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Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in Gauteng South Africa

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Klebsiella pneumoniae **multidrug-resistant (MDR) high-risk clones drive the spread of antimicrobial resistance (AMR) associated infections, resulting in limited therapeutic options. This study described the genomic characteristics of *K. pneumoniae* MDR high-risk clones in Gauteng, South Africa. Representative carbapenem-resistant [*K. pneumoniae* carbapenemase (KPC)-2, New-Delhi metallo-beta (β)-lactamase (NDM)-1, oxacillinase (OXA)-181, OXA-232, OXA-48, Verona integron-encoded metallo-β-lactamase (VIM)-1] *K. pneumoniae* isolates (n = 22) obtained from inpatient and outpatient's urine (n = 9) and inpatients rectal carriage (n = 13) were selected for short-read whole genome sequencing. *Klebsiella pneumoniae* population include sequence type (ST)-307 (n = 3), ST2497 (n = 5) and ST17 (n = 4). The ST17 strains were exclusively obtained from rectal screening. Ten isolates co-harboured carbapenemase genes including β-lactamase gene encoding KPC-2 + OXA-181, NDM-1 + OXA-48 and NDM-1 + OXA-181. One ST307 isolate (UP-KT-73CKP) co-harboured three carbapenemase genes (bla_{NDM-1} + bla_{OXA-48} + $bla_{OXA-181}$), while all the ST2497 strains co-harboured (bla_{NDM-1} + $bla_{OXA-232}$). Phenotypically, hypermucoviscosity was observed in a single ST307 isolate. The ST307 isolate UP-KT-151UKP harboured colibactin genotoxins. The following mobile genetic elements were detected: plasmids [incompatibility group (Inc)-FIB(K), IncX3], and bacteriophages [e.g. Klebsi_ST16_OXA48phi5.4_NC_049450, Klebsi_3LV2017_NC_047817(36)]. The study highlights the importance of local genomic surveillance systems to characterise *K. pneumoniae* MDR high-risk clones. This data will aid in designing infection and prevention measures for limiting the spread of carbapenemase-producing *K. pneumoniae* in Gauteng, South Africa.**

Keywords Carbapenemase-producing *Klebsiella pneumoniae*, High-risk clones, ST307, IncX3, $bla_{OXA-181}$, Virulence determinants

Klebsiella pneumoniae is one of the clinically relevant priority pathogens responsible for hospital- and community-acquired infections such as liver abscesses, and urinary tract infections (UTIs)[1,2]. Multidrug resistant (MDR) K. *pneumoniae* high-risk clones are important in the spread of carbapenemases locally and globally, revealing a significant public health threat[3–6]. The major well-studied *K. pneumoniae* high-risk clones include those from the multi-locus sequence typing (MLST) clonal group 258 especially, sequence type (ST)-11, ST258, and ST512[7].

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Klebsiella pneumoniae normally colonises mucosal surfaces such as the gastrointestinal tract, which can harbour numerous *K. pneumoniae* clones with antimicrobial resistance (AMR), virulence determinants, and diverse plasmids[8,9]. Classical *K. pneumoniae* (cKp) mostly include MDR high-risk clones, some of which have been reported to co-harbour carbapenemase genes, which limit therapeutic options for *K. pneumoniae* infections[10–12]. Hypervirulent *K. pneumoniae* (hvKp) are known to be susceptible to most antimicrobials however, it is more virulent compared to the cKp[13]. Though the MDR-cKp and hvKp populations remain non-overlapping, traits associated with both pathotypes have been described in *K. pneumoniae* high risk clones (ST101, ST147)[14,15]. The ongoing global convergence of AMR, especially carbapenemase genes and virulence determinants in *K. pneumoniae* epidemic clones indicates an alarming evolution, which deserves continuous monitoring to control and prevent further spread[16]. Whole genome sequencing (WGS) is able to provide a more accurate understanding of the evolution of these epidemic clones[17].

