished (Bush and Bradford, 2019). CTX-M-type enzymes which are normally produced by *Enterobacterales* species including *Shigella* spp., *Salmonella* spp., *C. freundii*, *S. marcescens,* and *Enterobacter* spp. as well as some non-fermentative bacteria (Cantón, González-Alba and Galán, 2012) are commonly inhibited by tazobactam, newer BLIs, including avibactam, relebactam, vaborbactam, and by clavulanate and sulbactam to varying degrees (Lomovskaya et al., 2017). Other variants of TEM and SHV, called inhibitor-resistance β-lactamases (functional group 2br), are not inhibited by clavulanic acid and sulbactam, however, most of them remain sensitive to inhibition by tazobactam and avibactam (Lahiri et al., 2016).

Serine carbapenemases belong to Ambler class A enzymes and are capable of hydrolysing carbapenems (serine carbapenemases) while MBLs distinguished by the presence of Zn2+ at the active site are members of Ambler class B and are classified in functional group Bush–Jacoby–Medeiros 2f, and group 3 respectively. These will be discussed in section (1.2.5.1).

AmpC β-lactamases belong to Ambler class C, functional group 1. These contribute resistance to most cephalosporins, including broad-spectrum cephalosporins such as ceftazidime, cefotaxime, and ceftriaxone, and cephamycins such as cefoxitin. They also hydrolyse penicillins and other monobactams to a lesser scale (Bush and Jacoby, 2010). The basal expression of chromosomal AmpC-type β-lactamases is usually small, but increased expression levels can be produced after exposure to certain β-lactams, which are especially important in clinical isolates of *E. cloacae, C. freundii, E. aerogenes, S. marcescens* and *P. aeruginosa*. AmpC β-lactamases may also be plasmid-mediated are disseminated widely. Many of these variants are CMY-1, originating from C. *freundii*, MIR and ACT families of *Enterobacter* spp., and DHA family, of *Morganella morganii* (Bush and Jacoby, 2010). When analysing the induction of β-lactamase in bacterial cells, the most important factors are the differentiation of chromosomal-encoded β-lactamases into constitutive and inducible. In both conditions, the former is present at a consistent level, while the latter is suppressed in the absence of antibiotics and triggered in the presence of β-lactam agents (Kong, Schneper and Mathee, 2010). Last in this classification, are oxacillin-hydrolysing β-lactamases (OXA-type) grouped as functional group 2d β-lactamases of molecular class D. OXA enzymes are often more hydrolytic to oxacillin than penicillin G, and some members of this group can hydrolyse cephalosporins or carbapenems. There are many OXA β-lactamases associated with an ESBL phenotype, such as OXA-11, OXA-14, and OXA-20 (Evans and Amyes, 2014). However, they are inhibited only slightly by clavulanate, while others are inhibited by tazobactam and avibactam (Ehmann et al., 2013).

The clinical effect of β-lactams is threatening by mobile β-lactamases in Gram-negative bacteria include ESBLs and serine and metallo-carbapenemases. β-lactamase-encoding genes can be acquired horizontally via plasmids, transposons, gene cassettes, integrons, and insertion sequences, with few species barriers for their transmission (Bush, 2018).

1.2.5 Carbapenem resistance mechanisms

Among the β-lactams, carbapenems are the most effective against Gram-positive and Gram-negative bacteria, offering a broad spectrum of antibacterial activity. A particular molecular structure, along with the β-lactam ring, gives added stability against most β-lactamases, including ESBLs. Consequently, carbapenems are established as one of the most effective drugs for

managing bacterial infections, and the advent and spread of resistance to these antibiotics is a significant public health issue (Nordmann, Naas and Poirel, 2011; Meletis, 2016).

Carbapenems are often referred to as "last-resort antibiotics" or "last-line agents" when infected patients become critically ill or are suspected of harbouring resistant bacteria. The extensive use of carbapenems for empirical and direct treatment of serious infections has led to the development of carbapenem hydrolysing β-lactamases, also known as carbapenemases, as the most recognized mechanism of carbapenem resistance (Papp-Wallace et al., 2011; Ramsamy et al., 2020). Carbapenem resistance may also result from altered PBPs, or reduced cell entrance due to upregulated efflux pumps or modifications through the outer membrane proteins (OMPs) together with ESBL and AmpC β-lactamase production (Bush and Bradford, 2020).

Infections generated from extensively drug-resistant (XDR) Gram-negative bacteria have emerged as one of the serious threats in the world, not least of which are the globally rising carbapenem-resistant bacteria (Prestinaci, Pezzotti and Pantosti, 2015; Logan and Weinstein, 2017; Bonomo et al., 2018). Resulting in limited treatment choices and an unavoidably high mortality rate, they are becoming a major global concern (Poirel et al., 2011; Cui, Zhang and Du, 2019; Fasciana et al., 2019).

Enterobacterales is a large group of rod-shaped, Gram-negative bacilli bacteria. They were by far the most isolated bacteria from clinical samples and accounted for up to 80% of all clinically relevant Gram-negative bacilli isolates in 2016, and up to 50% of all clinically significant bacteria. *Enterobacterales* cause nosocomial and community-acquired diseases, and progressively became MDR (Perovic et al., 2016). Due to this, WHO compiled a list of antibiotic-resistant bacteria for which new antibiotics urgently need to be developed in 2017. Based on the urgency in which new antibiotics are needed, this list is categorized into three categories: critical, high, or medium priority. Within the critical priority category are *Enterobacterales* resistant to carbapenems and 3rd generation cephalosporins (Tacconelli et al., 2018).

Enzyme-mediated carbapenem resistance is attributable to carbapenemases capable of inactivating carbapenems along with other β-lactam antibiotics. This remains the most frequent resistance mechanism within CREs. Carbapenemase-producing *Enterobacterales* (CPE) is CRE, which are carbapenem-resistant due to the production of carbapenemases. This form of resistance is the most

important clinically since these enzymes hydrolyse all or nearly all of the β-lactams, resulting in high minimum inhibitory carbapenem concentrations (MICs), are encoded by genes that are horizontally transferable by plasmids or transposons, and are usually associated with genes encoding other determinants of resistance (Meletis, 2016).

Initially, the carbapenemases were mediated chromosomally within few species. However, they are plasmid-mediated and sometimes chromosomally- and plasmid-mediated, resulting in horizontal transmission between different bacterial species and genera. The capacity of different MGEs to develop, harbour, and disseminate various resistance genes leads to resistant species being effectively and actively spread (Singh-Moodley and Perovic, 2016).

1.2.5.1 Carbapenemases

1.2.5.1.1 Class A Carbapenemases

Class A β-lactamases include the carbapenemase genes encoded by chromosomes such as the imipenem-hydrolysing β-lactamase-1(IMI-1), *Serratia marcescens* enzyme (SME), not metallo-enzyme carbapenemase A (NMC-A), *Serratia fonticola* carbapenemase-1(SFC-1), and those encoded by plasmids such as the variants of IMI (IMI-1 to IMI-3), *Klebsiella pneumoniae* carbapenemases (KPC-2 to KPC-13), and derivatives of Guiana extended spectrum (GES-1 to GES-20) (Nordmann and Poirel, 2014; Naas, Dortet and Iorga, 2016; Bonomo, 2017). Serine-based carbapenemases can hydrolyse all β-lactams including aztreonam but are inhibited by clavulanic acid, sulbactam, avibactam and tazobactam BLIs, as well as by derivatives of boronic acid (Sekyere, Govinden and Essack, 2016; Codjoe and Donkor, 2018). Among these, the KPCs are the most widespread, having spread across the globe and induced outbreaks in several North American, Asian, and European countries and in Africa within a few years of its detection. KPC producers are multidrug-resistant thereby reducing the therapeutic options for KPC-related infections (Kazmierczak et al., 2016; Codjoe and Donkor, 2018). Recently, at least 79 variants of KPC enzymes have been identified from various pathogens, 40 of which differ from the initial KPC-2 and KPC-3 enzymes by fewer than five amino acids (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#KPC). KPC was first described from an isolate of *K. pneumoniae* from a single patient in North Carolina, United States, in 1996. Although the next steps in propagation were not known, in the following decade, *K. pneumoniae* expressing KPC-2 becoming endemic in New York City (Bush and Bradford, 2020). KPC-producing pathogens have subsequently spread well, exceeding the northeast of the United States, so that

KPC enzymes have become reported globally (Castanheira et al., 2017). KPCs are not inhibited by clavulanate or tazobactam, but are significantly inhibited by avibactam, relebactam, and vaborbactam (Kazmierczak et al., 2016; Lomovskaya et al., 2017; Hackel et al., 2018; Carpenter et al., 2019).

GES enzymes were originally identified as ESBLs, but a single-point mutation extended the spectrum of these enzymes, adding carbapenem-hydrolysing activity to GES-2, GES-4, GES-11 and GES-14 (Nordmann and Poirel, 2014). NMC-A and IMI enzymes are chromosomally coded carbapenem-hydrolysing β-lactamases present in *E. cloacae*. Similarly, SME is a chromosomal enzyme originating primarily from *S. marcescens* shows the highest amino acid sequence similarity (70%) to NMC-A. SME enzymes develop resistance to carbapenems, but have minimal hydrolytic activity against extended-spectrum cephalosporins (Bush and Bradford, 2019).

Compared to a zinc metal in MBLs, this class harbours the serine amino acid at its active site, thus being called serine carbapenemases. Therefore boronic acid derivatives are inhibitors used to detect such enzymes in CPE (Sekyere, Govinden and Essack, 2015; Sekyere, Govinden and Essack, 2016).

1.2.5.1.2 Class B Metallo-β-lactamases (MBLs)

Class B metallo-β-lactamases comprise of NDM-1 (New-Delhi metallo-β-lactamase-1), VIM (Verona integron-encoded metallo-β-lactamase), IMP (Imipenem-resistant Pseudomonas type carbapenemases), SIM (Seoul imipenemase) and GIM (German imipenemase). MBLs hydrolyse most β-lactams, including carbapenems, but monobactams are not hydrolysed. Chelating agents such as ethylene diamine tetra acetic acid EDTA can inhibit them, but they are not inhibited by inhibitors in the latest FDA-approved BLI combinations. Plasmid-encoded MBLs pose a major clinical threat as they are embedded inside mobile genetic structures that can distribute and harbour genes of resistance to other classes of antimicrobials (Bush and Bradford, 2019). However, within acquired variants of MBLs, IMP, VIM, and NDM are the most common and have now been distributed throughout the world (Kazmierczak et al., 2016). At their active site, they incorporate one / two zinc atoms, hence the term MBLs. The addition of a chelating agent, such as EDTA to the enzyme solution, impedes the enzyme as the chelator absorbs all the zinc atoms, rendering it difficult for the enzyme to work (Sekyere, Govinden and Essack, 2016).

MBLs were described in various Gram-negative clinical isolates, including many species of *Enterobacterales*, *P. aeruginosa* and *Acinetobacter* spp. IMP enzymes were first to be identified as acquired MBLs. Enzymes of the type VIM have become widespread and are especially prevalent in the Mediterranean region (Bush and Bradford, 2019). Based on variations in their primary zinc coordination shell and their amino acid sequence, MBLs are divided into three subclasses: B1, B2, and B3. Sequence identity varies from 14 to 24% between subclass B1 and B2 enzymes, while sequence identity varies from 2% to 14 % between subclass B3 and subclasses B1 and B2 (Karsisiotis, Damblon and Roberts, 2014). These structural differences in established and emerging MBLs presents a challenge to the development of effective MBL inhibitors (Somboro et al., 2019). The NDM-1 gene may well have been acquired from the population by mid-2010 and transmitted by tourists traveling worldwide to other countries such as Europe and the United States, while NDM-1 was also identified in environmental samples of the same strains. Up to 89 and 73 varieties of carbapenemases of the type IMP and VIM have been identified recently (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#IMP;https://www.ncbi.nlm.nih.gov/pathogens/refgene/#VIM). The VIM and IMP families have certain overlap with regard to which plasmids they are borne on and that they are aligned with certain integrons (Codjoe and Donkor, 2018). The amino acid sequence similarity range of the VIM family is up to 10%, about 15% for IMP, and 70% among the two families. Both hydrolyse all β-lactams, but for monobactams (Marsik and Nambiar, 2011).

Less frequently acquired MBLs tend to be constrained to certain geographic areas or have only been identified in a few instances, including Sao-Paolo metallo-β-lactamase (SPM) and German imipenemase (GIM), found in Brazilian *P. aeruginosa* isolates (Sao Paulo) and Germany, respectively (Wendel et al., 2013).

1.2.5.1.3 Class D OXA-48-Type carbapenemases

Some other serine-based carbapenemases belong to class D, mostly the OXA-48-type. These are called oxacillinases because of their preferred oxacillin hydrolysis instead of penicillin. They are now considered a broad group of enzymes displaying variability in their substrate profiles and sequences. OXA carbapenemases are therefore distinguishable from other molecular classes where amino acids sequences of class D and class A or class C β-lactamases share less than 20% similarity (Poirel, Potron and Nordmann, 2012).This class of enzymes is not inhibited by EDTA

but is affected variably by the β-lactamase inhibitors tazobactam, sulbactam and clavulanic acid (Sekyere, Govinden and Essack, 2015). Within the Class D OXA family of β-lactamases, only a small number have functional roles as carbapenemases. OXA-23, OXA-40, and the frequently prevalent OXA-48, with their related versions, OXA-181, OXA-162, and OXA-232, are among these (Mairi et al., 2018). They usually have hydrolytic activity toward penicillins and carbapenems and slightly inhibited by β-lactamase inhibitors, with the exception of avibactam (Haidar et al., 2017; Lomovskaya et al., 2017).

Class D carbapenemases are reported to have high temocillin resistance that is used in their identification in enterobacterial hosts (Sekyere, Govinden and Essack, 2016; Codjoe and Donkor, 2018). The main concern regarding OXA carbapenemases is their capability to mutate and increase their activity spectrum quickly. Reports by Mathers et al. (2013) reported widespread identification of Class D in the *Enterobacterales* species rendering this class of carbapenemases a global threat and a significant public health concern (Mathers et al., 2013). OXA-48 was first observed amidst Turkish clinical isolates of *K. pneumoniae* and afterward spread to Europe and the Mediterranean region but were less commonly detected in the Americas (Mairi et al., 2018). However, several outbreaks have recently been reported in several countries in South America, including Colombia, Brazil, and Argentina (Villacís et al., 2020).

Similar enzymes such as OXA-162, OXA-163, OXA-181, and OXA-232 have been found in various species of *Enterobacterales* and have spread into Europe and the Mediterranean regions (Kazmierczak et al., 2018).

Because of their point mutant analogs with ESBLs, OXA-48-type producers are one of the most challenging carbapenemase producers to predict and their prevalence rates are difficult to estimate accurately. Moreover, 102 distinct OXA sequences were identified by 2011, 9 of which are extended-spectrum β-lactamases and a minimum of 37 of which were classified as carbapenemases (Nordmann, Naas and Poirel, 2011). More recently, 943 variants were detected in different pathogens (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#OXA).

1.2.5.2 Other Resistance Mechanisms

Multiple mechanisms of resistance can occur, including target alteration (mutation or expression of alternative PBPs), restriction of cellular uptake by downregulation of the porins needed for the

entry of carbapenems, over-expression of efflux pumps, and development of enzymes that change or degrade (Matlashewski et al., 2017). While carbapenem resistance is mostly mediated by β-lactamases in Gram-negative bacteria, for both Gram-negative and Gram-positive bacteria, other resistance mechanisms may also occur. The most evident mechanism is the production of low-affinity PBPs that require substantial inhibitory concentrations of carbapenem for bacterial killing (Bush and Bradford, 2020).

The access of carbapenems into periplasmic space and their subsequent interactions with PBPs in the cell membrane, may be restricted by manipulating porin expression or alterations in the porin-encoding gene, leading to either total loss or deficiencies in the corresponding porin. For instance, in *K. pneumoniae*, the altered expression of ompk35 and ompk36 induces a high degree of ertapenem resistance (Elshamy and Aboshanab, 2020). Mutations in OMPs in *Enterobacterales*, can involve changes in regulatory mechanisms affecting the expression of OMPs or result in non-functional or deleted OMPs (Bush and Bradford, 2020). Also, the expression of non-carbapenemase β-lactamases in conjunction with the loss of porins is an alternative mechanism of developing resistance to carbapenems. For instance, this mechanism was observed in clinical *E. coli* isolate harbouring β-lactamase plasmid-encoded AmpC (*bla*CMY-2) that increased meropenem minimum inhibitory concentrations. Two mutations were required, resulting first in the loss of expression of porin and then in increased expression of CMY-2 β-lactamase (van Boxtel et al., 2017). AmpC-type β-lactamases cannot degrade carbapenems or barely do but can bind to them covalently in the periplasm and prevent them from reaching their targets (Goessens et al., 2013). Overproduction of ESBLs and AmpCs paired with decreased outer-membrane permeability and/or overexpression of the efflux pump can also confer resistance to carbapenems (Meletis, 2016; Sekyere, Govinden and Essack, 2016).

Ertapenem is most significantly affected by porin manipulation among the carbapenems, while isolates can remain sensitive to other carbapenems. The decreased sensitivity to ertapenem primarily depends on AmpC/ESBL and level of porin mutations (Codjoe and Donkor, 2018).

Another mechanism which may lead to carbapenem resistance is the active expulsion of carbapenems after their entry into the periplasmic space. This is regulated by tripartite efflux systems consisting of a cytoplasmic membrane protein transporter, a periplasmic connective protein, and an outer membrane. The overexpression of efflux pumps that remove carbapenems,

mainly meropenem, result in resistance to carbapenems and confers a MDR phenotype since quinolones, β-lactams and aminoglycosides are also popular substrates for efflux pumps (Meletis, 2016). As affinity is dependent on physiochemical properties (e.g., electric charge, aromatic, or hydrophobic properties) rather than chemical structures, efflux pumps are generally capable of identifying various substrates. This explains the existence of pumps conferring for MDR, which can eject many structurally unrelated antibiotics (Elshamy and Aboshanab, 2020).

1.2.6 Epidemiology of carbapenemase-producing *Enterobacterales* (CPE) in South Africa

The burden of antibiotic resistance among *Enterobacterales* globally is substantial, continuously evolving and a significant challenge to public health (Tängdén and Giske, 2015). Nosocomial infections caused by these bacteria are associated with prolonged hospitalization, combined with exceedingly high mortality rates, particularly in carbapenemase-producing *K. pneumoniae* CPKP (Tzouvelekis et al., 2014).

The global distribution of CPE isolates includes African countries, with NDM-1 and OXA-48 being the most common carbapenemases, reported in Nigeria, Algeria, Angola, Gabon, Mali and South Africa (Rubin et al., 2014). In August 2011, the first NDM was identified in South Africa and the first KPC was identified in Africa in *E. cloacae* and *K. pneumoniae* in South Africa in private hospitals, in Pretoria and Johannesburg respectively. Ironically, neither this first NDM-1 nor the subsequent NDM-1 in *K. pneumoniae* was isolated from patient with a travel history within or around the Indian continent (as origin of NDM first detection), suggesting local evolution (Lowman et al., 2011; Brink et al., 2012). This indicates that the epidemiology of such enzymes in South Africa is more complicated than the reports of cases from several other countries. Thus, bacteria with NDM in South Africa may consist of local and imported cases of NDM-producing bacteria co-existing within the country.

In addition, Brink and colleagues (2012) reported carbapenemases such as OXA-48 and its' derivatives among hospitalized patients with infections associated with *Enterobacterales* in Johannesburg, Port Elizabeth and Cape Town after screening isolates from a private laboratory network in the country. Moreover, VIMs were detected in *K. pneumoniae* in Johannesburg and GES in Cape Town (*K. pneumoniae*), Witbank (*E. cloacae*), Bloemfontein (*K. oxytoca*) and Port Elizabeth (*Serratia marcescens*) (Brink et al., 2012). Several CPEs cases have been registered in the country since, with *K. pneumonia*, *Citrobacter* spp., *Enterobacter* spp. and *E. coli* implicated

as primary hosts. KPCs, NDM, OXA-types, IMP, GES and VIM were the major carbapenemases identified so far, while SMEs, GIM, SIM, NMCs and other carbapenemases were nominal with the vast majority reported from the regions of Gauteng and Western Cape (Sekyere, Govinden and Essack, 2016).