Klebsiella pneumoniae high-risk clones have been linked to several outbreaks in South African hospitals, particularly ST307 associated with incompatibility group (Inc)-X3 plasmids carrying beta (β)-lactamase gene encoding oxacillinase (bla_{OXA})$_{-181}$[4,5,15]. Studies investigating *K. pneumoniae* high-risk clones in South Africa focused on a single clone, and associated AMR genes[4–6]. There is paucity of information on the distribution of *K. pneumoniae* high-risk clones linked to AMR, and virulence determinants. This study aimed to describe the genomic characteristics of *K. pneumoniae* MDR high-risk clones in Gauteng, South Africa using WGS.

Methods

Bacterial isolates, identification, susceptibility and genotyping

This descriptive study analysed clinical isolates of carbapenemase-producing *K. pneumoniae* collected between February 2021 and May 2022. Each isolate was resistant to at least one carbapenem (ertapenem, imipenem and meropenem). Isolates were selected from a total sample of 446 obtained from inpatient and outpatient's urine (n = 194) as well as rectal screening (n = 252) at hospital admission. The susceptibility profiles of all 446 isolates were obtained. Urine isolates were identified using matrix assisted laser desorption ionization-time of flight mass spectrometry [MALDI-TOF MS, Bruker Daltonics, United States (US)]. Antimicrobial susceptibility testing (AST) was performed by disk diffusion method using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints[18]. The rectal carriage isolates were cultured on Chrom-ID CARBA SMART (bioMérieux, Marcy l'Étoile, France) according to manufacturer's instructions. Microbial identification of cultured isolates was confirmed using MALDI-TOF MS (Bruker Daltonics, US) at the Ampath National Reference Laboratory (Ampath-MDRC), Pretoria. Further analysis was performed at the Department of Medical Microbiology, University of Pretoria. The AST of rectal carriage isolates was performed using the VITEK® 2 system (bioMérieux-Vitek, Marcy-l'Étoile, France). String test was performed to determine mucoid phenotype[19]. Isolates were subjected to rapid polymyxin Nordmann/Poirel (NP) test, microbroth dilution testing, and PCR assays for phenotypic and genotypic detection of colistin resistance respectively. Carbapenemase genes [beta (β)-lactamase gene encoding New Delhi metallo-β-lactamase (bla_{NDM}), *K. pneumoniae* carbapenemase (bla_{KPC}), Verona integron-encoded metallo-β-lactamase (bla_{VIM}) and $bla_{OXA\text{-}48\text{-}like}$] were detected using PCR assays with published primers[20,21]. Selection criteria are described in Fig. 1. Since ST307 *K. pneumoniae* high-risk clone is a well-studied AMR clone, endemic to South African hospitals, representative ST307 (n = 36) and all non-ST307 (n = 287) isolates were typed using repetitive extragenic palindromic PCR (REP-PCR) to determine genetic relatedness among isolates. Patients' demographic data were collected. Twenty-two carbapenem-resistant *K. pneumoniae* (CRKp) isolated from urine (n = 9) and rectal carriage (n = 13) were selected based on the presence of the PCR detected carbapenemase genes for short-read WGS to explore the genomic context of carbapenemase-producing *K. pneumoniae* isolates representing different *K. pneumoniae* lineages circulating in Gauteng, South Africa.

Genomic DNA extraction

Genomic DNA (gDNA) was extracted from *K. pneumoniae* cultures using the Zymo Quick-DNA™ Fungal/Bacteria Mini prep Kit [ZYMO Research, US] according to the manufacturer's instructions. To ensure adequate quantity and quality of extracted gDNA, the yield, concentration, and purity were evaluated using the Nanodrop spectrophotometer (ND-1000). The gDNA was stored at – 20 °C.

Whole genome sequencing

The Qubit® 3.0 fluorometer (Invitrogen, Oregon, US) was used for quantification of the extracted gDNA to a concentration of > 10 nanogram/microlitre (ng/µL). Multiplexed, paired-end libraries (2 × 150 bp) were prepared using the Illumina DNA Prep kit (Illumina, San Diego, US), followed by sequencing on the Illumina NextSeq 550 platform (Illumina, San Diego, US) with 100 × coverage.

Bioinformatics analysis

Raw sequencing reads were analysed using the Jekesa pipeline v1.0 (https://github.com/stanikae/jekesa), which included the filtering of reads (Q, ≥ 20; length, ≥ 50) with Trim Galore v0.6.10 (https://github.com/FelixKrueger/TrimGalore), and de novo assembly with SPAdes v3.13.2 (https://github.com/ablab/spades)[22–24]. Sequence types were assigned using the BIGSdb platform curated by the Institute Pasteur (https://bigsdb.pasteur.fr/klebsiella)[25]. Raw reads were deposited in the National Center for Biotechnology Information (NCBI) under the Bio-Project number: PRJNA922902.

Pathogenwatch was used to infer isolates core genome (cg) MLST classification, capsular serotypes, and plasmid replicons[26–32]. The AMR and virulence determinants of the genomes were determined using Kleborate[30,31].

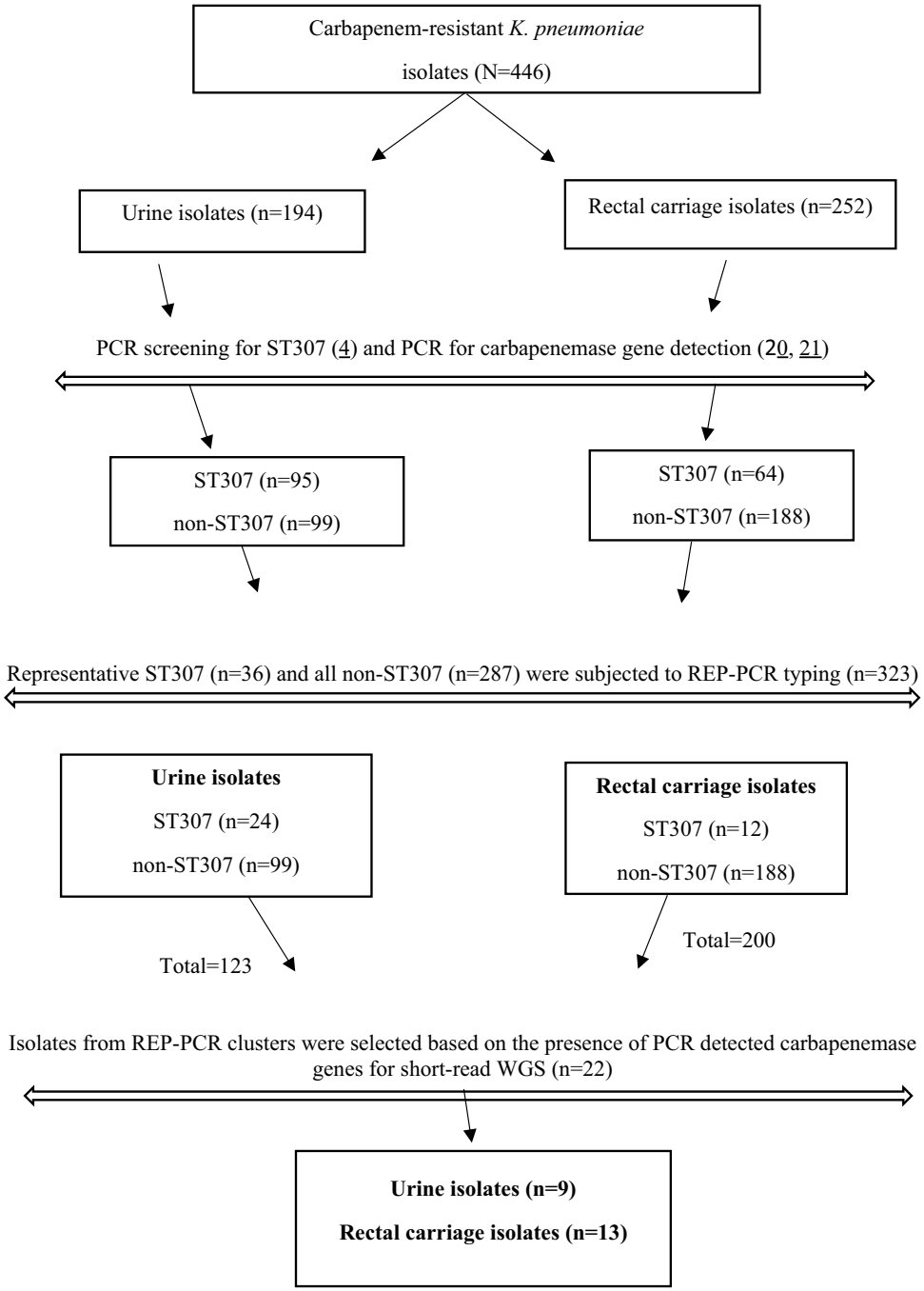

Fig. 1. Description of selection criteria for the representative isolates for whole genome sequencing from the primary data.

Phage prediction was performed with the PHAge Search Tool (PHASTER) for the identification, annotation, and visualisation of prophage sequences[33]. The 22 genomes were run as a query on the Centre for Genomic Epidemiology MobileElementFinder v.1.0.2 to determine the mobile genetic elements (MGEs) associated with AMR and virulence determinants[34]. ResFinder database was used to determine the molecular basis of chromosomal mutations causing resistance on genomes[35].

Phylogenetic analysis of the *Klebsiella* pneumoniae high-risk clones

Klebsiella pneumoniae publicly available reference genomes corresponding to the STs in this study from Australia, India, South Africa, United Kingdom, and the US, retrieved from Pathogenwatch were used to construct a phylogenetic tree[27]. The 22 genomes, reconstructed with ROARY, and aligned with MAFFET, were compared against *K. pneumoniae* reference genomes within the Pathogenwatch[27]. This was performed via BLASTn with species-specific parameter by default based on core gene pairwise SNP distances between genomes[27]. This was

used to construct the neighbour joining trees to demonstrate the phylogenetic relatedness of the isolates[27]. The phylogenetic tree was visualised in FigTree v1.4.4, and annotated using the interactive tree of life (iTol) (https://itol.embl.de)[36,37]. The cgMLST and cg life identification number (cgLN) code information of the genomes were used to confirm the clonality of each ST[26].