A comprehensive analysis to raise awareness of South Africa's precarious state of public health was conducted in 2016, aiming to promote government action to implement policies that decrease resistance to reserve antibiotics (carbapenems, colistin, and tigecycline). Assessment of carbapenem resistance-reporting publications from early January 2000 and 20 May 2016 revealed that there were an estimated 2315 cases/infections resistant to carbapenem. Most of these recorded cases (n=1220) were from Gauteng province, followed by a smaller number from KwaZulu-Natal province (n = 515). The most common carbapenem-resistant isolates were the family members of *Enterobacterales* such as *K. pneumoniae*, *E. cloacae*, and *S. marcescens*. NDM (n=860) and OXA-48 (n=584) were among the most reported carbapenemases intimating the potential endemicity of NDM and OXA-48 among *Enterobacterales* and other bacterial species in South Africa (Sekyere, 2016). Perovic and colleagues also published similar findings in 2016 from private sector laboratories in South Africa, where a high prevalence of *bla*NDM-1 was observed in Gauteng province compared to *bla*OXA-48 through an evaluation of 9029 Gram-negative ESKAPE (*E. faecium, E. faecalis, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, E. cloacae,* and *E. coli*) isolates (Perovic et al., 2018). However, carbapenem resistance determinants may change as the same province reported an exponential increase in *bla*OXA-48–like producing *K. pneumoniae* strain where 471 *K. pneumoniae* isolates were positive for OXA-48–like enzymes among 1,247 unique clinical isolates collected from a private laboratory network a year later (Lowe et al., 2019).

Adopting advanced sequencing technologies allowed Pedersen and colleagues to investigate the molecular epidemiology of CRE clinical samples (n=45) collected by a private laboratory in Durban from patients hospitalized in 10 separate private hospitals between 2012 and 2013. Focusing on the carbapenem-resistance encoding determinants and their genetic support, they used WGS analyses to demonstrate complex patterns of dissemination of carbapenemase-encoding genes (*bla*NDM-1, *bla*GES-5, *bla*OXA-232, and *bla*NDM-5) via MGEs (e.g., integrons and insertion sequences) embedded in five different plasmids that appeared to mediate their horizontal

transfer, together with clonal transmission, between various species of *Enterobacterales* (Pedersen et al., 2018).

Further research in the same province has also used WGS to study the genomic epidemiology of extensively drug-resistant *K. pneumoniae* isolates, focussing on determinants encoding carbapenem resistance, clonality, mobile genetic support, and epidemiological linkages. Between May 2016 and May 2017, rectal swabs were collected from patients admitted to the surgical, medical, and trauma ICU of a quaternary public hospital where a total of 263 patients were screened for CPE. In addition, *K. pneumoniae* clinical isolates involved in bloodstream infections were collected from the same wards during the same time. Five of the 263 rectal swabs were confirmed as carbapenem-resistant *K. pneumoniae* (CRKP), as were five blood culture samples collected from infected patients for comparison, and CPEs were collected from admitted patients. All isolates had the same capsular serotypes (KL149-wzc:928, wzi:110) and all except one (ST3136) belonged to the ST152 clone. Assessment of the genetic backbone of the carbapenemase as well as its flanking sequences placed *bla*NDM-1 to the multi-replicon plasmid that was identified in several other CRE isolates Comparative phylogenomic evaluation of the 10 CRKP isolates confirmed their similar epidemiological profiles (Ramsamy et al., 2020).

1.2.7 The role of whole genome sequencing (WGS)

The earliest application of WGS was the ability to assess resistance to antimicrobials from a single assay. WGS has the potential to generate data on any resistance gene or mutation present, and the data may then be evaluated to produce a genotypically inferred antibiotic resistance profile (antibiogram) or, possibly, to predict susceptibility (Ellington et al., 2017).

Over existing gold-standard techniques that describe CRE, WGS is considered a powerful approach with significantly improved resolution capacity that provides extensive insights into strain-relatedness, molecular epidemiology, carbapenemase-containing plasmid replicon groups, prediction of factors affecting carbapenem resistance, point mutation, and the existence of other resistance mechanisms (Lynch et al., 2016). Moreover, it is now possible to describe the genetic environment surrounding these genes including MGEs such as integrons, insertion sequences (IS), and the methylome, in addition to the virulence repertoire in one assay (Kopotsa, Mbelle and Sekyere, 2020).

WGS improves the ability to characterize and address CRE outbreaks, recognize, and anticipate epidemiological patterns, and develop new machine learning techniques to detect novel variants quickly (Taggar et al., 2020).

Thus, scientists and public health professionals can now identify closely the spread, outbreak, infection epidemiology, and resistant bacteria using genomics and different bioinformatics tools (Kleinheinz, Joensen and Larsen, 2014). Large-scale and comparative analyses using these tools on human, animal, and environmental samples offered unparalleled insights into the global dissemination of antibiotic resistance genes and disseminating multidrug-resistant bacteria (Pesesky et al., 2015), resistance transmission networks (Pehrsson et al., 2016), and how diverse environments and phylogeny impact the evolutionary mechanisms of antibiotics resistance globally (Gibson, Forsberg and Dantas, 2015). As new algorithms for monitoring the spread of carbapenem-resistant bacteria in diverse fields, namely health care facilities and agricultural products, are being established, they are becoming more efficient and can to be interpreted with the degree of accuracy necessary to direct the improvement of infection control procedures and food safety protocols to limit the spread of infection (Lynch et al., 2016; Taggar et al., 2020).

This study describes the molecular epidemiology of CPE infection and colonization in patients from ICU in a single public hospital in the KwaZulu-Natal area using WGS and bioinformatics analysis. Delineating the molecular signatures of CPEs will direct containment strategies to improve patient outcomes.

1.3 Aim and objectives of the study

1.3.1 Aim of the study

The aim of the study was to delineate the molecular epidemiology of CPEs colonizing patients in ICU in a public hospital in the uMgungundlovu district.

1.3.2 Objectives

The objectives were to:

1. Obtain rectal swabs from all consenting ICU patients on day 1, 3, 7 and weekly thereafter until death or discharge from the ICU.
2. Screen the rectal swabs for CPE using Chrom-ID selective media.

3. Speciate and determine the antibiotic susceptibility profile of CPE using Vitek II according to EUCAST guidelines.
4. Undertake ERIC-PCR to determine the clonality and relatedness of the CPE within the ICU.
5. Undertake whole genome sequencing on representative isolates, and using bioinformatics tools delineate:
    - Antibiotic resistance genes.
    - Virulence genes.
    - Mobile genetic elements such as plasmids, transposons and integrons putatively associated with resistance and virulence.
    - MLST profiles.
    - Phylogeny of CPEs circulating in South Africa and beyond.

1.4 Summary of methodology

1.4.1 Ethical considerations

This study has been ethically reviewed and approved by the UKZN Biomedical Research Ethics Committee (BREC) (Ref No BE709/18 and BREC/00001723/2020). Permission was sought to extend this study to include molecular biological investigations as described above.

Permission was granted by the KwaZulu-Natal Department of Health, the uMgungundlovu District Manager, and the hospital Chief Executive Officer and ICU Manager. Voluntary informed consent was obtained from patients themselves, from their next of kin, or proxy consent from ICU manager.

1.4.2 General methodology

This was a descriptive sub-study on isolates collected from a larger PhD study, conducted in the public healthcare system at a general ICU, of a regional hospital that admitted both medical and surgical adult patients, to ascertain incidence and transmission of CPEs among this category of patients from February 2020 and March 2020. This study's population were all consenting adult patients admitted over one month. The researcher obtained rectal swabs from patients on day 1, 3, 7 and weekly thereafter until transfer (to another ward, another healthcare institution), discharged from the hospital (to home) or death.

Firstly, rectal swabs were sent for the identification of CPE positive samples using chromogenic screening media at National Health Laboratory Service (NHLS). Antibiotic susceptibility testing was also conducted to evaluate the antibiogram of CPEs and confirm the minimum inhibitory

concentrations (MICs) using the VITEK II automated method, and the findings were interpreted according to EUCAST guidelines (https://eucast.org/clinical_breakpoints/).

Genomic DNA was extracted using GenElute bacterial genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions for further molecular tests.

Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) was used to determine the genetic fingerprints to assess the relatedness of CPEs in the ICU. Representative isolates showing a similar antibiogram and also belonging to the same ERIC profile from each patient, were sent for WGS applying an Illumina MiSeq platform at the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa. Assembled genomes were annotated using the Centre for Genomic Epidemiology (CGE) tools to reveal resistance genes, type plasmid replicons, and draw phylogenetic trees, using ResFinder, PlasmidFinder, and CSIPhylogeny respectively. The presence of transposases, integrons, and other genomic characteristics were detected by RAST SEED viewer. MobileElementFinder was used to infer insertion sequences (ISs). Multi-locus sequence type (MLST) was established using PubMLST server and serotypes were predicted using Kaptive website. Virulence genes and virulence factors were detected using BacWGSTdb and Pathogenwatch servers, respectively.

1.5 Dissertation Structure

This study aimed to investigate the molecular epidemiology of CPEs colonizing and/or infecting patients in a general ICU of a public sector regional hospital, in the uMgungundlovu district. The research is presented in three chapters as follows:

Chapter 1 provides the introduction, literature review, aims, and objectives as well as summary of the methodology used.

Chapter 2 presents the investigations and findings in manuscript format prepared for submission to the Journal of Hospital Infection.

Chapter 3 includes conclusions, limitations, and recommendations for future research.

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CHAPTER TWO

This dissertation is in a manuscript format as follows:

Madni, O., Amoako, D.G., Abia, A.L.K., Rout, J. and Essack, S.Y. Molecular Epidemiology of Carbapenem-Resistant *Klebsiella pneumoniae* Colonization in an Intensive Care Unit, uMgungundlovu District, South Africa. To be submitted to the Journal of Hospital Infection.

AUTHOR CONTRIBUTIONS

- **Osama Madni**, as the principal investigator, co-conceptualized the study, undertook the laboratory work analysed the data and drafted the manuscript.
- Dr. Daniel Gyamfi Amoako, as a co-supervisor assisted with laboratory protocols, facilitated data acquisition, data analysis, and undertook a critical revision of the manuscript.
- Dr. Akebe L. K. Abia, as a co-supervisor assisted with laboratory work, vetted the results, and undertook a critical revision of the manuscript.
- Ms. Joan Rout, as the co-supervisor, facilitated data collection, supervised literature review and undertook a critical revision of the manuscript.
- Sabiha Y. Essack, as principal supervisor, co-conceptualized the study, guided the literature review and ethical clearance application, facilitated data collection and analysis, and undertook critical revision of the manuscript.

MOLECULAR EPIDEMIOLOGY OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* COLONIZATION IN AN INTENSIVE CARE UNIT, UMGUNGUNDLOVU DISTRICT, SOUTH AFRICA

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Abstract

Background: Due to the high association with mortality and morbidity, carbapenem-resistant *Enterobacterales* (CRE) in general, and carbapenem-resistant *Klebsiella pneumoniae*, in particular, have been listed as high-priority pathogens by the World Health Organization (WHO) for the research and development of new antibiotics. Concomitant resistance to multiple antibiotics of different classes, impedes efficient clinical management of CRE infections. We characterized carbapenemase-producing *K. pneumoniae* (CPKP) isolates from sequential rectal screening of patients in a single intensive care unit (ICU) in a public hospital in the uMgungundlovu District of Kwazulu-Natal, South Africa, collected over one month. **Method:** Ninety-seven rectal swabs collected from all consenting adult patients (n=31) on day 1, 3, 7 and weekly thereafter were screened for carbapenemase-production using Chrome-ID selective media. Fourteen CPKP were subjected to speciation and antibiotic susceptibility testing using the VITEK 2® automated system and their clonality was ascertained by ERIC/PCR. A sub-sample of eight isolates from five patients underwent whole genome sequencing (WGS) on the Illumina MiSeq platform followed by bioinformatics analysis to delineate the resistome, virulome, mobilome, clonality and phylo-geography. **Results:** All isolates (100%) were resistant to ertapenem and meropenem and 71.4% (n=10) were resistant to imipenem. All isolates harbored the *bla*OXA-181 carbapenemase (100%, n=8) and also carried other β-lactamase genes such as OXA-1, CTX-M-15, TEM-1B and SHV-1. IncF, IncX3, and Col plasmid replicons groups and class I integrons (ln191 and ln27) were detected. All isolates belonged to the same sequence type ST307 and capsular serotypes (K102, O2v2) and several were associated with a single bed located in the ICU. All but one isolate carried the same plasmid multilocus sequence type [K7:A-:B-] and the same virulence repertoire was identified reflecting the epidemiological relationships between isolates. *Bla*OXA-181 were presumably located on a multi-replicon plasmid similar to that of *E. coli* p010_B-OXA181, and isolates were aligned with several South African and international clades, demonstrating horizontal and vertical transboundary distribution. **Conclusion:** OXA-181-producing *K. pneumoniae* belonging to ST307 was found to be potentially endemic in the hospital ICU environment of a public hospital in KwaZulu-Natal South Africa. The presence of a myriad of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in different permutations and combinations presents challenges to clinical management and infection prevention and control measures. This

necessitates a CRE screening programme and strict infection prevention and control measures to detect and eliminate this endemic clone.

Keywords

Carbapenem resistance; *Enterobacterales*; *Klebsiella pneumoniae;* whole genome sequencing; South Africa

Introduction

β-lactams are one of the most prescribed antibiotics globally (Klein et al., 2018), in part because they are flexible and can be structurally modified to result in several active forms, a malleability not possessed by other antibiotic classes (Bush and Bradford, 2016). Carbapenems, among the β-lactams, are effective against Gram-positive and Gram-negative bacteria, providing a broad spectrum of antibacterial activity. A particular molecular structure, along with the β-lactam ring, gives added stability to most β-lactamases including extended-spectrum β-lactamases (ESBLs), making carbapenems "last-resort antibiotics" or "last-line agents" for critically ill patients suspected of drug-resistant infections treatment (Ramsamy et al., 2020). The widespread use of carbapenems for the multidrug-resistant bacterial infections has contributed to the development of resistance, most frequently mediated by β-lactamases, called carbapenemases (Pitout, Nordmann and Poirel, 2015). Less common resistance mechanisms include altered target penicillin binding proteins (PBPs) or reduced cell entry due to upregulated efflux pumps or modifications in the outer membrane proteins (Bush and Bradford, 2020).

Carbapenemases belong to the Ambler class A (e.g., KPC, SME, IMI, and GES), class B (e.g., NDM, VIM, and IMP), and class D (OXA-48–like). The carbapenemases of class A and class D are serine β-lactamases, and the carbapenemases of class B are metallo-β-lactamases (MBLs) (Cui, Zhang and Du, 2019). Class B carbapenemases, particularly *bla*NDM, are more potent than other classes and cannot be inhibited by β-lactamase inhibitors (BLIs) such as clavulanic acid, tazobactam, or sulbactam (Somboro et al., 2018). OXA carbapenemases, and specifically *bla*OXA-48 has a more restricted spectrum of β-lactam hydrolysis with clinically significant penicillin and imipenem hydrolysis and lower meropenem hydrolysis (Bush and Bradford, 2020). OXA carbapenemases are poorly inhibited by BLIs except for avibactam (Lomovskaya et al., 2017).

Carbapenem-resistant *Enterobacterales* (CRE), *Pseudomonas aeruginosa and Acinetobacter baumannii* are listed by the World Health Organization (WHO) as high priority pathogens for the research and development of new antibiotics (World Health Organization, 2017). Serious CRE infections have a higher risk of fatality than those caused by carbapenem-sensitive isolates (Martin et al., 2018). This threat is considerably higher when the carbapenem resistance in *Enterobacterales* is due to carbapenemase production (CPEs). Compared with the non-CPE infections (n=37,45%), CPE infections (n=46, 55%) were reported to cause four times the risk of death within 14 days among hospitalized patients in an academic hospital in the USA between March 2013 and April 2016 (Tamma et al., 2017).

Carbapenemase resistance genes are associated with diverse mobile genetic elements (MGEs) including but not limited to plasmids, transposons, and integrons (Lynch et al., 2016). IncF plasmid replicons are the most common and are primarily reported to bear the *bla*KPC and *bla*NDM genes in the United States, Greece, Canada, Taiwan, and South Africa (Pedersen et al., 2018). The transposon Tn125, harbouring the insertion element ISAba125, is recognized as the primary vehicle for disseminating NDM-1 enzymes in *Acinetobacter baumannii* (Khan, Maryam and Zarrilli, 2017). The successful propagation of OXA-48 is linked with different Tn1999 variants on highly transferable IncL plasmids, strengthened by the global distribution of specific high-risk clones such as *K. pneumoniae* ST307 (Pitout et al., 2019).

Klebsiella pneumoniae is an invasive and virulent bacterium among *Enterobacterales* that harbours multiple antibiotic resistance genes (ARGs) (Partridge, 2011). *K. pneumoniae* easily assimilate ARGS by horizontal gene transfer (HGT) of MGEs (Mathers, Peirano and Pitout, 2015). The increase in multidrug-resistant (MDR) *K. pneumoniae* and especially carbapenem-resistant *K. pneumoniae* (CRKP), is of significant medical concern. A recent meta-analysis approximated mortality associated with *K. pneumoniae* health care associated infections (HAI) at 42% for CRKP compared with 21% for carbapenem-susceptible strains (Xu, Sun and Ma, 2017).

We used whole genome sequencing (WGS) and bioinformatics analysis to characterize CPE implicated in the colonization of patients in an intensive care unit (ICU) of a public hospital in the uMgungundlovu district, KwaZulu-Natal, South Africa. Isolates were a sub-sample of a larger study investigating the incidence and transmission of CPEs among ICU patients receiving carbapenem treatment in three hospitals.

Materials and methods

Ethical considerations

This study was approved by the UKZN Biomedical Research Ethics Committee (BREC) (Ref No BE709/18 and BREC/00001723/2020).

Permission was granted by the KwaZulu-Natal Department of Health, the uMgungundlovu District Manager, the hospital Chief Executive Officer and ICU Manager. Voluntary informed consent was obtained from patients themselves, from their next of kin, or proxy consent was obtained from the ICU manager.

Study population and settings

This was a descriptive sub-study on isolates collected from a larger PhD study, conducted in three hospitals in the public and private healthcare system in uMgungundlovu district that investigated the incidence and transmission of CPEs among ICU patients receiving carbapenem treatment. The sub-study focused on isolates obtained from ICU patients of one study hospital, from February 2020 and March 2020. This study's participants were all consenting adult patients admitted over one month into the general ICU (medical and surgical) of a regional public hospital.

Sample collection

Patients were screened for CPE using rectal swabs on day 1, 3, 7 and weekly thereafter until transfer (to another ward or healthcare institution), discharge or death.

Laboratory analysis

Screening for CPEs

Ninety-seven rectal swabs from 31 patients were screened for CPE using chromogenic screening media Chrom-ID CARBA SMART according to manufacturer instructions (BioMérieux, Marcy l'Étoile, France). Fourteen samples that tested positive for CPEs (14.4% of total samples) from five patients (16% of total patients enrolled) formed the final sample.

Antimicrobial susceptibility testing (AST)

Identification of the isolates to species levels and antibiotic susceptibility testing was conducted using the VITEK 2® automated system (BioMérieux-Vitek, Marcy-l'Étoile, France), and results

were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. The antibiotic panel consisted of 18 antibiotics: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), piperacillin-tazobactam (TZP), cefuroxime (CXM), cefuroxime-axetil (CXM-AX), cefoxitin (FOX), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), ertapenem (ERT), imipenem (IPM), meropenem (MEM), amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), tigecycline (TGC), nitrofurantoin (NIT), and trimethoprim-sulfamethoxazole (SXT).