Ethics

Ethical approval was obtained from the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria, South Africa (Ethics Reference No: 819/2020). Since clinical bacterial isolates were used for this study, the need for informed consent to participates was waived by the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria, South Africa (Ethics Reference No: 819/2020). The study was performed according to the Declaration of Helsinki. The study methods adhered to the relevant guidelines and regulations.

Results

Baseline characteristics of sequenced isolates

A total of 22 CRKp isolates were analysed. Antibiograms of isolates are shown in Table 1. Among the CRKp isolates, 13 (59%) were obtained from rectal carriage and 9 (41%) from urine (Table 2). Urine isolates were collected from one (11%) outpatient and eight (89%) inpatients, of which five (63%) were admitted to the intensive care unit (ICU). Four of the CRKp isolates were resistant to colistin: minimum inhibitory concentrations (MICs) ≥ 64 µg per millilitre (µg/mL).

Genomic assembly, quality control and phylogenetic tree of the *Klebsiella* pneumoniae high-risk clones

Genome sequencing of isolates generated a collective length of contigs from assembled genomes within a length range of 5.5 mega bp (Mbp) to 6.0 Mbp, with the total assembly genome length (N50) ranging from 34 kilo bp (Kbp) to 215 Kbp. Neighbour-joining phylogenetic trees are shown in Figs. 2 and 3. Core genome SNPs grouped the genomes into eight clonal groups and eight sublineages. The phylogenetic tree (Fig. 2) showed that the CRKp isolates clustered into nine distinct STs, five of which represented ≥ 3 isolates: ST2497 (n = 5), ST17 (n = 4) and ST307 (n = 3). Comparison of the virulence determinants, plasmids, and bacteriophage diversity of the isolates is shown in Fig. 3.

Among the 22 sequenced isolates, three (14%) were ST307 (Table 2). Commonly detected non-ST307 *K. pneumoniae* clones were ST2497 (5/22; 23%), followed by ST17 (4/22; 18%). The ST17 clones were only identified from rectal carriage isolates. Most of the isolates were assigned to capsular serotype KL64 (*wzi64*) (ST147, ST2497), and six O1 (ST2497, ST530) antigen types were identified (Table 2). Isolates belonging to ST307 clustered with ST2975 [ST307 single-locus variant (SLV)].