Genomic characterization

DNA extraction of CPKP isolates

Genomic DNA was extracted using GenElute bacterial genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions for further molecular tests. IncoTherm digital incubator (Labotec, Cape Town, South Africa) and Heraeus Biofuge Pico centrifuge (DJB Labcare Ltd, Bucks, England) were used in this process. Extracted DNA was checked for suitable concentrations and purity using Nanodrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Genomic fingerprinting

Clonality was investigated using enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). The primers ERIC1 (52-ATGTAAGCTCCTGGGATTCAC-32) and ERIC2 (52-AAGTAAGTG ACTGGGTGAGCG-32) (Inqaba Biotech (Pty.) Ltd., Pretoria, South Africa), were used to perform ERIC-PCR reactions (Chukwu et al., 2019) on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) with a total volume of 25 µL consisting of 12.5 µL of Dream Taq Green PCR Master Mix (2X) (ThermoFisher Scientific, Waltham, MA USA), 0.1 µL (100 µM) of each primers ERIC1 and ERIC2, 8.3 µL of nuclease-free H_2O and 4 µL of total genomic DNA. The PCR conditions were initial denaturation at 94°C for 3 min, followed by 29 cycles denaturation at 94°C (30 s), annealing at 50°C (1 min), an extension at 65°C (8 min), and a final extension at 65°C (16 min). PCR amplicons were resolved horizontally by electrophoresis on 1.5% (w/v) on SeaKem® LE agarose gel at 90V for two hours (Lonza, Rockland, ME, USA) followed by staining in 0.1 mg/ml of ethidium bromide for 15 minutes. The Quick-load®1-kb DNA ladder (New England Biolabs, Ipswich, Massachusetts, USA), a positive control (*K. pneumoniae* ATCC BAA-

1705) and a "no template control (NTC)" were included in these reactions. The gel was visualized, and the images captured under ultraviolet light using the Gel Doc™ XR+ system (Bio-Rad, Hercules, CA, USA). Data was exported to Bionumerics software (version 7.6, Applied Maths, TX, USA) for cluster analysis. Strains were allocated to various clusters by evaluating the similarity coefficient from the homology matrix using the Jaccard approach. Dendrograms were assembled using the Unweighted Pair-Group Method (UPGMA). A 1% optimization and band tolerance (version 7.6, Applied Maths, TX, USA) were set, and 80% cut-off similarity was used to identify clusters.

Whole-genome sequencing of CPKP isolates

A sub-sample of ten isolates, one per patient, was chosen on the basis of similar antibiograms and ERIC profiles and subjected to whole genome sequencing on the Illumina MiSeq platform. Using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA, USA), multiplexed paired-end libraries (2300 bp) were prepared, and sequences were measured on 100-coverage. Utilizing CLC Genomics Workbench version 10, the resulting raw reads were screened for quality, trimmed, and de novo assembled into contigs (CLC, Bio-QIAGEN, Aarhus, Denmark) (Bankevich et al., 2012). Two isolates were removed for poor DNA quality.

Genomic analyses and annotation

The National Centre for Biotechnology Information (NCBI) prokaryotic genome annotation pipeline (PGAP) and RAST 2.0 server (Tatusova et al., 2016) were used to annotate assembled reads. All contiguous sequences were subsequently submitted to GenBank and assigned accession numbers under the Bioproject (PRJNA674742). ARGs were detected using ResFinder (Zankari et al., 2012) and plasmids were delineated using PlasmidFinder (Carattoli et al., 2014), respectively. The RAST SEED viewer (Overbeek et al., 2014) was employed to detect the genomic characteristics of the contigs, and the presence of transposases flanking the β-lactamase genes (or carbapenemases). The INTEGRALL database was used to assess integrons present (http://integrall.bio.ua.pt/). Insertion sequences (IS) residing in genomes were inferred by uploading contigs to the MobileElementFinder (Johansson et al., 2021). The BacWGSTdb and Pathogenwatch servers were employed to infer Virulence genes and factors (http://bacdb.cn/BacWGSTdb/, https://pathogen.watch/). Kaptive-web, the reference online site tool for Klebsiella WGS data, was used to predict isolate serotypes (K types, wzc and wzi allelic

types, and O types) (Wyres and Holt, 2016). Multi-locus sequence type (MLST) analyses were performed to determine sequence types (STs) of our *K. pneumoniae* isolates on the PubMLST database (https://pubmlst.org/).

Detection of *Bla*OXA-181 plasmid-encoding

The carbapenemase genes and their flanking sequences retrieved from the RAST SEED viewer were searched on the NCBI microbial nucleotide basic local alignment search tool (BLAST). Fully sequenced plasmids with the closest synteny obtained from the BLAST search were used as a reference input to GView Server (https://server.gview.ca/) to visualize the presumed presence/absence of specific plasmid DNA.

Phylogenetics

Phylogenomic analysis was undertaken to determine how our isolates compare to *K. pneumoniae* genomes from South Africa. All *K. pneumoniae* genomes reported in South Africa (n=89) were downloaded from the PATRIC website (https://www.patricbrc.org/), annotated (Table S1) and included in the analysis. The phylogenetic tree was constructed based on the maximum likelihood method using the CSIPhylogeny (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) (Kaas et al., 2014) which performs single nucleotide polymorphism (SNP) calling, filters the SNPs, and infers phylogeny based on the concatenated alignment of the high-quality SNPs, using the assembled contigs. The analysis was performed on the platform using default parameters as follows: minimum depth at SNP positions, 10X; minimum relative depth at SNP positions of 10%; minimum distance between SNPs (prune) at 10bp; minimum SNP quality, 30; minimum read mapping quality of 25 and minimum Z-score of 1.96. The *Escherichia coli* ATCC 25922 was used as the outgroup strain (reference genome), facilitating the configuration of the phylogenetic distance between the isolates on the branches. The Figtree software (http://tree.bio.ed.ac.uk/software/figtree/) was used visualize edit and annotate the generated phylogenetic tree.

Nucleotide sequence accession numbers

The nucleotide sequences of the eight isolates analysed in this study were deposited in NCBI GenBank database in the Bioproject number (PRJNA674742): under the Accession numbers; JADKPL000000000.1, JADOEH000000000.1, JADOEG000000000.1, JADKPK000000000.1,

JADKPJ000000000.1, JADKPI000000000.1, JADKPH000000000.1, and JADKPG000000000.1, respectively.

Results

Population demographics

Of the five consenting adult patients that screened positive for CPE, three were males, while two patients were females, with age ranging from 26 to 64 years old and a mean age of 45. Although they were admitted to the ICU with different diagnoses, almost all had the same invasive devices. The relevant patient's data appears in Supplementary Table S2.

Antimicrobial resistance profiles

Isolates showed a similar resistance patterns to the panel of antibiotics. All isolates (100%) were resistant to 14 antibiotics, viz., ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefuroxime-axetil, cefoxitin, cefotaxime, ceftazidime, cefepime, ertapenem, meropenem, ciprofloxacin, nitrofurantoin, and trimethoprim-sulfamethoxazole. However, they were fully susceptible to tigecycline and amikacin. Twelve (85,7%) of isolates were susceptible to gentamycin and 28.6% (n=4) were susceptible to imipenem. All isolates were MDR and the resistance phenotype was confirmed by the ARGS detected by WGS (Figure 2).

Genomic fingerprint

The ERIC dendrogram showed five main clusters. Isolates belonging to the same ERIC cluster generally had similar antibiograms, except for ERIC cluster 2 where isolates exhibited two antibiograms. Patients generating more than one CPE sample carried isolates belonging to 2-4 different ERIC clusters (e.g., isolates 13, 22 and 30) (Figure 1, Table 1).

Genomes characteristics

The draft genomes sizes of the eight isolates subjected to WGS and bioinformatics analysis were similar and ranged from 5.58 Mb to 5.72 Mb. The (G+C) content was the similar for all isolates (57.1) except 2 that differed slightly in terms of median contig lengths (N50, L50), contigs, RNAs present, and number of coding sequences (Table S3).

In silico ARGs

The genomic data (resistomes) confirmed the resistance phenotypes in most isolates (Table 1). Anomalies were detected for aminoglycosides where the presence of the aadA1 (n=2), aadA2 (n=1), aac(6')-Ib-cr (n=8), aph(3")-Ib (n=5), aph(3')-Ia (n=2), aph(3')-Via (n=2), armA (n=2), aph(6)-Id (n=5), aac(3)-Ia (n=1) aminoglycoside ARGs did not translate into phenotypic aminoglycoside resistance on AST. A single carbapenemase gene (*bla*OXA-181) was common to all isolates. Five other β-lactamase were detected [*bla*CTX-M-15, *bla*OXA-1, *bla*SHV-106] (n=8), *bla*TEM-1B (n=7), and *bla*TEM-1C (n=1). Carbapenemases from classes A, B, and C were not detected. Other ARGs detected conferred resistance to tetracyclines [tet(A)] (n=7), fluoroquinolones [aac(6')-lb-cr, oqxA, oqxB, qnrB1, qnrS1] (n=8), trimethoprim [dfrA12 (n=1), dfrA14 (n=7)], sulphonamides [sul1 (n=1), sul2 (n=5)], fosfomycin (fosA) (n=8), macrolides [msr(E) (n=2), mph(E) (n=2)], phenicols [catB3 (n=8), cmlA1 (n=2)], rifamycin (ARR-2) (n=1) and streptogramin b [msr(E) (n=2)].

Sequence types analysis (MLST) and in silico mobile genetic elements (Mobilome)

All isolates belonged to ST307 (Table 1). Plasmid analysis showed that each isolate harboured at least two replicon types simultaneously (Table 2). All isolates carried ColKP3 and 6, 7 and 6 isolates carried the IncX3, IncFIB (K) and IncFII (K) replicons respectively. One isolate carried the IncFIB (pNDM-Mar) from the same group. The search for carbapenemase (OXA-181) and its flanking sequences using NCBI microbial nucleotide BLAST showed that it was located on a multi replicon plasmid *E. coli* p010-B-OXA181 (accession no.: CP048332.1). The presence of genome assemblies with similar genetic synteny and 99.98% coverage and identity to the *E. coli* p010 B-OXA181 reference was confirmed by comparative analyses through the GView server (Figure 3) intimating the location of OXA-181 on a similar plasmid in our CPKP isolates.

We tried to determine the location of ARGs & MGEs in synteny in our isolates on plasmids and chromosomes on the NCBI databases, which led us to assume their location on the plasmid replicons we identified except for one β-lactamase gene, blaSHV-106, that was associated with a chromosomal location (Table 3). It is noteworthy that the ISKpn19 insertion sequence containing the OXA-181 concurrently harboured the EreA and QnrS1 genes in seven of the eight isolates together with recombinase and transposases. One isolate carried the same insertion sequence but was located on transposon (Tn3-like IS3000 transposase), found in the same plasmid and with the same companions (Table 3, Figure 4).

We further identified *bla*SHV-106 on all isolates in parallel with other β-lactamase genes viz., *bla*OXA-1, *bla*CTX-M-15, *bla*TEM-1B, and *bla*TEM-1C in various permutations and combinations, the synteny of which was closely related to plasmid sequences already described in Genbank and summarised in Table 3. Across the isolates, *bla*CTX-M-15 (bracketed by Tn3 transposon, IS6100 and ISEc9), most of *bla*TEM-1B genes (bracketed by IS91, and a recombinase), and most of aph(6)-Id, aph(3")-Ib, sul2 genes (bracketed by IS91, IS5070 and IS5) were found in close synteny on the same plasmids, except for one isolate (EC03612985) that encoded both *bla*CTX-M-1 and *bla*TEM-1C on one insertion sequence (ISEc9) showing a slightly different genetic environment (Table 3).

All isolates had trimethoprim and aminoglycoside resistance genes located on a class 1 integron (In191) in six isolates with five isolates also containing the dfra14 genes on gene cassettes. Another class 1 integron (In27), was detected in only one isolate and contained the dfrA12-gcuF-aadA2 cassette arrays. Only one isolate did not have any integrons with aminoglycosides or trimethoprim resistance genes (Table 2).

Virulome

All isolates carried the identical repertoire of 65 virulence genes. The virulome was not clone specific in that isolates clustered in different clades had the same virulence genes.

The K- loci and O- loci results (KL102, 939, 173, and O2v2) were identical and showed no clonal specificity in our isolates.

Phylogenetic analysis

Although all eight isolates belonged to the same sequence type, ST307, small differences were evident from their phylogenetic tree, (Figure 5). When compared to genomes of *K. pneumoniae* isolates from studies conducted in Durban, Pretoria, Pietermaritzburg, Ozwatini, and Cape Town (Table S1), our isolates showed greatest similarity to the clade of ST307 from Pretoria and Cape Town (clade VIII) with one ST25 clustered close to the ST307 clade (Figure 5).

Discussion

The global spread of CPE presents a significant challenge to public health and clinical practice as these bacteria are resistant to last-resort antibiotics (carbapenems) and cause high fatality rates

(Sheu et al., 2019). WGS data for eight CPKP strains isolated between February 2020 and March 2020 from patients at a general ICU of a regional hospital in uMgungundlovu District in Durban, South Africa, was analysed to delineate the genetic context of carbapenem resistome and associated mobilome together with the broader resistome, virulome, clonality and phylogeny.

K. pneumoniae is an established and significant pathogen in nosocomial infections. MDR and extremely drug-resistant (XDR) *K. pneumoniae* is closely linked to ARGs acquired via plasmids and other MGEs, resulting in a 'super resistome' (Navon-Venezia, Kondratyeva and Carattoli, 2017).

CRKP has been implicated in outbreaks in South Africa and appears to be endemic to certain hospitals and regions. In May 2012, a tertiary academic hospital in Cape Town reported one of the first laboratory-confirmed outbreaks of *K. pneumoniae* expressing OXA-181 in South Africa linked to two patients admitted to the haematology ICU. Laboratory records before that date and the results of 340 rectal swabs or stool specimens collected from patients and staff members revealed an “outbreak” involving eight patients. Seven of them were admitted to the haematology unit during their hospitalization period. The remaining patient was epidemiologically linked to another patient because he was admitted to a bed adjacent to him (Jacobson et al., 2015).

The identification of ARGs from WGS data correlated with the phenotypic resistance observed in most instances in our study, with resistance ranging from 14% to 100%. However, some anomalies were observed, in particular, for aminoglycosides, where the presence of aminoglycoside ARGs on WGS were not phenotypically evident, intimating transcriptionally silent or unexpressed ARGs. All isolates belonged to the same ST type and the same phylogenetic clade suggesting an endemic strain in the ICU. Additionally, all patients occupying a particular bed (bed 5) in the ICU belonged to the same ERIC cluster indicating the hospital environment as a potential source of colonization (Figure 1, Table S2).

Our findings showed that the CPKP hospital population was not diverse and belonged to a single sequence type (ST307) (Table 1), clade and capsular serotype and contained identical repertoires of virulence genes). The phylogeography analysis with South African *K. pneumonia* isolates showed our isolates clustering in clade VIII similar to isolates from Pretoria and Cape Town (Figure 5). The Pretoria study characterized the resistome, mobilome, methylome, virulome, and

phylogeography of 56 CRKP with reduced susceptibility to carbapenems from six public hospitals and centers in the Tshwane District in 2018. The *K. pneumoniae* isolates belonged to ST307 and had similar resistance determinants, particularly, OXA-181 along with OXA-1, TEM-1B, CTX-M-15, and blaSHV-28 genes. Moreover, similar plasmid (Inc) types [ColKP3,IncX3,IncFIB (k),IncFII (k)] and serotypes (KL102, O2v2) were evident (Kopotsa, Mbelle and Sekyere, 2020), suggesting that the same strain is circulating in the Kwazulu-Natal area.

Klebsiella pneumoniae ST307 is a relative newcomer to successful high-risk antibiotic resistant strains contributing to a significant global public health burden. It possibly originated in Europe during the early to mid-1990s but was first published in the MLST database from the Netherland in 2008 (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The earliest published reports came from the USA (Castanheira et al., 2013) and Pakistan (Habeeb et al., 2013) in 2013 and were followed by reports from different countries including from Russia (Lazareva et al., 2016), Spain (Ruiz-Garbajosa et al., 2016), Brazil (Dropa et al., 2016), and Japan (Harada et al., 2016) during 2016. The global distribution of *K. pneumoniae* ST307 has been recorded from all continents, except Antarctica and has been implicated in many nosocomial (Baek et al., 2020) and long-term care centre outbreaks. Findings from Colombia (Ocampo et al., 2016), Texas, the United States (Long et al., 2017), Argentina (Cejas et al., 2019), and Italy (Bonura et al., 2015) have also shown that the incidence of ST307 among CPKP has been rising over the years, even replacing other high-risk antibiotic resistant strains , including ST258 in regions such as Italy and Colombia (Bonura et al., 2015; Ocampo et al., 2016).

A recent study examined major nationwide nosocomial outbreaks of *bla*OXA-181 and *bla*CTX-M-15 within several South African provinces. *bla*OXA-181 was reported in 471 *K. pneumoniae* isolates belonging to ST307, isolated from 1,247 unique clinical isolates from a private laboratory network repository over the period 2014–2016. Bayesian evolution analysis showed that ST307 isolates have evolved into six clades with clades I - IV restricted to Texas in the United States and clade VI confined to South Africa. Clade V is, in contrast, globally distributed. Clade VI in South Africa originated in 2013, and developed into two distinct lineages during 2014 spreading over a span of 15 months, across 23 cities/towns in six provinces of South Africa (Lowe et al., 2019).

Several carbapenemases have been identified in *K. pneumoniae* in South Africa. Lowe and colleagues (2019) reported an exponential increase in *bla*OXA-48 like producing *K. pneumoniae*

in contrast to two studies in 2016 that detected *bla*NDM-1 in higher amounts than *bla*OXA-48. In 2016, Perovic and colleagues reported data from private sector laboratories in South Africa, where a high prevalence of *bla*NDM-1 was observed in Gauteng province compared to *bla*OXA-48 through an evaluation of 9029 Gram-negative ESKAPE (*E. faecium*, *E. faecalis*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E. cloacae* and *E. coli*) isolates (Perovic et al., 2018). A review in the same year (2016), assessing carbapenem resistance-reporting publications from January 2000 and May 2016, showed that most common carbapenem-resistant isolates were isolated in *Enterobacterales* such as *K. pneumoniae*, *E. cloacae*, and S. *marcescens* with NDM (n=860) and OXA-48 (n=584) among the most reported carbapenemases (Sekyere, 2016). A recent report described another increment in the *bla*OXA-48-like producing *K. pneumoniae* strains, where *bla*OXA-48 (65%), followed by *bla*NDM-1(29%), was the most common carbapenemases detected (Kopotsa, Mbelle and Sekyere, 2020), in contrast to our study where OXA-181 was dominant. *bla*NDM-1 to *bla*OXA-48 that has also been identified in the provinces of Gauteng and Eastern Cape (Perovic et al., 2018).

Our isolates carried carbapenemase *bla*OXA-181, other β-lactamases, plasmid-mediated quinolone resistance (PMQR) genes and other ARGs conferring resistance to several antibiotic classes (Table 3, Figure 4), suggesting co-selection of resistance genes, and more importantly, HGT (Asante and Sekyere, 2019) via plasmids and other MGEs (Partridge et al., 2018; Kopotsa, Sekyere and Mbelle, 2019).

*bla*CTX-M-15 was located on ISEc9 in all isolates, and most of *bla*TEM-1B on IS91 (Table 3). This observation that was detected globally, demonstrates the clonal and plasmid-mediated distribution of these ARGs within the same genetic context and on the same plasmid replicons in the same and different species. For example, IncF plasmids and ISEc9 have been shown to promote the global spread of *bla*CTX-M-15, alongside *bla*OXA-10, aac(6′)Ib-cr, and *bla*TEM, across different species (Kopotsa, Sekyere and Mbelle, 2019). The presence of these genes in similar genetic contexts were reported in another South African study describing 20 consecutive MDR *E. coli* isolates collected from a referral laboratory serving two secondary and three tertiary academic hospitals in Gauteng province. The study reported ARGs mediating resistance to fluoroquinolones, aminoglycosides, and tetracyclines in *E. coli* isolates alongside β-lactam resistance mediated by OXA, CTX-M, and TEM-1B. Many of these resistance determinants were located on contigs

containing multiple plasmid replicons and bracketed by composite transposons (Tn3), various ISs, and class 1 integrons. IncF was the most common plasmid replicon, class 1 integrons were the only integron type identified and *bla*CTX-M genes was frequently detected in ISEc9 (Mbelle et al., 2019), similar to our findings.

The diverse mobilome consisting of different plasmid replicons [ColKP3, IncFIB(K), IncFII(K), IncFIB(pNDM-Mar), and IncX3] (Table 2) and insertion sequences (Table 3) intimates the potential mobility of this *bla*OXA-181 by HGT among isolates. Furthermore, the global dominance of the IncF plasmid, an HGT-associated MGE, is verified in previous studies. For instance, Pedersen and colleagues confirmed the role of MGEs in the potential distribution of carbapenemases. They investigated the molecular epidemiology of CRE clinical samples (n=45) collected by a private laboratory in Durban from hospitalized patients in 10 separate private hospitals between 2012 and 2013. Focusing on the carbapenem-resistance encoding determinants and their genetic support, they demonstrated patterns of dissemination of CPE gens (*bla*NDM-1, *bla*GES-5, blaOXA-232, and *bla*NDM-5) via MGEs (e.g., integrons and insertion sequences) embedded in five different plasmids have been revealed to mediate their horizontal transfer, together with clonal transmission, between various *Enterobacterales* species (Pedersen et al., 2018).