Klebsiella pneumoniae high-risk clones harbouring multiple carbapenemases and other antimicrobial resistance determinants

Among the 22 sequenced isolates, 10 isolates carried two or more carbapenemase genes (Fig. 2). The ST307 UP-KT-73CKP isolate harboured $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-181}}$. All the ST2497 strains carried $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-232}}$. The most prevalent carbapenemase genes were $bla_{\text{NDM-1}}$ (n = 11) and $bla_{\text{OXA-181}}$ (n = 11). Eight out of the 10 isolates co-harbouring carbapenemases have carbapenem MICs of 16 to > 32 µg/mL (Table 1).

All isolates also harboured extended-spectrum β-lactamase (ESBL) genes (e.g., $bla_{\text{CTX-M-15}}$) with high prevalence as shown in Fig. 2. Two of the four colistin-resistant CRKp isolates carried mutations in the *mgrB* gene, while the other isolates possess an unknown colistin resistance mechanism. Plasmid-mediated colistin resistant genes *mcr-1* to *mcr-9* were not detected. Chromosomal mutations were observed for *parC* encoding topoisomerase IV, and *gyrA* (n = 21) encoding DNA gyrase. Defects in the outer membrane proteins [e.g., *ompK37*(n = 22)] were identified.

Klebsiella pneumoniae high-risk clones ST307 harbouring unusual virulence traits

Isolate ST307 UP-KT-139UKP *K. pneumoniae* strain exhibited a hypermucoviscosity phenotype. The ST307 UP-KT-151UKP harboured siderophore genes encoding yersiniabactin [*ybt17* on integrative and conjugate element (ICEKp1)], and colibactin genotoxin (*clb3*) associated with hvKp (Fig. 3).

Plasmid replicons and bacteriophages associated with *Klebsiella* pneumoniae high-risk clones

Several plasmid replicons (Fig. 2), most commonly IncFIB(K) (n = 14), IncX3 (n = 13), and ColKP3 (n = 13) were identified. Each isolate harboured at least two intact bacteriophages, most commonly Klebsi_ST16_OXA48phi5.4_NC_049450 (n = 8) and Klebsi_3LV2017_NC_047817(36) (n = 8).

Genetic environment of the antimicrobial resistance and virulence genes

Antimicrobial resistance gene clusters, conferring resistance to multiple antimicrobial classes, flanked by MGEs were identified in 12 isolates (Table 3). In ST147 UP-KT-105CKP isolate, $bla_{\text{OXA-181}}$ and *qnrS1* encoding quinolone resistance on ColKP3 plasmid was coupled with virulence gene cluster *irp2*; *fyuA* on ICEKp1in the same contig. Further details regarding the genomic characteristics of the study isolates can be found in the supplementary file 1-Table S1.