Conclusion

OXA-181-producing *K. pneumoniae* belonging to ST307 was found to be potentially endemic in the hospital ICU environment of a public hospital in KwaZulu-Natal South Africa. The presence of a myriad of ARGs and/or MGEs in different permutations and combinations presents challenges to clinical management and infection prevention and control measures. This necessitates a CRE screening programme and strict infection prevention and control measures to detect and eliminate this endemic clone.

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LIST OF TABLES

FIGURE LEGENDS

files. Resistance genes and their genetic context, i.e., transposons, insertion sequences, integrons, resolvases, and recombinases/integrases, are shown with their orientation (arrow direction), synteny, and immediate surroundings in yellow-colored arrows on the plasmid and with yellow labels. To make the image less cluttered, other genes with unknown functions are masked. There is a circular variant of the plasmid shown in (A). The liner version of the large section of the plasmid that focuses on resistance genes is shown (B). ISKpn19 insertion sequence carried *bla*OXA-181 along with recombinase and transposase in our isolates and concurrently harbored the EreA and QnrS1 genes.

Figure 5. Phylogeography and resistance profiles of *K. pneumoniae*. The phylogeographic relationship between our eight *K. pneumoniae* isolates with other South African *K. pneumoniae* isolates is shown in.

SUPPLEMENTAL MATERIAL:

Table S1: Comparison of *K. pneumoniae* genomes with South African genomes, downloaded from NCBI and PATRIC servers.

Table S2: Patients demographics.

Table S3: General genomic features of eight sequenced *K. pneumoniae* isolates.

Table 1. Patient demographics, antibiograms, sequence types, carbapenemase, other β-lactamases and ARGs in *K. pneumoniae isolates*.

Isolate	Demographic Information				Antibiogram	ERIC Type	MLST	Resistome		
	Patient	Gender	Date	Study Day				Carbapenemase	Other β-lactamases	Other ARGs
EC03607709	P2	Male	19.2.20	Week 2	A	2	ST307	*bla*OXA-181	*bla*CTX-M-15, *bla*OXA-1, *bla*SHV-106, *bla*TEM-1B	aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,qnrS1,qnrB1,oqxB,oqxA,dfrA14,sul2,fosA,catB3,tet(A)
EC03605938	P13	Male	18.2.20	48 hrs	B	2	ST307	*bla*OXA-181		aac(6')-Ib-cr, aph(3')-VIa,armA,qnrS1,oqxB,oqxA,dfrA14,fosA, msr(E),mph(E),catB3,tet(A),cmlA1,ARR-2
EC03607707	P13	Male	19.2.20	72 hrs	A	1	ST307	*bla*OXA-181		aac(6')-Ib-cr, aph(3')-VIa,armA,qnrS1,oqxB,oqxA,dfrA14,fosA, msr(E),mph(E),catB3,tet(A),cmlA1
EC03612985	P22	Female	24.2.20	Discharge	C	5	ST307	*bla*OXA-181	*bla*CTX-M-15, *bla*OXA-1, *bla*SHV-106, *bla*TEM-1C	aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,qnrS1,qnrB1,oqxB,oqxA,dfrA14,sul2,fosA, catB3,tet(A)
EC03623002	P30	Male	3.3.20	48 hrs	A	1	ST307	*bla*OXA-181	*bla*CTX-M-15, *bla*OXA-1, *bla*SHV-106, *bla*TEM-1B	aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,qnrS1,qnrB1,oqxB,oqxA,dfrA14,sul2,fosA, catB3,tet(A)
EC03629993	P30	Male	8.3.20	Mero D4	A	2	ST307	*bla*OXA-181		aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,qnrS1,qnrB1,oqxB,oqxA,dfrA14,sul2,fosA, catB3,tet(A)
EC03632007	P30	Male	10.3.20	Mero D6	A	4b	ST307	*bla*OXA-181		aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,qnrS1,qnrB1,oqxB,oqxA,dfrA14,sul2,fosA, catB3,tet(A)
EC03638959	P30	Male	14.3.20	Week 2	A	4a	ST307	*bla*OXA-181		aac(6')-Ib-cr,aph(3'')-Ib,aph(6)-Id,qnrS1,qnrB1,oqxB,oqxA,dfrA14,sul2,fosA, catB3,tet(A)

Abbreviations: Mero D6; Delivering meropenem on day 6, Mero D4; Delivering meropenem on day 4

A; AMP-AMC-TZP-CXM-CXM-AX-FOX-CTX-CAZ-FEP-ERTA-IPM-MEM-CIP-NIT-SXT.

B; AMP-AMC-TZP-CXM-CXM-AX-FOX-CTX-CAZ-FEP-ERTA-MEM-CIP-NIT-SXT.

C; AMP-AMC-TZP-CXM-CXM-AX-FOX-CTX-CAZ-FEP-ERTA-MEM-GEN-CIP-NIT-SXT.

Table 2. Plasmid replicon types, class 1 integrons, and gene cassettes found in *K. pneumoniae* isolates.

Isolate	Plasmids replicons	pMLST	Integron class	Integron	Cassette arrays					
					GC1	GC2	GC3	GC4	GC5	GC6
EC03607709	ColKP3,IncFIB(K),IncFII(K),IncX3	[K7:A-:B-]	Integron integrase IntI1	In191	dfrA14	—	—	—	—	—
EC03638959	ColKP3,IncFII(K)	[K7:A-:B-]	Integron integrase IntI1	In191	dfrA14	—	—	—	—	—
EC03632007	ColKP3,IncFIB(K),IncFII(K)	[K7:A-:B-]	ND	ND	dfrA14	—	—	—	—	—
EC03629993	ColKP3,IncFIB(K),IncFII(K),IncX3	[K7:A-:B-]	Integron integrase IntI1	In191	dfrA14	—	—	—	—	—
EC03607707	ColKP3,IncFIB(K),IncFII(K),IncX3	[K7:A-:B-]	Integron integrase IntI1	In191	dfrA14	—	—	—	—	—
EC03623002	ColKP3,IncFIB(K),IncFII(K),IncX3	[K7:A-:B-]	Integron integrase IntI1	In191	dfrA14	—	—	—	—	—
EC03605938	ColKP3,IncFIB(K),IncFII(K),IncX3	[K7:A-:B-]	Integron integrase IntI1	In191	dfrA14	—	—	—	—	—
EC03612985	ColKP3,IncFIB(pNDM-Mar),IncX3	[F-:A-:B-]	Integron integrase IntI1	In27	dfrA12	gcuF	aadA2	—	—	—

Abbreviations: ND; Not detected.

Table 3. ARGs and MGEs in synteny and their plasmidic/chromosomal locations.

Isolate	Contig	Synteny of resistance genes and MGEs	Plasmid/chromosomal sequence with closest nucleotide homology (accession number)
EC03607709	49	blaOXA-181:EreA::ISKra4 (ISKpn19) recombinase::::recombinase:QnrS1:transposase	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	34	IS5:IS5075 (IS110)::Sul2:aph(3")-Ib:aph(6)-Id:IS91:TEM-1B:recombinase:: IS1380 (ISEc9):blaCTX-M-15::Tn3 family:IS6 (IS6100)	p72_FIBkpn (100%), accession (CP034282.1)
	54	CatB3:blaOXA-1:aac(6')-Ib-cr5	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	15	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	50	IS6 (IS6100)::dfrA14:IntI1	
EC03638959	95	transposase:QnrS1 recombinase::::recombinase:ISKra4 (ISKpn19)::EreA:blaOXA-181	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	98	blaTEM-1B:recombinase::IS1380 (ISEc9):blaCTX-M-15::Tn3 family:IS6 (IS6100)	p72_FIBkpn (100%), accession (CP034282.1)
	109	aac(6')-Ib-cr5:blaOXA-1:CatB3	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	24	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	102	dfrA14::IS6 (IS6100)	
EC03632007	93	blaOXA-181:EreA::ISKra4 (ISKpn19) recombinase::::recombinase:QnrS1:transposase	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	96	Tn3 family::blaCTX-M-15:IS1380 (ISEc9)::recombinase:TEM-1B	p72_FIBkpn (100%), accession (CP034282.1)
	112	CatB3:blaOXA-1:aac(6')-Ib-cr5	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	23	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	101	dfrA14::IS6 (IS6100)	
EC03629993	47	blaOXA-181:EreA::ISKra4 (ISKpn19) recombinase::::recombinase:QnrS1:transposase	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	35	IS5:IS5075 (IS110)::Sul2:aph(3")-Ib:aph(6)-Id:IS91:TEM-1B:recombinase:: IS1380 (ISEc9):blaCTX-M-15::Tn3 family:IS6 (IS6100)	p72_FIBkpn (100%), accession (CP034282.1)
	55	aac(6')-Ib-cr5:blaOXA-1:CatB3	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	16	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	49	IntI1:dfrA14::IS6 (IS6100)	
Continued			

EC03607707	50	blaOXA-181:EreA::ISKra4 (ISKpn19):recombinase::::recombinase:QnrS1:transposase	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	36	IS6 (IS6100):Tn3 family::blaCTX-M-15:IS1380 (ISEc9):: recombinase:TEM-1B:IS91:aph(6)-Id:aph(3")-Ib:Sul2::IS5075 (IS110):IS5	p72_FIBkpn (100%), accession (CP034282.1)
	56	CatB3:blaOXA-1:aac(6')-Ib-cr5	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	13	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	52	IS6(IS6100)::dfrA14:IntI1	
EC03623002	45	blaOXA-181:EreA::ISKra4 (ISKpn19):recombinase::::recombinase:QnrS1:transposase	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	32	IS5:IS5075 (IS110)::Sul2:aph(3")-Ib:aph(6)-Id:IS91:TEM-1B:recombinase::IS1380 (ISEc9):blaCTX-M-15::Tn3 family:IS6 (IS6100)	p72_FIBkpn (100%), accession (CP034282.1)
	54	aac(6')-Ib-cr5:blaOXA-1:CatB3	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	16	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	47	IntI1:dfrA14::IS6 (IS6100)	
EC03605938	48	transposase:QnrS1 recombinase::::recombinase:ISKra4 (ISKpn19)::EreA:blaOXA-181	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	34	IS6 (IS6100):Tn3 family::blaCTX-M-15:IS1380 (ISEc9): recombinase:TEM-1B:IS91:aph(6)-Id:aph(3")-Ib:Sul2::IS5075 (IS110):IS5	p72_FIBkpn (100%), accession (CP034282.1)
	53	aac(6')-Ib-cr5:blaOXA-1:CatB3	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	14	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	50	IntI1:dfrA14::IS6 (IS6100)	
EC03612985	48	Tn3-like IS3000:blaOXA-181:EreA::ISKra4 (ISKpn19):recombinase::::recombinase:QnrS1:transposase	*E. coli* p010_B-OXA181 (99.98%), accession (CP048332.1)
	54	::blaCTX-M-15:IS1380 (ISEc9)::recombinase:blaTEM-1C::	*E. coli* str. 473 pRCS52 (99.9%), accession (LO017736.1)
	61	aac(6')-Ib-cr5:blaOXA-1:CatB3	*E. coli* pYJ3-a DNA (100%), accession (AP023228.1)
	2	::::blaSHV-106::::	*K.p* F16KP0053 chromosome (100%), accession (CP052727.1)
	49	recombinase::IntI1:dfrA12:gcuF:aadA2:: Sul1::::::::IS6 (IS6100)::::Mph(A)	
	59	IS3:aac(3)-IIa	

Table S1: Comparison of *K. pneumoniae* genomes with South African genomes, downloaded from NCBI and PATRIC servers.

No.	Genome ID	Strain	MLST	BioProject Accession	BioSample Accession	Isolation Source	Collection Date	Geographic Location	Host Name	TEM-1	SHV	SCO	CTX-M-15	OXA	GES	NDM	IMP	LEN	ble	VIM	aac(6')-Ib3	aac(3)-II	aadA/StrA	aph(3'')-Ib	aph(6)-Id	aph(3')-Ia	arr-3	catA/B	dfrA	emrD	fosA	mph(A)	oqxA/B	oqxB9/19/25	qnrB/S	fosA7	sul1/2	tet	rmtC	gyrA	parC
1	573.15255	ST-152:K021	ST152	PRJNA355910	SAMN06106841	urine	25.04.2013	Pretoria	Human				+					+			+						+		+	+	+		+	+	+					
2	573.15256	ST-1552:K089	ST1552	PRJNA355910	SAMN06106843	hospitals	30.05.2013	Pretoria	Human				+					+			+						+		+	+	+		+	+	+					
3	573.15257	ST-39:K0179	ST39	PRJNA355910	SAMN06106845	sputum	14.11.2013	Pretoria	Human	+			+										+					+	+						+						
4	573.15258	ST-15:E041	ST15	PRJNA355910	SAMN06106848	urine	24.08.2013	Pretoria	Human				+					+			+						+	+	+	+	+		+	+	+					
5	573.15259	ST-1414:K058	ST1414	PRJNA355910	SAMN06106842	blood	09.05.2013	Pretoria	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+		+	+			
6	573.1526	ST-152:KO25	ST152	PRJNA355910	SAMN06106855	urine	25.04.2013	Pretoria	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+		+	+			
7	573.15261	ST-152:K031	ST152	PRJNA355910	SAMN06106856	hospitals	29.04.2013	Pretoria	Human	+	+		+								+	+	+	+	+		+		+	+	+		+	+	+		+			
8	573.15263	ST-234:K059	ST234	PRJNA355910	SAMN06106858	blood	09.05.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
9	573.15264	ST-1414:K078	ST1414	PRJNA355910	SAMN06106862	urine	22.05.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
10	573.15265	ST-323:K090	ST323	PRJNA355910	SAMN06106863	blood	30.05.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
11	573.15266	ST-1414:K141	ST1414	PRJNA355910	SAMN06106868	blood	18.08.2013	Pretoria	Human		+		+								+	+	+	+	+			+	+	+	+		+	+			+		+	+
12	573.15267	ST-1552:K117	ST1552	PRJNA355910	SAMN06106867	hospitals	19.07.2013	Pretoria	Human	+	+	+	+		+						+	+								+	+		+	+	+				+	+
13	573.15268	ST-1552:K077	ST1552	PRJNA355910	SAMN06106870	urine	22.05.2013	Pretoria	Human	+	+		+			+			+		+	+	+	+	+			+	+	+	+		+	+			+	+	+	+
14	573.15269	ST-152:K181	ST152	PRJNA355910	SAMN06106872	urine	14.04.2013	Pretoria	Human		+		+								+	+	+	+	+			+	+	+	+		+	+			+		+	+
15	573.1527	ST-152:K071	ST152	PRJNA355910	SAMN06106869	blood	17.05.2013	Pretoria	Human		+		+								+	+	+	+	+			+	+	+	+		+	+			+		+	+
16	573.15271	ST-101:K085	ST101	PRJNA355910	SAMN06106883	urine	26.05.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
17	573.15272	ST-25:K161	ST25	PRJNA355910	SAMN06106881	urine	02.10.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
18	573.15273	ST-1552:K061	ST1552	PRJNA355910	SAMN06106859	hospitals	15.05.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+	+	+
19	573.15274	ST-1552:K086	ST1552	PRJNA355910	SAMN06106861	hospitals	27.05.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
20	573.15276	ST-15:K087	ST15	PRJNA355910	SAMN12738925	blood	29.05.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+		+	+
21	573.15277	ST-234:K080	ST1552	PRJNA355910	SAMN06106871	urine	23.05.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+	+	+
22	573.15278	ST-152:K110	ST152	PRJNA355910	SAMN06106866	missing	09.07.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+	+	+
23	573.15279	ST-15:K125	ST15	PRJNA355910	SAMN06106873	blood	30.07.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+	+	+
24	573.1528	ST-39:K145	ST39	PRJNA355910	SAMN06106874	urine	22.08.2013	Pretoria	Human	+	+		+								+	+	+	+	+			+	+	+	+		+	+			+	+	+	+
25	573.15281	ST-15:K104	ST15	PRJNA355910	SAMN06106884	hospitals	17.06.2013	Pretoria	Human	+	+		+								+	+	+	+	+			+	+	+	+		+	+			+	+	+	+
26	573.15288	K123	missing	PRJNA355910	SAMN06106899	blood	28.07.2013	Pretoria	Human	+	+	+	+		+						+	+	+				+		+	+	+		+	+			+		+	+
27	573.1529	ST-234:K053	ST234	PRJNA355910	SAMN06106894	blood	07.05.2013	Pretoria	Human	+	+		+			+			+		+	+	+	+	+			+	+	+	+		+	+			+	+	+	+
28	573.15291	ST-152:K069	ST-152	PRJNA355910	SAMN06106897	sputum	16.05.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+		+	+
29	573.15292	ST-234:K054	ST234	PRJNA355910	SAMN06106895	blood	07.05.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+		+	+
30	573.15293	ST-234:K062	ST234	PRJNA355910	SAMN06106896	blood	15.05.2013	Pretoria	Human		+		+			+			+		+	+		+	+			+	+	+	+	+	+	+			+	+		+	+
31	573.15294	ST-643:K129	ST643	PRJNA355910	SAMN06106885	urine	07.08.2013	Pretoria	Human	+	+		+			+			+		+	+	+	+	+			+	+	+	+	+	+	+			+	+	+	+	+
32	573.15295	ST-14:K118	ST14	PRJNA355910	SAMN06106887	urine	20.07.2013	Pretoria	Human	+	+		+								+	+	+					+	+		+		+				+				

33	573.15297	ST-607:K014	ST607	PRJNA355910	SAMN06106892	sputum	21.04.2013	Pretoria	Human	+	+		+								+	+	+					+	+		+		+				+				
34	573.15298	ST-1552:K038	ST1552	PRJNA355910	SAMN06106893	blood	01.05.2013	Pretoria	Human	+	+		+								+	+		+	+			+	+	+	+		+	+	+	+	+	+			
35	573.15301	ST-182:K137	ST182	PRJNA355910	SAMN06106901	sputum	14.08.2013	Pretoria	Human	+	+		+								+	+							+	+			+	+		+	+				
36	573.15302	ST-1414:K146	ST1414	PRJNA355910	SAMN06106902	sputum	24.08.2013	Pretoria	Human	+	+		+			+			+		+	+		+	+			+	+	+	+		+	+		+	+		+		
37	573.15303	ST-101:K169	ST101	PRJNA355910	SAMN06106903	urine	18.10.2013	Pretoria	Human	+	+		+											+	+			+	+	+	+		+	+			+	+			
38	573.16409	ST-17:K120	ST17	PRJNA355910	SAMN06106898	blood	22.07.2013	Pretoria	Human	+	+	+	+								+	+	+					+	+	+	+		+	+	+		+				
39	573.1641	ST-101:K126	ST101	PRJNA355910	SAMN06106900	hospitals	30.07.2013	Pretoria	Human		+		+								+	+	+	+	+			+	+	+	+	+			+		+	+			
40	573.20016	AA03064255	ST152	PRJNA477444	SAMN09469783	Rectum	04.2017	Durban	Human	+	+		+								+	+	+					+	+	+	+	+					+				
41	573.20017	KZ00371410	ST152	PRJNA477444	SAMN09469776	Rectum	07.2016	Durban	Human		+		+								+	+	+	+	+			+	+	+	+				+		+	+			
42	573.20018	KZ00370220	ST152	PRJNA477444	SAMN09469778	Rectum	06.2016	Durban	Human	+	+		+								+	+	+					+	+		+	+			+		+	+			
43	573.20019	AA03509400	ST152	PRJNA477444	SAMN09469780	Rectum	01.2017	Durban	Human																																
44	573.2002	AA03814079	ST152	PRJNA477444	SAMN09469782	Rectum	04.2014	Durban	Human	+	+		+											+	+				+	+	+		+	+	+		+	+			
45	573.20021	KZ00369997	ST152	PRJNA477444	SAMN09469777	Rectum	07.2016	Durban	Human	+	+		+								+	+						+	+	+	+	+	+	+	+	+	+				
46	573.20022	AA03610795	ST152	PRJNA477444	SAMN09469781	Rectum	03.2017	Durban	Human	+	+	+	+			+			+		+	+	+	+	+		+	+	+	+	+		+	+			+				
47	573.20023	AA03535901	ST152	PRJNA477444	SAMN09469779	Rectum	01.2017	Durban	Human	+	+	+	+								+	+	+	+	+		+		+	+	+		+	+	+		+				
48	573.20024	KZ00372150	ST152	PRJNA477444	SAMN09469775	Rectum	07.2016	Durban	Human																																
49	573.20025	KZ00361153	ST3136	PRJNA477444	SAMN09469774	Rectum	05.2017	Durban	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+			
50	573.25658	ED01500733	ST983	PRJNA429538	SAMN08358012	Rectum	06.2017	Pietermaritzburg	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+			
51	573.25659	A105R2B2	ST607	PRJNA429538	SAMN08358017	Rectum	05.2017	Ozwatini	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+			
52	573.2566	A111R1B2	ST17	PRJNA429538	SAMN08358019	Rectum	05.2017	Ozwatini	Human	+	+	+	+								+	+	+	+	+			+	+	+	+		+	+	+	+					
53	573.25661	G702R3B2	ST152	PRJNA429538	SAMN08358021	Rectum	06.2017	Pietermaritzburg	Human	+	+		+								+	+		+	+			+	+	+	+		+	+	+		+	+		+	+
54	573.25662	ED01503757	ST152	PRJNA429538	SAMN08358024	Rectum	05.2017	Pietermaritzburg	Human	+	+		+								+	+	+	+	+				+	+	+		+	+	+		+	+			
55	573.25663	G702R1B5	ST152	PRJNA429538	SAMN08358044	Rectum	06.2017	Pietermaritzburg	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
56	573.25664	A105R1B5	ST983	PRJNA429538	SAMN08358041	Rectum	05.2017	Ozwatini	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
57	573.25679	G702R2B5	ST152	PRJNA429538	SAMN08358052	Rectum	06.2017	Pietermaritzburg	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
58	573.2568	ED01502268	ST432	PRJNA429538	SAMN08358015	Rectum	06.2017	Pietermaritzburg	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
59	573.27948	Kp32	ST429	PRJNA565241	SAMN12738927	urine	2018	Pretoria	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
60	573.27949	Kp33	ST39	PRJNA565241	SAMN12738928	urine	2018	Pretoria	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
61	573.27953	Kp15	ST607	PRJNA565241	SAMN12738925	Catheter tip	2018	Pretoria	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
62	573.27954	Kp29	ST17	PRJNA565241	SAMN12738926	swab	2018	Pretoria	Human	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
63	573.27955	Kp10	ST39	PRJNA565241	SAMN12738924	urine	2018	Pretoria	Human	+	+		+								+	+		+	+			+	+	+	+		+	+	+		+				
64	573.27956	Kp8	ST307	PRJNA565241	SAMN12738923	Pus swab	2018	Pretoria	Human	+	+		+	+							+		+	+	+		+	+	+	+	+		+	+	+		+			+	+
65	573.29132	Kpn01733	ST307	PRJEB34702	SAMEA5988754	clinical material	2016	Cape Town	Human	+	+		+	+							+		+	+	+		+	+	+	+	+		+	+	+		+			+	+
66	573.29133	Kpn01734	ST307	PRJNA355910	SAMN06106864	urine	03.07.2013	Pretoria	Human	+	+		+								+	+	+	+	+			+	+	+	+		+	+			+	+		+	+
67	573.34076	EC03612985	ST307	PRJNA674742	SAMN16674263	Rectum	24.02.2020	Durban	Human	+	+	+	+	+							+	+	+	+	+			+	+	+	+		+	+	+		+				
68	573.34077	EC03605938	ST307	PRJNA674742	SAMN16674262	Rectum	18.02.2020	Durban	Human	+	+		+	+							+	+		+	+			+	+	+	+		+	+			+			+	+
69	573.34078	EC03629993	ST307	PRJNA674742	SAMN16674259	Rectum	08.03.2020	Durban	Human	+	+		+	+							+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+			
70	573.34079	EC03607709	ST307	PRJNA674742	SAMN16674256	Rectum	19.02.2020	Durban	Human	+	+		+	+							+	+	+	+	+			+	+	+	+		+	+	+		+				
71	573.3408	EC03607707	ST307	PRJNA674742	SAMN16674260	Rectum	19.02.2020	Durban	Human	+	+		+	+							+	+	+	+	+			+	+	+	+		+	+	+		+				

72	573.34081	EC03623002	ST307	PRJNA674742	SAMN16674261	Rectum	03.03.2020	Durban	Human	+	+		+	+							+	+	+	+	+			+	+	+	+		+	+	+		+				
73	573.34172	EC03632007	ST307	PRJNA674742	SAMN16674258	Rectum	10.03.2020	Durban	Human	+	+		+	+							+	+		+	+			+	+	+	+		+	+	+		+				
74	573.34173	EC03638959	ST307	PRJNA674742	SAMN16674257	Rectum	14.03.2020	Durban	Human	+	+		+	+							+	+	+	+	+			+	+	+	+		+	+	+		+				
75	573.15274	ST-1552:K086	ST1552	PRJNA355910	SAMN06106861	missing	27.05.2013	Pretoria	Human																																
76	72407.1	ST101:U44822	ST101	PRJNA287968	SAMN04011447	missing	26.07.2013	Durban	Human	+	+		+								+	+	+					+	+	+	+		+	+			+				
77	72407.101	ST1478:939997103	ST1478	PRJNA287968	SAMN04011450	art line	missing	Durban	Human	+	+		+								+	+	+					+	+	+	+		+	+			+				
78	72407.102	ST101:939996824	ST101	PRJNA287968	SAMN04011454	pus	29.01.2013	Durban	Human	+	+		+								+	+		+	+			+	+	+	+		+	+	+		+				
79	72407.103	ST101:951363981	ST101	PRJNA287968	SAMN04011455	urine	04.04.2013	Durban	Human	+	+	+	+								+	+	+					+	+	+	+		+	+	+	+					
80	72407.104	ST101:945154233	ST101	PRJNA287968	SAMN04011446	sputum	missing	Durban	Human	+	+		+			+			+		+			+	+			+	+	+	+		+	+	+		+	+	+		
81	72407.84	ST101:950171785	ST101	PRJNA287968	SAMN04011419	missing	missing	Durban	Human	+	+		+			+			+		+			+	+			+	+	+	+		+	+	+		+	+	+		
82	72407.85	ST101:960186733	ST101	PRJNA287968	SAMN04011426	missing	missing	Durban	Human	+	+		+	+							+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	+		+	+
83	72407.86	ST101:951373950	ST101	PRJNA287968	SAMN04011433	blood	03.07.2013	Durban	Human	+	+		+	+							+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+
84	72407.87	ST101:945165838	ST101	PRJNA287968	SAMN04011436	pus swab from leg	25.06.2013	Durban	Human	+	+		+						+		+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+
85	72407.88	ST2017:950118422	ST2017	PRJNA287968	SAMN04011441	abdominal swab	12.12.2012	Durban	Human	+	+		+						+		+	+	+	+	+	+	+	+	+		+	+	+	+	+		+		+	+	+
86	72407.89	ST101:945169659	ST101	PRJNA287968	SAMN04011438	urine	19.07.2013	Durban	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+			+	+		+	+
87	72407.9	ST101:950173000	ST101	PRJNA287968	SAMN04011420	missing	missing	Durban	Human																																
88	72407.91	ST323:941530379	ST323	PRJNA287968	SAMN04011425	missing	missing	Durban	Human	+	+		+								+	+	+	+	+		+	+	+	+	+	+	+	+			+	+		+	+
89	72407.92	ST14:944535499	ST14	PRJNA287968	SAMN04011430	missing	missing	Durban	Human	+	+		+						+		+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+	+	+
90	72407.93	ST2016:951362657	ST101	PRJNA287968	SAMN04011434	missing	missing	Durban	Human	+	+		+						+		+	+	+	+	+		+	+	+	+	+	+	+	+	+		+		+	+	+
91	72407.94	ST2017:950117510	ST2017	PRJNA287968	SAMN04011440	urine	23.12.2012	Durban	Human	+	+		+						+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+	+	+
92	72407.95	ST2017:950142398	ST2017	PRJNA287968	SAMN04011439	tracheal fluid	missing	Durban	Human																												+				
93	72407.96	ST101:957083896	ST101	PRJNA287968	SAMN04011442	urine	03.08.2013	Durban	Human	+	+		+						+		+	+	+	+	+		+	+	+	+	+	+	+	+	+		+		+	+	+
94	72407.97	ST101:951384356	ST101	PRJNA287968	SAMN04011443	TIP	26.08.2013	Durban	Human	+	+		+						+		+	+	+	+	+		+	+	+	+	+	+	+	+	+		+		+	+	+
95	72407.98	ST101:957089165	ST101	PRJNA287968	SAMN04011444	pus swab (trachea)	missing	Durban	Human	+	+		+						+		+	+		+	+		+	+	+	+	+	+	+	+			+		+	+	+
96	72407.99	ST101:957083320	ST101	PRJNA287968	SAMN04011445	urine	21.07.2013	Durban	Human	+	+		+						+		+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	+		+
97	244366.105	ST-1791:KOO1	ST1791	PRJNA287968	SAMN04011450	missing	18.04.2013	Pretoria	Human	+	+		+						+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+

Table S2: Patients demographics.

Patient Study No.	Race	Gender	Age	Diagnosis	Invasive Devices	Date of isolation (Test Date)	Study Day	Bed No.	Isolate Number
P2	African	Male	64	Blunt abdominal injury – cow attack	Endotracheal tube Central line Arterial line Urinary Catheter Nasogastric tube Chest drains Abdominal wound drain Colostomy	19.2.20	Week 2	5	EC03607709
P12	African	Female	26	Overdose Atenolol and Amlodipine	Endotracheal tube Central line Arterial line Urinary Catheter Nasogastric tube	17.2.20	72 hrs	3	EC03604125
P13	African	Male	58	Traumatic brain injury - assault	Endotracheal tube Central line Arterial line Urinary Catheter Nasogastric tube	18.2.20	48 hrs	5	EC03605938
						19.2.20	72 hrs	5	EC03607707
						21.2.20	Discharge	5	EC03611456
P22	African	Female	42	Electrolyte imbalance – chronic renal disease	Endotracheal tube Central line Arterial line Urinary Catheter Nasogastric tube	22.2.20	Admission	5	EC03612372
						24.2.20	Discharge	5	EC03612985
P30	African	Male	39	Penetrating chest injury, crush injury – community assault	Endotracheal tube Central line Arterial line Urinary Catheter Nasogastric tube Chest drains Peritoneal dialysis	3.3.20	48 hrs	1	EC03623002
						6.3.20	Mero D2	1	EC03628574
						8.3.20	Mero D4	1	EC03629993
						10.3.20	Mero D6	1	EC03632007
						14.3.20	Week 2	1	EC03638959
						20.3.20	Week 3	1	EC03647661
						25.3.20	Week 4	1	EC03651429

Table S3: General genomic features of eight sequenced *K. pneumoniae* isolates.

Isolate	Accession number	Size (mb)	GC content	N50	L50	Contigs	Number of Coding Sequences	RNAs
EC03607709	JADKPL000000000.1	5,60	57.1	177788	10	111	5588	91
EC03638959	JADOEH000000000.1	5,72	56.7	177788	10	99	5738	85
EC03632007	JADOEG000000000.1	5,58	57.1	214866	9	99	5550	85
EC03629993	JADKPK000000000.1	5,60	57.1	177788	10	100	5589	89
EC03607707	JADKPJ000000000.1	5,61	57.1	186809	9	113	5588	90
EC03623002	JADKPI000000000.1	5,60	57.1	214866	9	101	5590	90
EC03605938	JADKPH000000000.1	5,60	57.1	214866	9	107	5591	93
EC03612985	JADKPG000000000.1	5,70	56.7	189938	9	116	5724	91

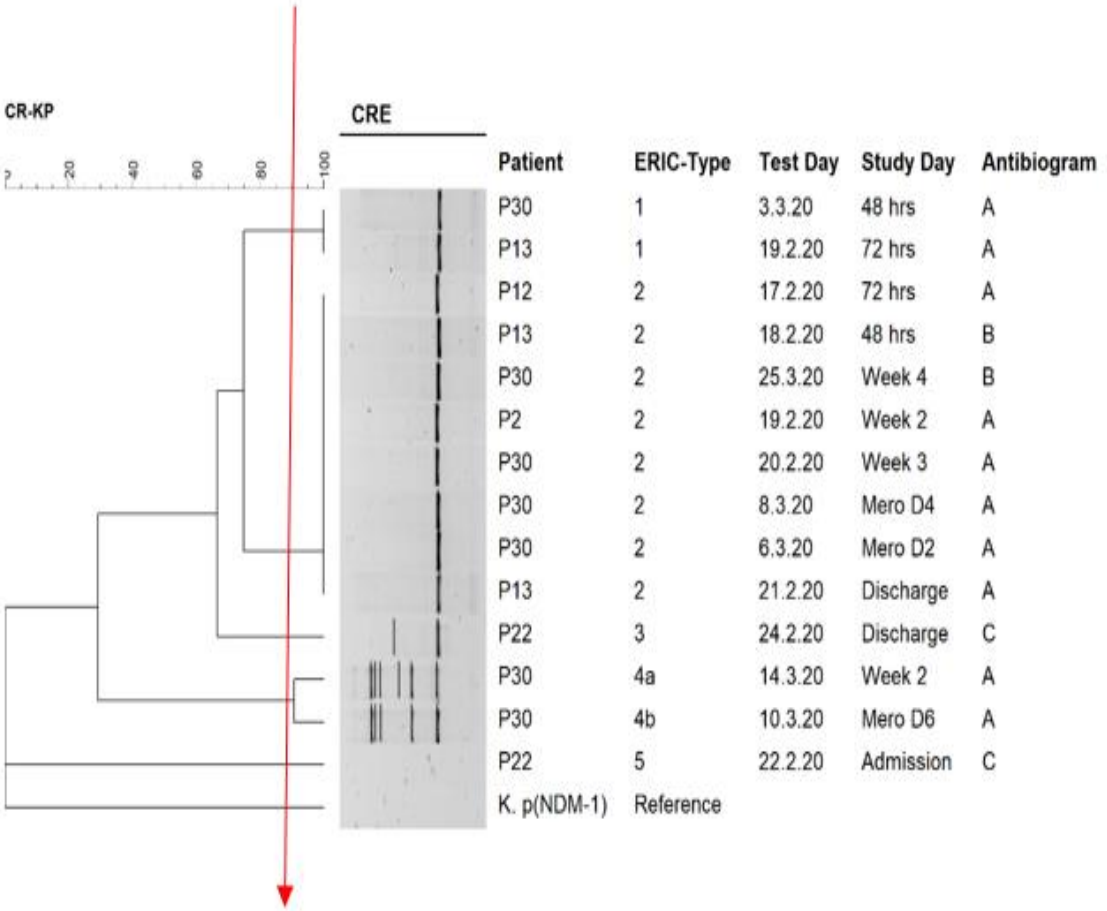


Figure 1. ERIC/PCR dendrogram for CPKP isolates (n=14).

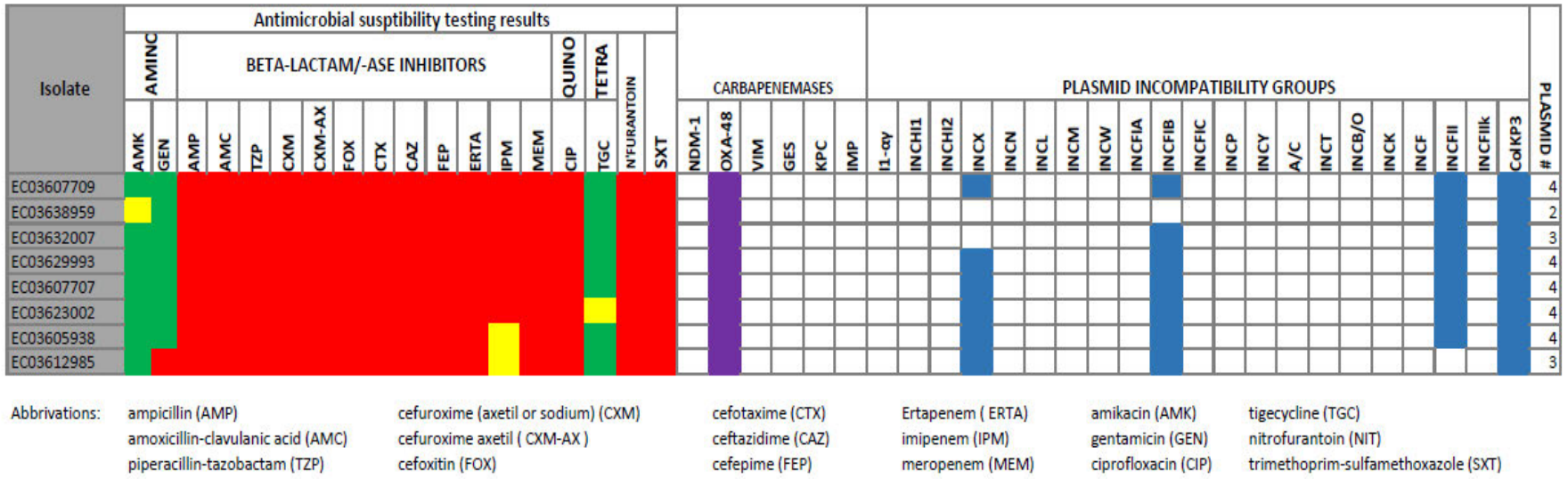


Figure 2. Antibiotic susceptibility patterns, carbapenemase genes and plasmid replicon groups of CPKP isolates (n=8).

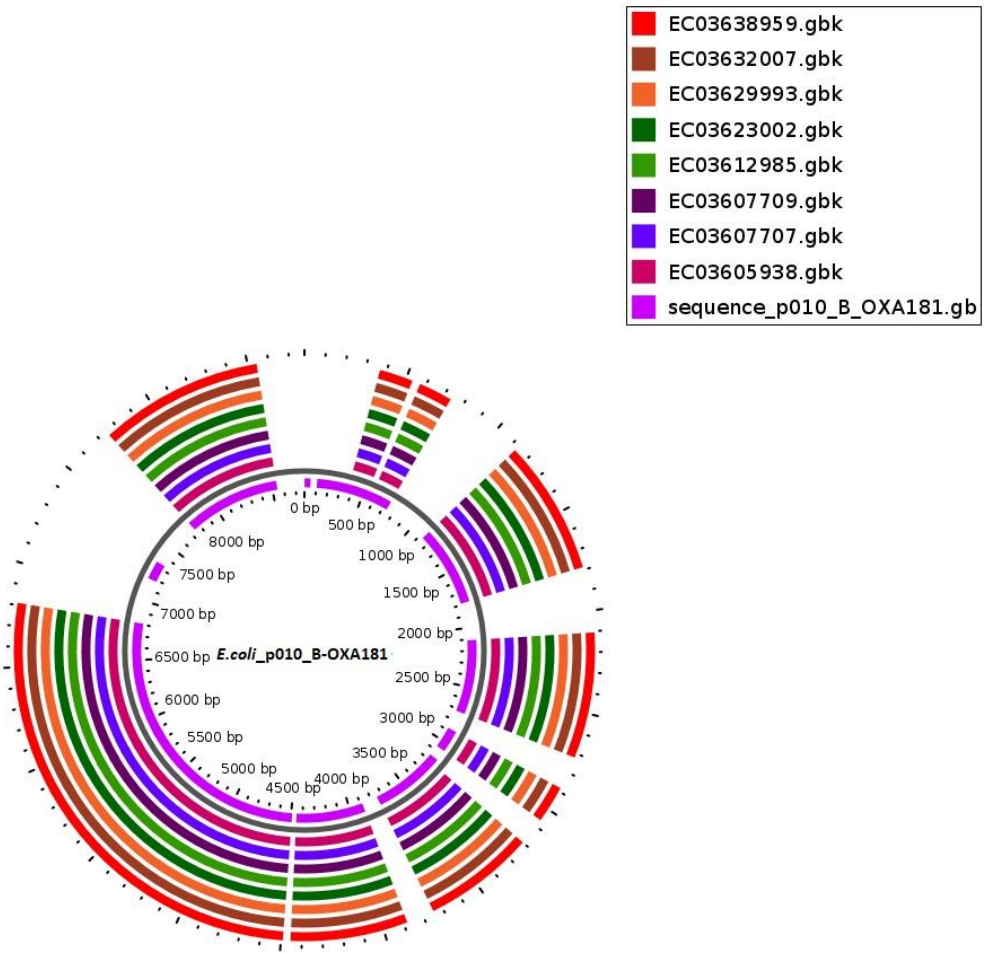
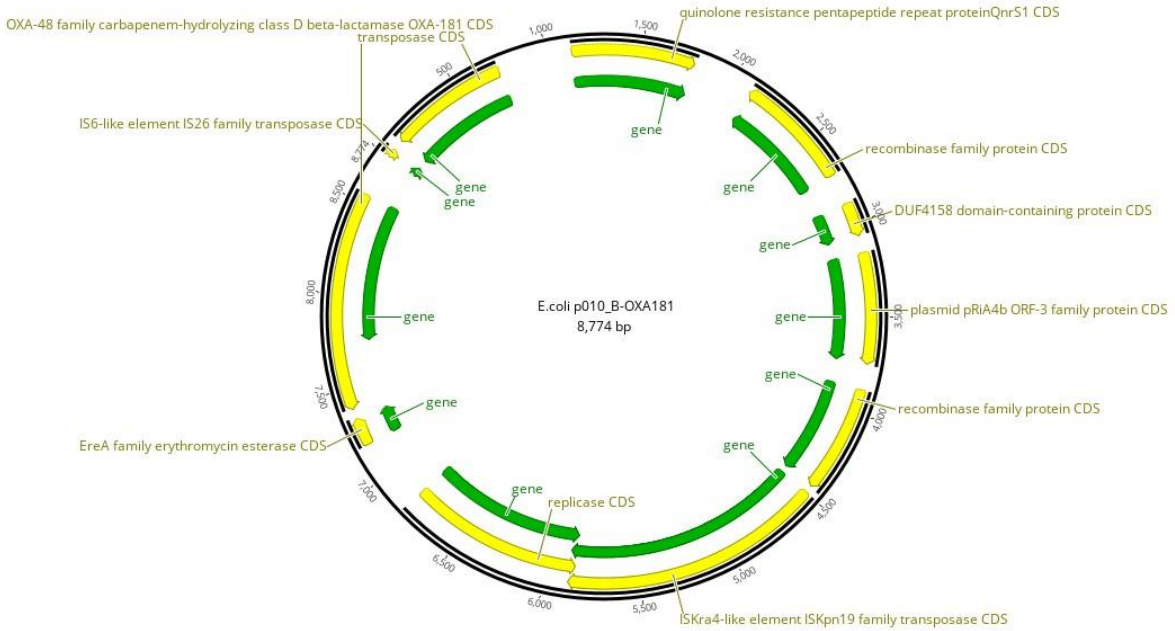


Figure 3. Comparison *E. coli* plasmid p010 B-OXA181 with genome assemblies containing OXA-181 in CPKP isolates (n=8).

(A)



(B)

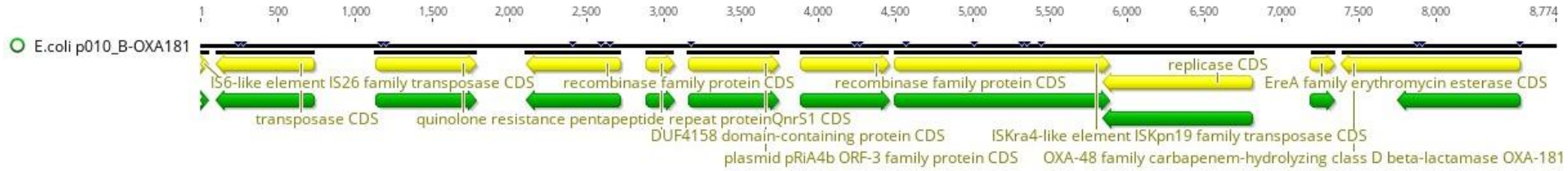
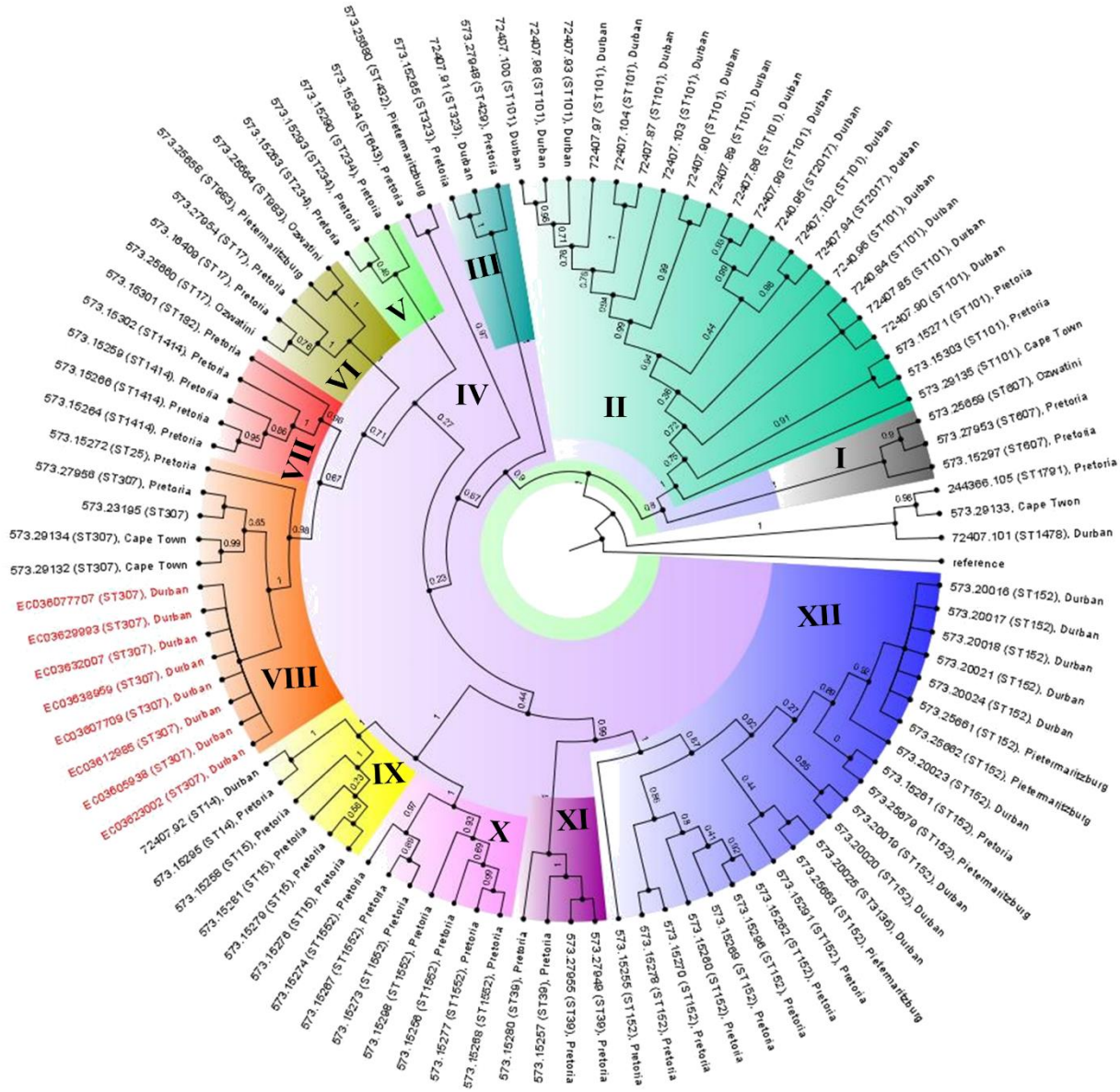


Figure 4. Genetic environment of *bla*OXA-181 found in the *K. pneumoniae* isolates.



CLADE	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Color Code												
Major ST	ST607	ST101	ST323	ST432/ST643	ST234	ST17	ST1414	ST307	ST14/ST15	ST1552	ST39	ST152

Figure 5. Phylogeography and resistance profiles of *K. pneumoniae*.

CHAPTER 3
CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

This study describes the molecular epidemiology of CPE colonization in patients from ICU in a single public hospital in the KwaZulu-Natal area using WGS and bioinformatics analysis. Delineating the molecular signatures of CPEs will inform containment strategies to improve patient outcomes.

Conclusions

The following conclusions were reached in relation to the objectives of the study:

- Fourteen CPEs (14.4% of total samples) from five patients (16% of total patients enrolled) formed the final sample.
- The isolates were 100% resistant to ertapenem and meropenem together with other β-lactams, i.e., ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefuroxime-axetil, cefoxitin, cefotaxime, ceftazidime, cefepime, ciprofloxacin, nitrofurantoin, and trimethoprim-sulfamethoxazole.
- Resistance to imipenem lower at 71.4%.
- The highest rate of susceptibility was to tigecycline and amikacin (100%), followed by gentamicin (85.7%).
- All isolates were MDR displayed one of three antibiograms.
- Five ERIC profiles were identified, with an endemic strain associated with bed 5 as evident from the colonization of all patients occupying this bed.
- Bioinformatics analysis revealed that all isolates belonged to ST307.
- ARGs identified from WGS corroborated the resistance phenotypes for all antibiotics except for aminoglycoside.
- *Bla*OXA-181 carbapenemase carried on ISKpn19 was evident in all isolates concurrently with other resistance genes.
- Other MGEs identified in the isolates included plasmid replicons, integrons (class I), and transposons.
- Phylogenetic analysis clustered the isolates in clade VIII similar to isolates from Pretoria and Cape Town, indicating the circulation of this sequence type between provinces here in South Africa.

Limitations and Recommendations

Limitation - The sample size was relatively small and limited to patients admitted into a general ICU during one month for one public hospital.

Recommendation - To reflect the actual prevalence of CPKP in South African hospitals, future research should be statistically powered to include multiple study sites from different provinces.

Limitation – Our sample was limited to carbapenemase-producing *Enterobacterales* and carbapenems resistance attributed to other mechanisms such as efflux pumps and porin profiles was not investigated here.

Recommendation - Prospective studies should explore all the mechanisms contributing to carbapenem resistance.

Limitation – The resistance phenomes was not corroborated with the resistomes in certain isolates.

Recommendation – Future studies should include gene expression analysis and transcriptomics.

Limitation – We employed short-read sequencing (Illumina MiSeq) method that precluded the definitive association of ARGs on MGEs.

Recommendation - Studies in the future should use long-read third generation approaches such as PacBio or Nanopore SMRT sequencing to provide details about the location of ARGs chromosomes or plasmids.

Further recommendations

To prevent the colonization and spread of CPEs in ICUs in hospitals, relevant infection control procedures should be applied. We recommend isolating patients with verified carbapenem-resistant *Enterobacterales* colonization/infection, ensuring good hand hygiene for medical staff, and the use of personal protective equipment together with frequent environmental decontamination. The implementation of active screening contact precautions for positive patients, decreased invasive procedures, and rational use of antibiotics for patients entering the ICU could further mitigate colonization and subsequent outbreaks. Regular disinfection of the environment and ICU equipment, frequent changes in bedding, and sterilization of medical waste from the patient may also significantly reduce residual bacteria in infected patients' beds. In summary, there

is a need for strengthening infection prevention and control policies and practices to reduce the colonization and spread of resistant bacteria.

APPENDICES

Appendix 1: Biomedical Research Ethics Committee approval letter.

11 October 2020

Mr Osama Madni (220081240)
School of Health Sciences
Westville

Dear Mr Madni,

Protocol reference number: BREC/00001723/2020
Project title: Molecular Epidemiology of Carbapenem-Resistant Enterobacterales Colonization and Infection in an Intensive Care Unit
Degree: Masters

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 11 October 2020. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations dated 26th August 2020, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Lockdown_Level_2_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 11 October 2020. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 10 November 2020.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

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

Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 2: Additional bioinformatics analyses

We further investigated assembled genomes using the Centre for Genomic Epidemiology (CGE) tools to determine pathogenicity, and ascertain restriction-modification systems (RMS), using PathogenFinder, and Restriction-ModificationFinder, respectively (https://cge.cbs.dtu.dk/services/PathogenFinder/, https://cge.cbs.dtu.dk/services/Restriction-ModificationFinder/). For identifying integrative and mobilizable elements (IMEs), and prophages incorporated, ICEberg and PHASTER servers were used, respectively https://db-mml.sjtu.edu.cn/ICEberg2/index.php, https://phaster.ca/).

Table I. Pathogenicity score, RMS, IMEs, ICEs, and intact prophages detected in *K. pneumoniae* isolates.

Isolate	Pathogenicity	RMS	ICE	IME	Prophages
EC03607709	0.887/(323)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	T4SS (2)	ND	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5, PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3
EC03638959	0.885/(377)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	ND	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5,PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3,PHAGE_Escher_HK639
EC03632007	0.887/(321)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	T4SS	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5,PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3,PHAGE_Escher_HK639
EC03629993	0.887/(323)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	T4SS	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5,PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3,PHAGE_Escher_HK639
EC03607707	0.887/(323)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	T4SS	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5,PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3
EC03623002	0.887/(323)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	T4SS	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5,PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3,PHAGE_Escher_HK639
EC03605938	0.887/(323)	Type II (M.EcoRII,M.Kpn34618Dcm,Eco128I)	T4SS	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5,PHAGE_Salmon_RE_2010,PHAGE_Salmon_118970_sal3
EC03612985	0.893/(307)	Type II (M.Kpn34618Dcm)	T4SS	1	PHAGE_Cronob_ENT47670,PHAGE_Salmon_SEN5, PHAGE_Salmon_118970_sal3

Abbreviation: ND; Not detected.

Table II: Methylome data of the *K. pneumoniae* isolates showing the restriction enzymes, type II methylases, DNA motifs, and their accession numbers.

Isolate	Gene	%Identity	HSP/Query length	Contig	Position in contig	Type	Function	Recognition Seq	Accession number
EC03607709	*Eco128I*	100.00	1095 / 1095	contig00050	3882..4976	Type II	restriction enzyme	CCWGG	AY618907,
	M.Kpn34618Dcm	99.23	1434 / 1434	contig00001	468096..469529	Type II	methyltransferase	CCWGG	CP010392,
	M.EcoRII	100.00	1434 / 1434	contig00050	5130..6563	Type II	methyltransferase	CCWGG	NEBM64,
EC03638959	*M.Kpn34618Dcm*	99.23	1434 / 1434	contig00001	12800..14233	Type II	methyltransferase	CCWGG	CP010392,
	Eco128I	100.00	1095 / 1095	contig00102	1957..3051	Type II	restriction enzyme	CCWGG	AY618907,
	M.EcoRII	100.00	1434 / 1434	contig00102	370..1803	Type II	methyltransferase	CCWGG	NEBM64,
EC03632007	*M.Kpn34618Dcm*	99.23	1434 / 1434	contig00001	12832..14265	Type II	methyltransferase	CCWGG	CP010392,
	Eco128I	100.00	1095 / 1095	contig00101	1957..3051	Type II	restriction enzyme	CCWGG	AY618907,
	M.EcoRII	100.00	1434 / 1434	contig00101	370..1803	Type II	methyltransferase	CCWGG	NEBM64,
EC03629993	*Eco128I*	100.00	1095 / 1095	contig00049	1956..3050	Type II	restriction enzyme	CCWGG	AY618907,
	M.EcoRII	100.00	1434 / 1434	contig00049	369..1802	Type II	methyltransferase	CCWGG	NEBM64,
	M.Kpn34618Dcm	99.23	1434 / 1434	contig00001	468134..469567	Type II	methyltransferase	CCWGG	CP010392,
EC03607707	*Eco128I*	100.00	1095 / 1095	contig00052	3882..4976	Type II	restriction enzyme	CCWGG	AY618907,
	M.Kpn34618Dcm	99.23	1434 / 1434	contig00001	42575..44008	Type II	methyltransferase	CCWGG	CP010392,
	M.EcoRII	100.00	1434 / 1434	contig00052	5130..6563	Type II	methyltransferase	CCWGG	NEBM64,
EC03623002	*M.Kpn34618Dcm*	99.23	1434 / 1434	contig00010	150687..152120	Type II	methyltransferase	CCWGG	CP010392,
	Eco128I	100.00	1095 / 1095	contig00047	1956..3050	Type II	restriction enzyme	CCWGG	AY618907,
	M.EcoRII	100.00	1434 / 1434	contig00047	369..1802	Type II	methyltransferase	CCWGG	NEBM64,
EC03605938	*Eco128I*	100.00	1095 / 1095	contig00050	1956..3050	Type II	restriction enzyme	CCWGG	AY618907,
	M.EcoRII	100.00	1434 / 1434	contig00050	369..1802	Type II	methyltransferase	CCWGG	NEBM64,
	M.Kpn34618Dcm	99.23	1434 / 1434	contig00001	468096..469529	Type II	methyltransferase	CCWGG	CP010392,
EC03612985	*M.Kpn34618Dcm*	99.23	1434 / 1434	contig00001	468135..469568	Type II	methyltransferase	CCWGG	CP010392,

Table III: Virulence analysis of the *K. pneumoniae* isolates showing the virulence genes repertoire (all isolates carried the same repertoire of 65 genes).

Isolate	Virulence gens
EC03607709 (P2)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03638959 (P30)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03632007 (P30)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03629993 (P30)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03607707 (P13)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03623002 (P30)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03605938 (P13)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.
EC03612985 (P22)	acrA, acrB, clpV/tssH, cpsACP, dotU/tssL, entA, entB, entC, entD, entE, entF, fepA, fepB, fepC, fepD, fepG, fes, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK, fyuA, galF, glf, gnd, hcp/tssD, icmF/tssM, impA/tssA, iroE, irp1, irp2, kfoC, KPHS_23120, manB, manC, mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, rcsA, rcsB, sciN/tssJ, tli1, tssF, tssG, ugd, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC, wbbM, wbbN, wbbO, wzi, wzm, wzt, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ybdA, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX, ykgK/ecpR.

Figure I (A-H): shows prophage DNA types and positions (regions) in the *K. pneumonia* isolates

Fig. I (A).

Fig. I (A).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypotheical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (B).


EC03638959 contigs
1
contig00003,len=367054,cov=25.0,corr=0,origname=NODE_3_length_367054_cov_24.993214_pilon,sw=shovill-spades/1.0.9,dat
2
contig00016,len=177030,cov=22.6,corr=0,origname=NODE_16_length_177030_cov_22.563187_pilon,sw=shovill-spades/1.0.9,da
3
contig00043,len=82654,cov=23.0,corr=0,origname=NODE_43_length_82654_cov_22.999576_pilon,sw=shovill-spades/1.0.9,date
4
contig00045,len=74607,cov=24.0,corr=0,origname=NODE_45_length_74607_cov_23.987195_pilon,sw=shovill-spades/1.0.9,date
5
contig00072,len=34128,cov=24.7,corr=0,origname=NODE_72_length_34128_cov_24.693990_pilon,sw=shovill-spades/1.0.9,date
6
contig00074,len=31710,cov=24.5,corr=0,origname=NODE_74_length_31710_cov_24.471446_pilon,sw=shovill-spades/1.0.9,date
7
contig00080,len=25733,cov=24.9,corr=0,origname=NODE_80_length_25733_cov_24.903629_pilon,sw=shovill-spades/1.0.9,date
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (B).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypotheical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (C).


EC03632007 contigs
contig00002,len=459036,cov=23.0,corr=0,origname=NODE_2_length_459036_cov_23.006814_pilon,sw=shovill-spades/1.0.9,dat
contig00015,len=177960,cov=21.3,corr=0,origname=NODE_15_length_177960_cov_21.347667_pilon,sw=shovill-spades/1.0.9,da
contig00038,len=82654,cov=21.7,corr=0,origname=NODE_38_length_82654_cov_21.682124_pilon,sw=shovill-spades/1.0.9,date
contig00041,len=74607,cov=22.5,corr=0,origname=NODE_41_length_74607_cov_22.529354_pilon,sw=shovill-spades/1.0.9,date
contig00070,len=34502,cov=22.6,corr=0,origname=NODE_70_length_34502_cov_22.579087_pilon,sw=shovill-spades/1.0.9,date
contig00073,len=31710,cov=22.5,corr=0,origname=NODE_73_length_31710_cov_22.504825_pilon,sw=shovill-spades/1.0.9,date
contig00080,len=25733,cov=35.6,corr=0,origname=NODE_80_length_25733_cov_35.598283_pilon,sw=shovill-spades/1.0.9,date
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (C).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypothetical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (D).


EC03629993 contigs
1
contig00003,len=367056,cov=26.2,corr=0,origname=NODE_3_length_367056_cov_26.178660_pilon,sw=shovill-spades/1.0.9,date=20201027
2
contig00011,len=177603,cov=22.8,corr=0,origname=NODE_11_length_177603_cov_22.779408_pilon,sw=shovill-spades/1.0.9,date=20201027
3
contig00025,len=82654,cov=23.9,corr=0,origname=NODE_25_length_82654_cov_23.883987_pilon,sw=shovill-spades/1.0.9,date=20201027
4
contig00026,len=74607,cov=26.8,corr=0,origname=NODE_26_length_74607_cov_26.767784_pilon,sw=shovill-spades/1.0.9,date=20201027
5
contig00035,len=36246,cov=66.6,corr=0,origname=NODE_35_length_36246_cov_66.588136_pilon,sw=shovill-spades/1.0.9,date=20201027
6
contig00038,len=34128,cov=26.1,corr=0,origname=NODE_38_length_34128_cov_26.087994_pilon,sw=shovill-spades/1.0.9,date=20201027
7
contig00039,len=31683,cov=27.1,corr=0,origname=NODE_39_length_31683_cov_27.141165_pilon,sw=shovill-spades/1.0.9,date=20201027
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (D).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypothetical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (E).

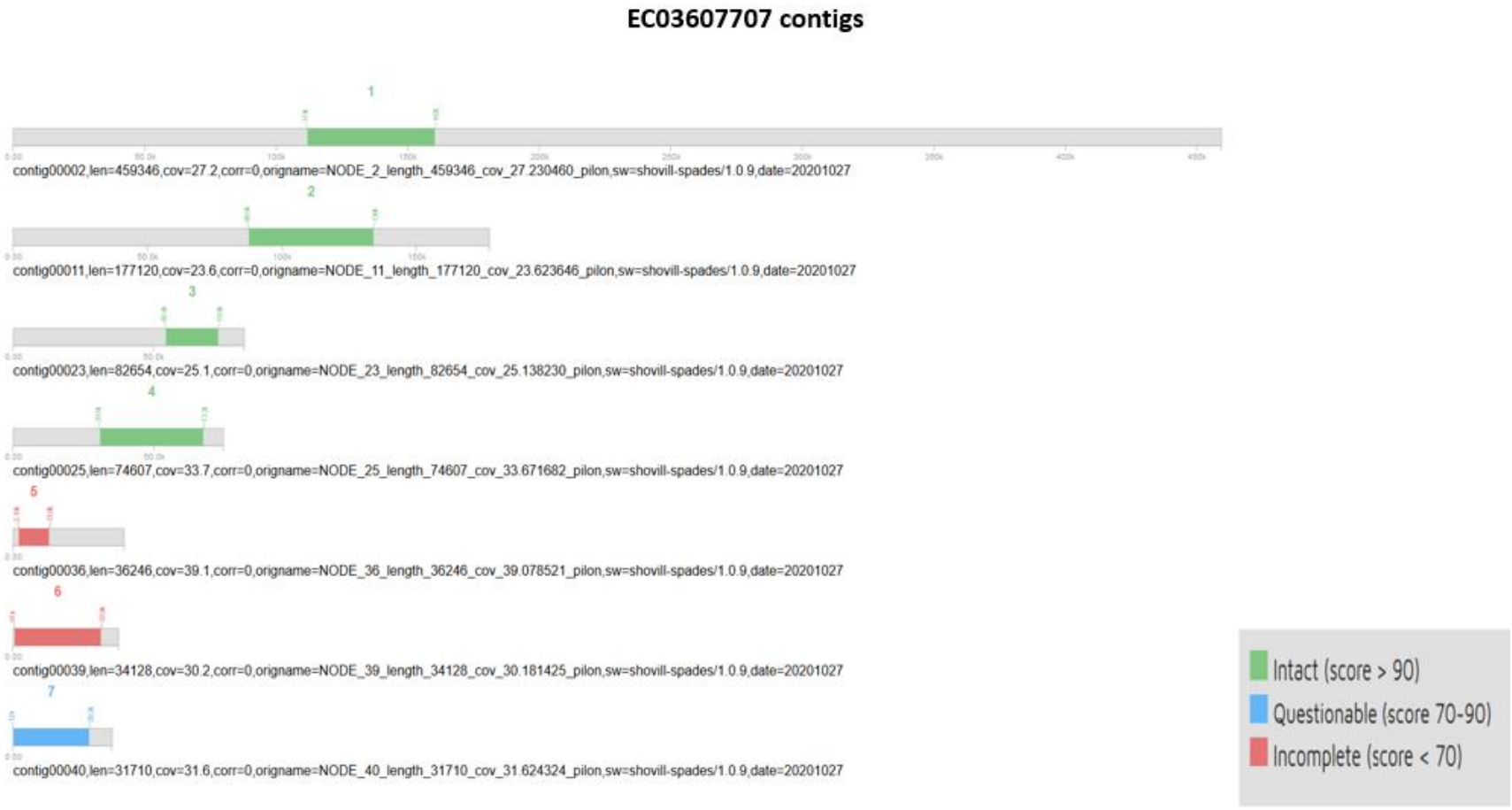

EC03607707 contigs
1
contig00002,len=459346,cov=27.2,corr=0,origname=NODE_2_length_459346_cov_27.230460_pilon,sw=shovill-spades/1.0.9,date=20201027
2
contig00011,len=177120,cov=23.6,corr=0,origname=NODE_11_length_177120_cov_23.623646_pilon,sw=shovill-spades/1.0.9,date=20201027
3
contig00023,len=82654,cov=25.1,corr=0,origname=NODE_23_length_82654_cov_25.138230_pilon,sw=shovill-spades/1.0.9,date=20201027
4
contig00025,len=74607,cov=33.7,corr=0,origname=NODE_25_length_74607_cov_33.671682_pilon,sw=shovill-spades/1.0.9,date=20201027
5
contig00036,len=36246,cov=39.1,corr=0,origname=NODE_36_length_36246_cov_39.078521_pilon,sw=shovill-spades/1.0.9,date=20201027
6
contig00039,len=34128,cov=30.2,corr=0,origname=NODE_39_length_34128_cov_30.181425_pilon,sw=shovill-spades/1.0.9,date=20201027
7
contig00040,len=31710,cov=31.6,corr=0,origname=NODE_40_length_31710_cov_31.624324_pilon,sw=shovill-spades/1.0.9,date=20201027
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (E).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypothetical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (F).

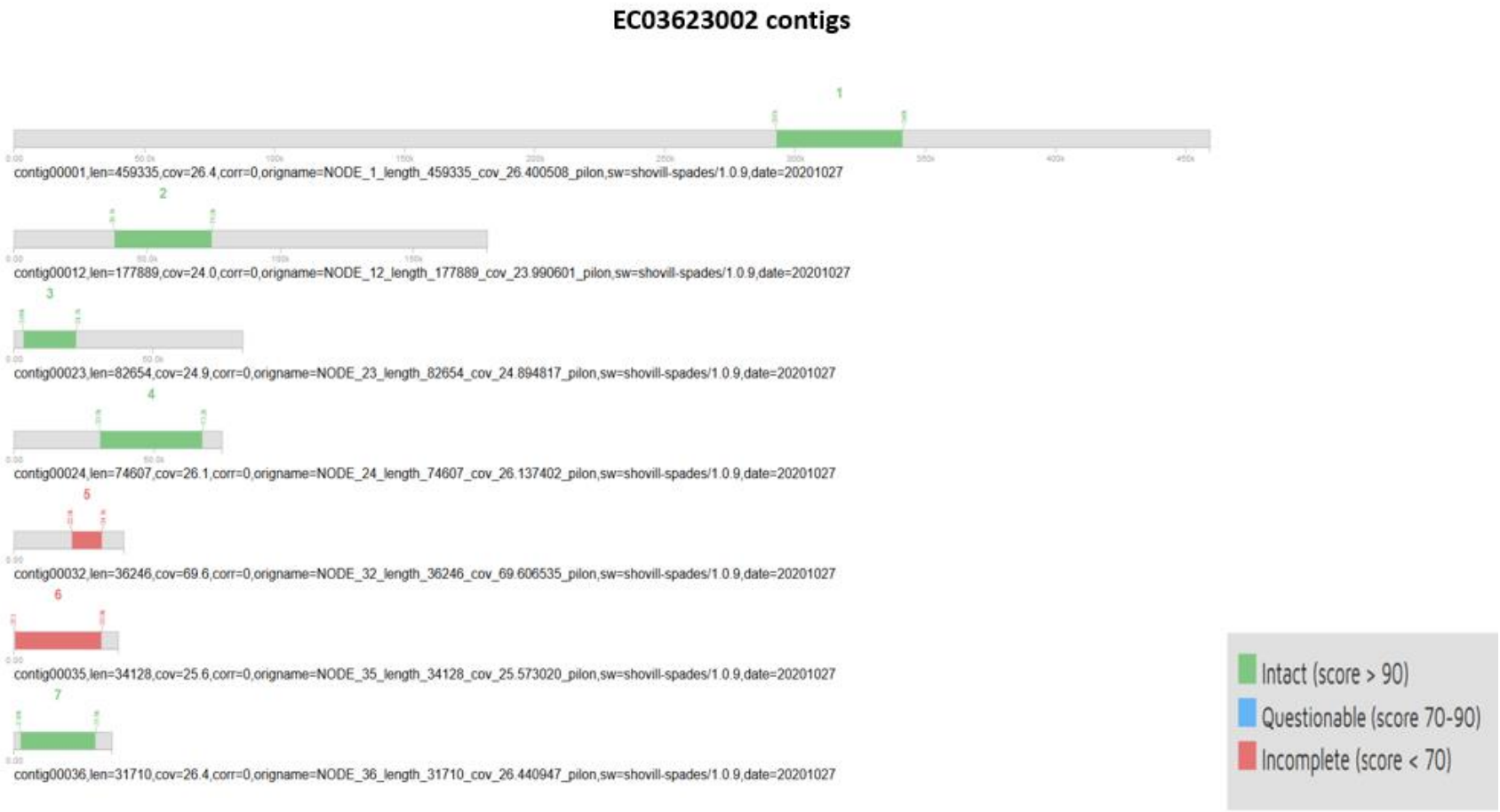

EC03623002 contigs
1
contig00001,len=459335,cov=26.4,corr=0,origname=NODE_1_length_459335_cov_26.400508_pilon,sw=shovill-spades/1.0.9,date=20201027
2
contig00012,len=177889,cov=24.0,corr=0,origname=NODE_12_length_177889_cov_23.990601_pilon,sw=shovill-spades/1.0.9,date=20201027
3
contig00023,len=82654,cov=24.9,corr=0,origname=NODE_23_length_82654_cov_24.894817_pilon,sw=shovill-spades/1.0.9,date=20201027
4
contig00024,len=74607,cov=26.1,corr=0,origname=NODE_24_length_74607_cov_26.137402_pilon,sw=shovill-spades/1.0.9,date=20201027
5
contig00032,len=36246,cov=69.6,corr=0,origname=NODE_32_length_36246_cov_69.606535_pilon,sw=shovill-spades/1.0.9,date=20201027
6
contig00035,len=34128,cov=25.6,corr=0,origname=NODE_35_length_34128_cov_25.573020_pilon,sw=shovill-spades/1.0.9,date=20201027
7
contig00036,len=31710,cov=26.4,corr=0,origname=NODE_36_length_31710_cov_26.440947_pilon,sw=shovill-spades/1.0.9,date=20201027
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (F).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypotheical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (G).


EC03605938 contigs
contig00002,len=459331,cov=26.4,corr=0,origname=NODE_2_length_459331_cov_26.391579_pilon,sw=shovill-spades/1.0.9,date=20201027
contig00018,len=103881,cov=24.8,corr=0,origname=NODE_18_length_103881_cov_24.804005_pilon,sw=shovill-spades/1.0.9,date=20201027
contig00023,len=82654,cov=25.0,corr=0,origname=NODE_23_length_82654_cov_25.043525_pilon,sw=shovill-spades/1.0.9,date=20201027
contig00024,len=74607,cov=30.3,corr=0,origname=NODE_24_length_74607_cov_30.317339_pilon,sw=shovill-spades/1.0.9,date=20201027
contig00034,len=36246,cov=40.9,corr=0,origname=NODE_34_length_36246_cov_40.891127_pilon,sw=shovill-spades/1.0.9,date=20201027
contig00037,len=34128,cov=28.0,corr=0,origname=NODE_37_length_34128_cov_28.040206_pilon,sw=shovill-spades/1.0.9,date=20201027
contig00038,len=31692,cov=29.0,corr=0,origname=NODE_38_length_31692_cov_29.048878_pilon,sw=shovill-spades/1.0.9,date=20201027
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (G).


Region 1
Region 2
Region 3
Region 4
Region 5
Region 6
Region 7
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypothetical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Fig. I (H).


EC03612985 contigs
1
contig00003,len=389178,cov=27.8,corr=0,origname=NODE_3_length_389178_cov_27.845076_pilon,sw=shovill-spades/1.0.9,date=20201027
2
contig00011,len=176976,cov=26.9,corr=0,origname=NODE_11_length_176976_cov_26.877969_pilon,sw=shovill-spades/1.0.9,date=20201027
3
contig00024,len=74607,cov=28.2,corr=0,origname=NODE_24_length_74607_cov_28.236457_pilon,sw=shovill-spades/1.0.9,date=20201027
4
contig00033,len=34129,cov=28.0,corr=0,origname=NODE_33_length_34129_cov_28.010345_pilon,sw=shovill-spades/1.0.9,date=20201027
5
contig00035,len=30270,cov=27.8,corr=0,origname=NODE_35_length_30270_cov_27.785163_pilon,sw=shovill-spades/1.0.9,date=20201027
Intact (score > 90)
Questionable (score 70-90)
Incomplete (score < 70)

Fig. I (H).


Region 1
Region 2
Region 3
Region 4
Region 5
Lysis
Terminase
Portal Protein
Protease
Coat Protein
Tail Shaft
Attachment Site
Integrase
Phage-like Protein
Hypothetical Protein
Other
Transposase
Fiber Protein
Plate Protein
tRNA

Figure II (A-H): Types and distribution of K and O capsule serotypes among *K. pneumoniae*

Fig. II (A)

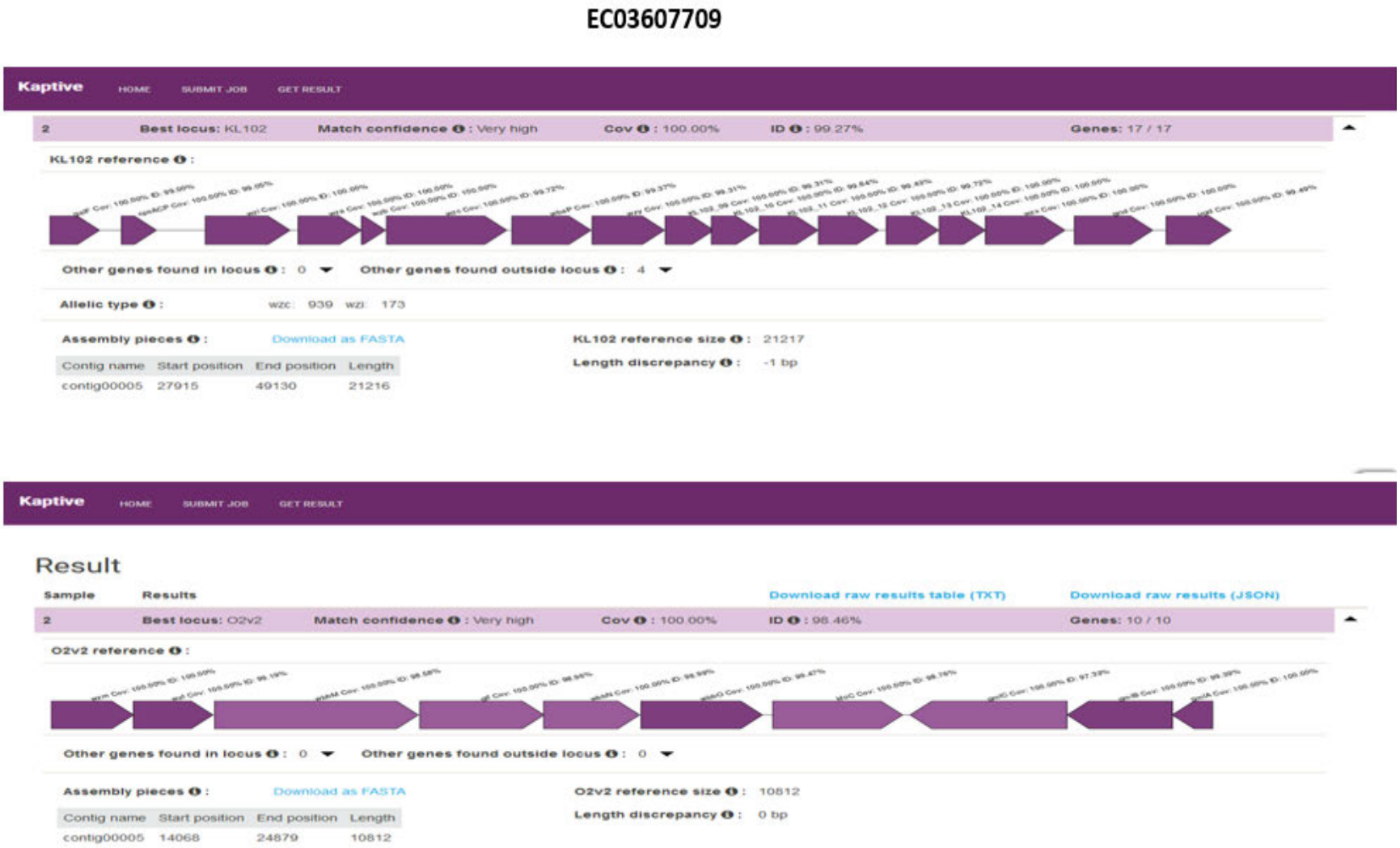


Fig. II (B)

EC03638959

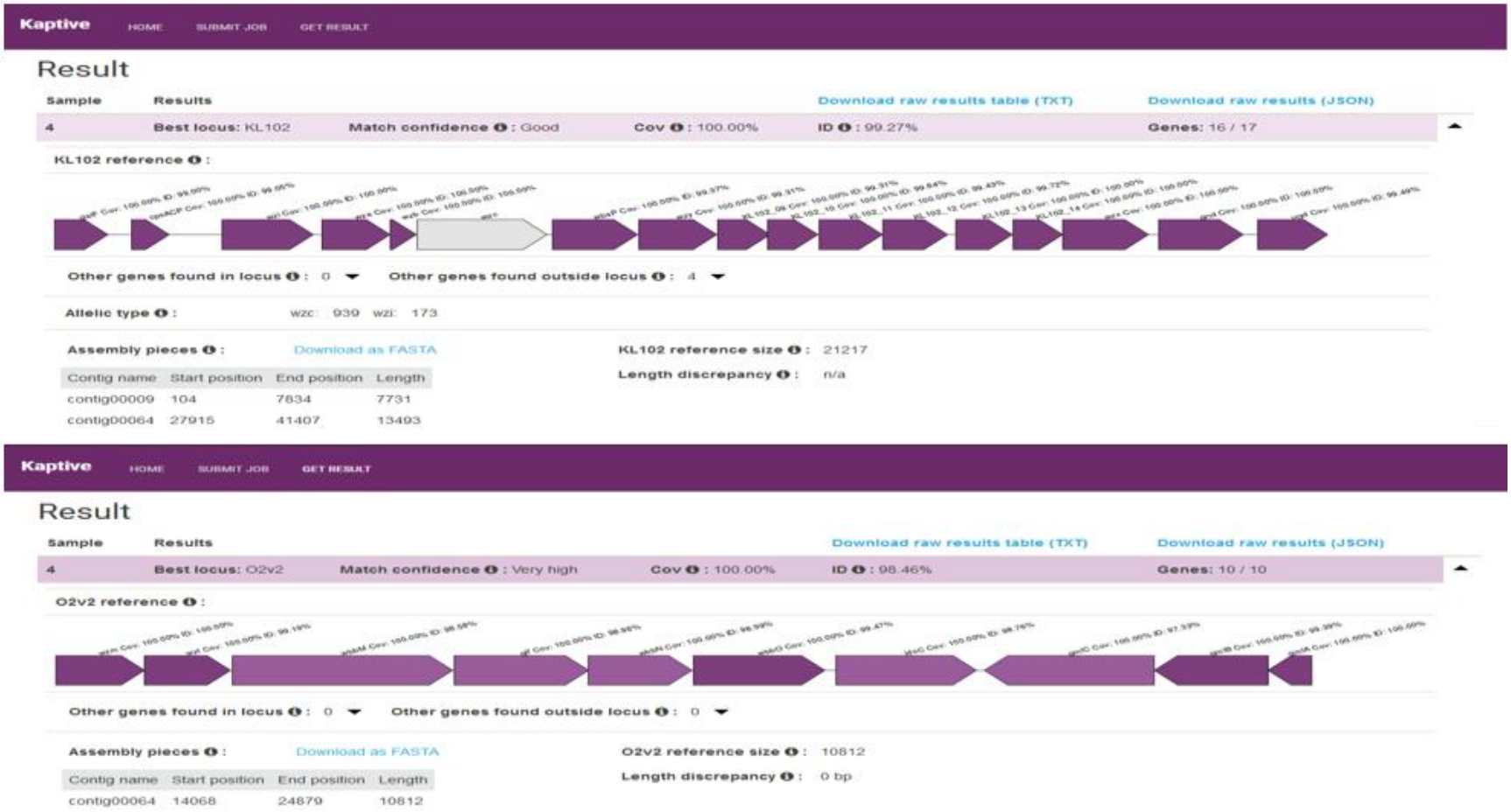


Fig. II (C)

EC03632007

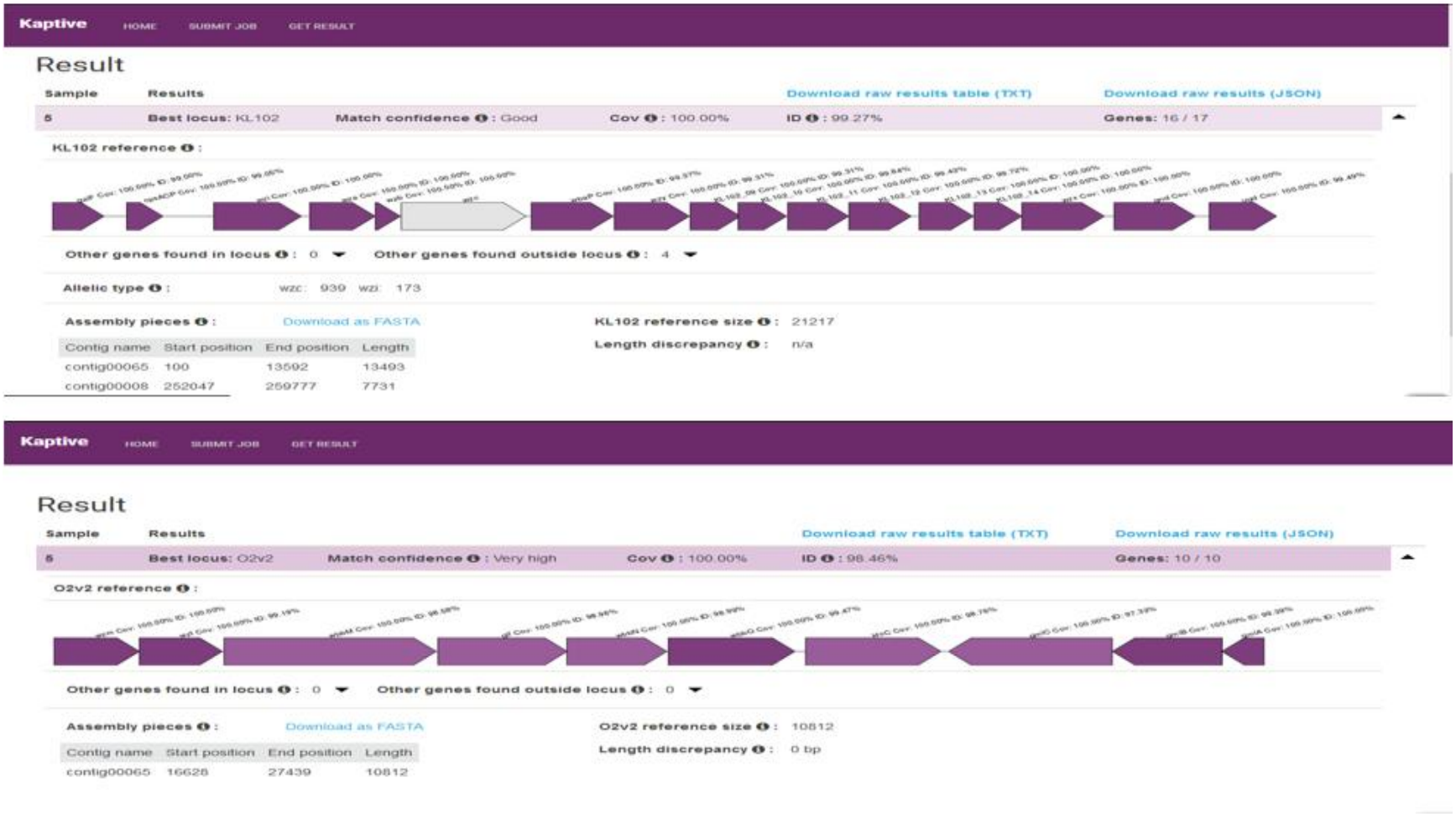


Fig. II (D)

EC03629993

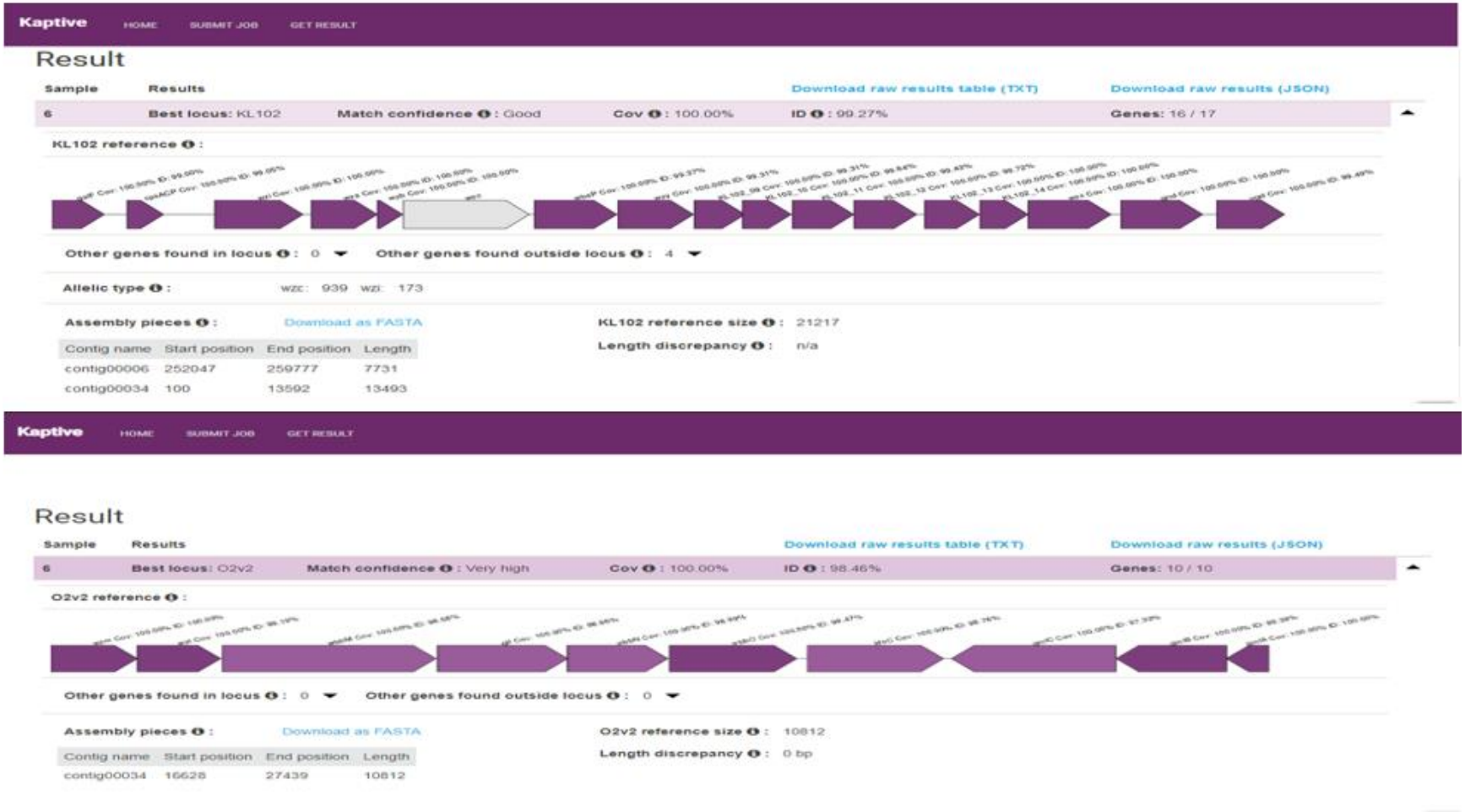


Fig. II (E)

EC03607707

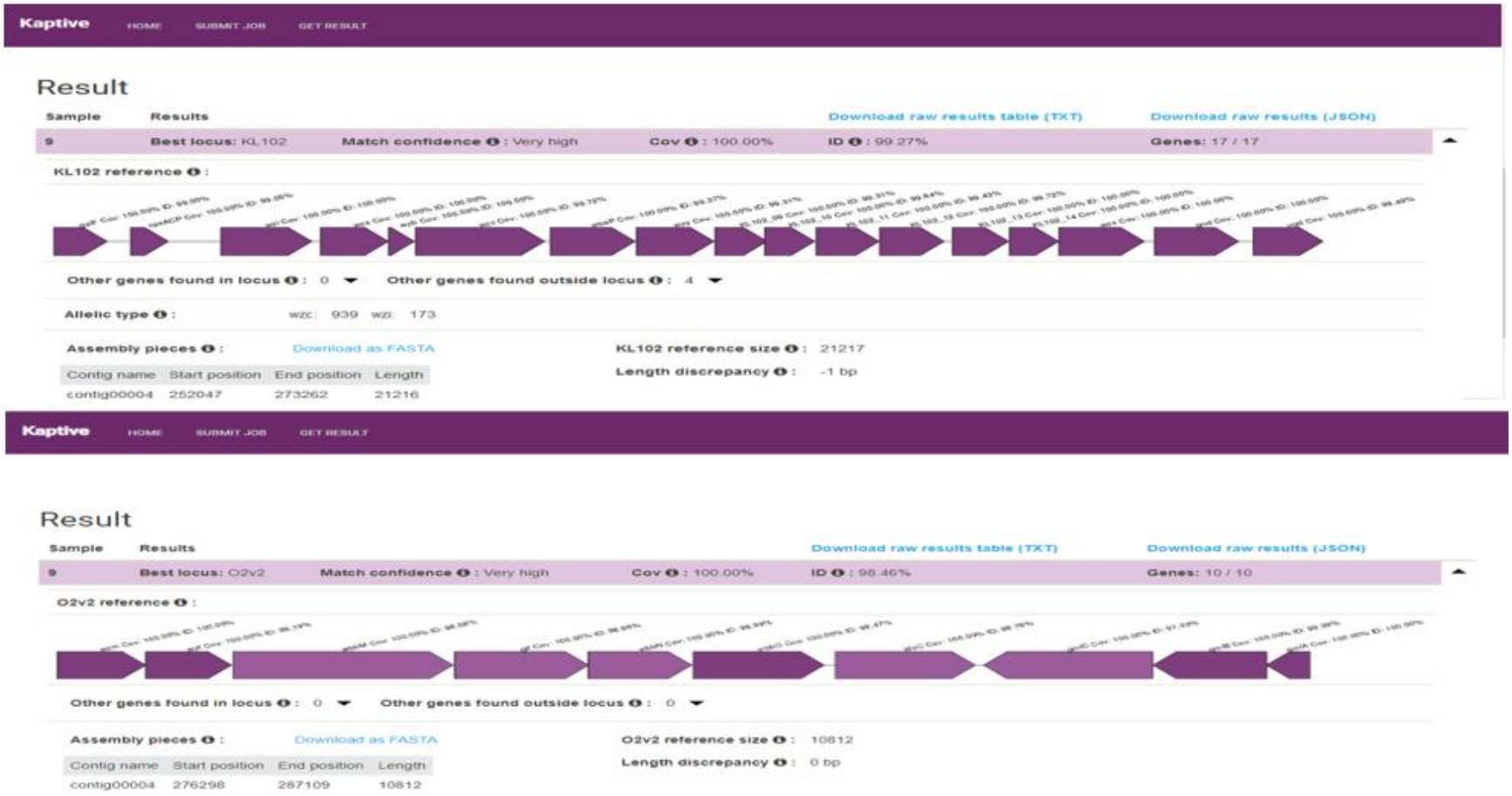


Fig. II (F)

EC03623002

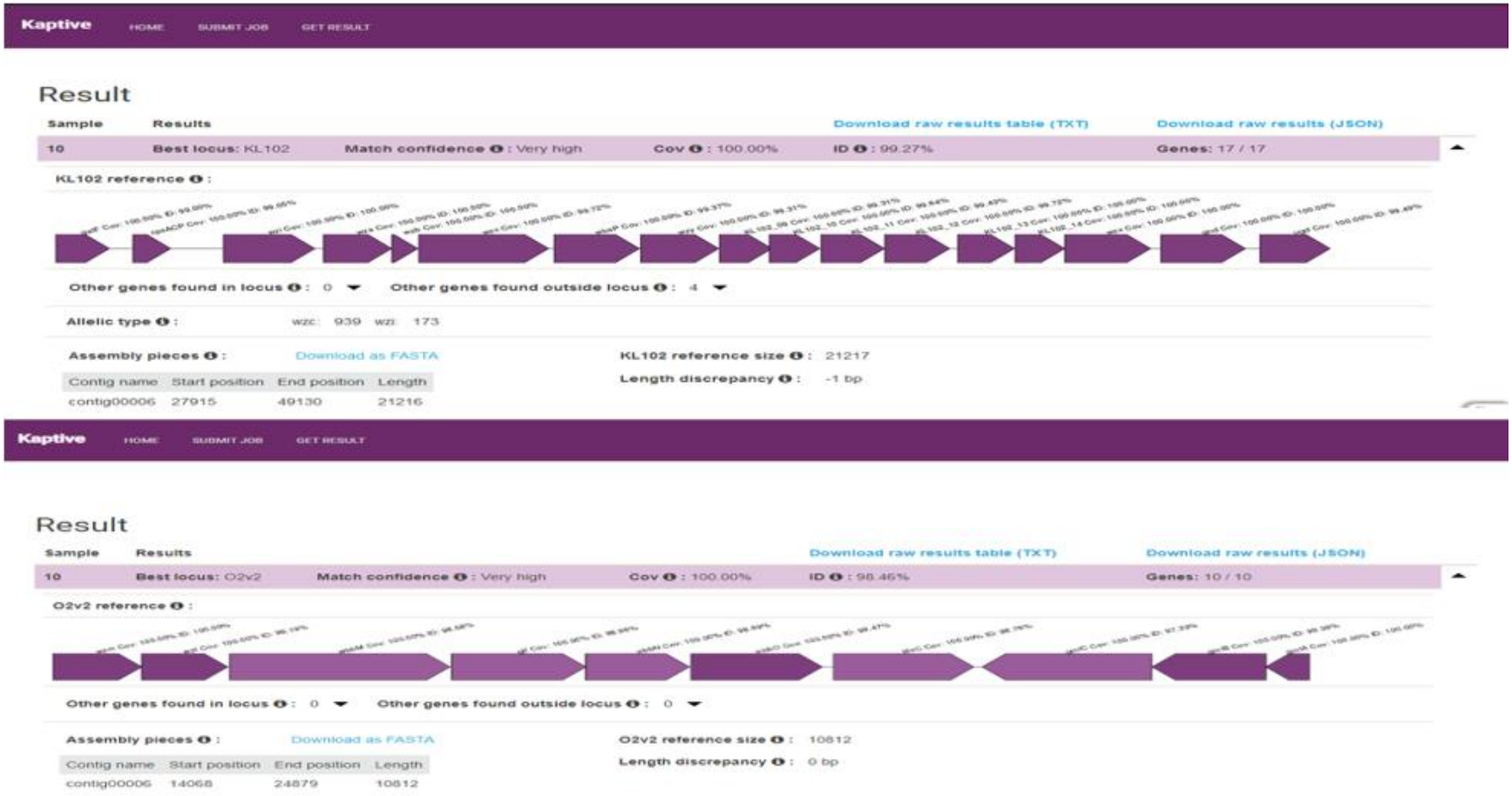


Fig. II (G)

EC03605938

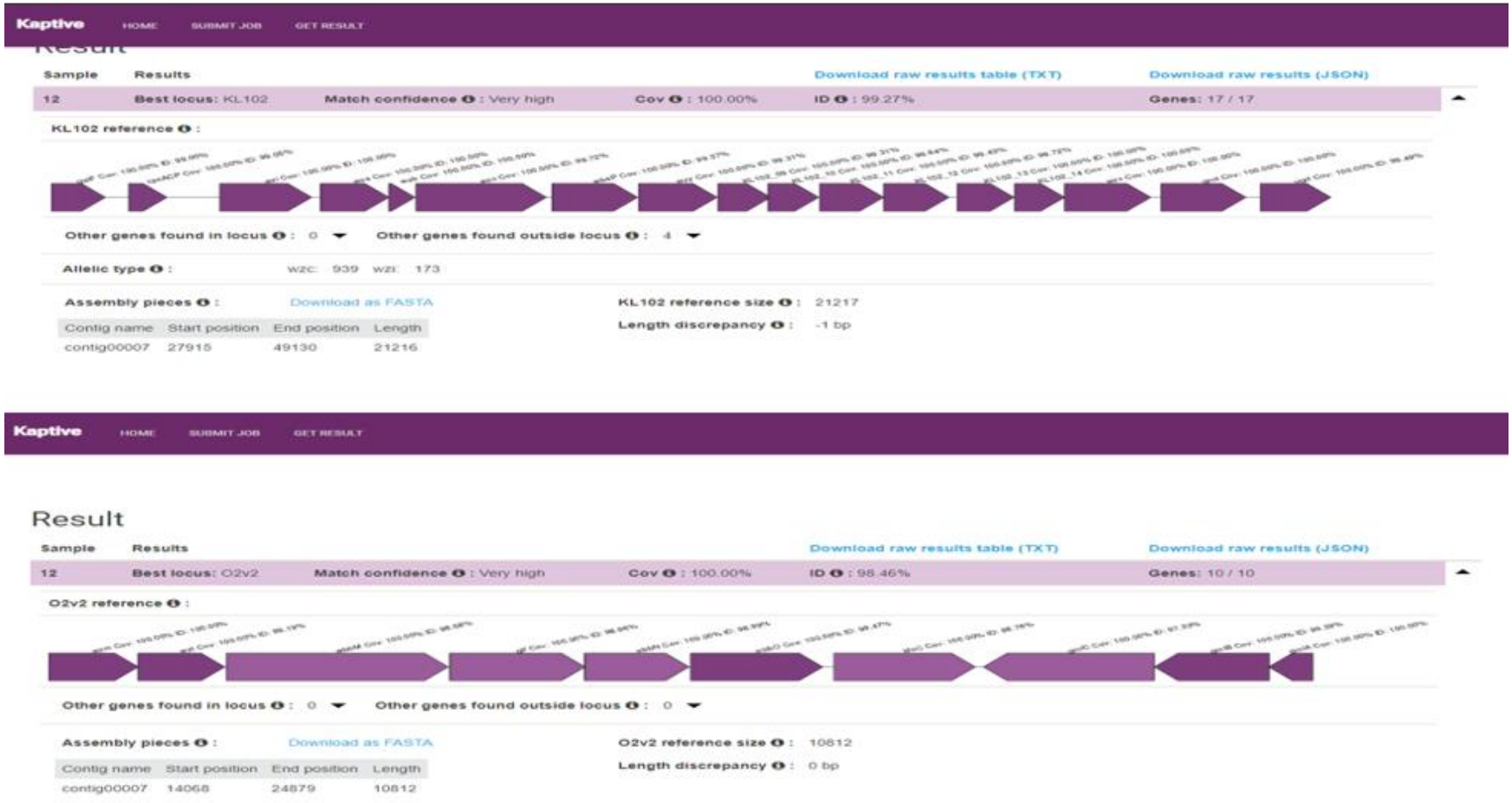


Fig. II (H)

EC03612985