Isolate	Sequence types	Patients	Antimicrobial resistance														Carbapenemase genes
Isolate ID		Population	AMP	AMC	TZP	CXM	CAZ	FEP	ETP	IPM[a]	MEM[b]	AMK	GEN	CIP	CST[c]	SXT	
UP-KT-9CKP	ST17	Inpatient/RS*	R	R	R	R	R	R	R	R	R	S	S	R	R	S	$bla_{OXA-181}$
UP-KT-18CKP	ST530	Inpatient/RS*	R	R	R	R	R	R	R	R	R	S	S	R	S	R	bla_{KPC-2}; $bla_{OXA-181}$
UP-KT-64CKP	ST34	Inpatient/RS*	R	R	R	R	R	R	I	R	R	S	S	R	S	S	bla_{VIM-1}
UP-KT-73CKP	ST307	Inpatient/RS*	R	R	R	R	R	R	R	R	R	R	R	R	S	R	bla_{NDM-1}; $bla_{OXA-181}$; bla_{OXA-48}
UP-KT-105CKP	ST147	Inpatient/RS*	R	R	R	R	R	R	R	R	R	R	R	R	R	R	$bla_{OXA-181}$
UP-KT-118CKP	ST2497	Inpatient/RS*	R	R	R	R	R	R	R	R	R	R	I	R	S	R	bla_{NDM-1}; $bla_{OXA-232}$
UP-KT-120CKP	ST147	Inpatient/RS*	R	R	R	R	R	R	R	R	R	I	S	R	S	R	bla_{NDM-1}
UP-KT-132CKP	ST17	Inpatient/RS*	R	R	R	R	R	R	R	R	R	I	R	R	S	R	$bla_{OXA-181}$
UP-KT-162CKP	ST152	Inpatient/RS*	R	R	R	R	R	R	R	R	R	R	S	R	S	R	bla_{NDM-1}; bla_{OXA-48}
UP-KT-190CKP	ST2497	Inpatient/RS*	R	R	R	R	R	R	R	R	R	R	I	R	S	R	bla_{NDM-1}; $bla_{OXA-232}$
UP-KT-202CKP	ST17	Inpatient/RS*	R	R	R	R	R	R	R	R	R	S	R	R	S	S	$bla_{OXA-181}$
UP-KT-221CKP	ST17	Inpatient/RS*	R	R	R	R	R	R	R	S	R	S	R	R	S	R	$bla_{OXA-181}$
UP-KT-228CKP	ST2497	Inpatient/RS*	R	R	R	R	R	R	R	R	R	R	I	R	S	R	bla_{NDM-1}; $bla_{OXA-232}$
UP-KT-23UKP	ST152	Inpatient/urine	R	R	R	R	R	R	R	I	R	R	R	R	S	R	bla_{NDM-1}; $bla_{OXA-181}$
UP-KT-25UKP	ST2497	Inpatient/urine	R	R	R	R	R	R	R	R	R	R	R	R	S	R	bla_{NDM-1}; $bla_{OXA-232}$
UP-KT-70UKP	ST147	Inpatient/urine	R	R	R	R	R	R	R	R	R	R	R	R	S	R	bla_{NDM-1}
UP-KT-74UKP	ST353	Inpatient/urine	R	R	R	R	R	R	R	S	S	S	S	S	S	R	bla_{KPC-2}
UP-KT-118UKP	ST2795	Inpatient/urine	R	R	R	R	R	R	R	R	R	R	R	R	R	R	$bla_{OXA-181}$
UP-KT-128UKP	ST152	Outpatient/urine	R	R	R	R	R	R	R	I	I	R	R	R	S	R	bla_{NDM-1}; bla_{OXA-48}
UP-KT-139UKP	ST307	Inpatient/urine	R	R	R	R	R	R	R	R	R	S	S	R	S	R	$bla_{OXA-181}$
UP-KT-151UKP	ST307	Inpatient/urine	R	R	R	R	R	R	R	S	R	S	S	R	S	R	$bla_{OXA-181}$
UP-KT-195UKP	ST2497	Inpatient/urine	R	R	R	R	R	R	R	R	R	R	R	R	R	R	bla_{NDM-1}; $bla_{OXA-232}$

Table 1. Antimicrobial susceptibility profile and carbapenemase genes associated with the sequenced isolates. *AMP* Ampicillin, *AMC* Amoxicillin/Clavulanate, *TZP* Piperacillin/tazobactam, *CXM* Cefuroxime, *CAZ* Ceftazidime, *FEP* Cefepime, *ETP* Ertapenem, *IPM* Imipenem, *MEM* Meropenem, *AMK* Amikacin, *GEN* Gentamicin, *CIP* Ciprofloxacin, *CST* Colistin, *SXT* Trimethoprim/Sulfamethoxazole. *Rectal screening (RS). **a**IPM MIC range of isolates: susceptible (S) (0.5 to 2 µg/mL); intermediate (I) (4 to 8 µg/mL); resistant (R) (16 to > 32 µg/mL). **b**MEM MIC range of isolates: susceptible (S) (0.5 µg/mL); intermediate (I) (8 µg/mL); resistant (R) (16 to > 32 µg/mL). **c**CST MIC range of isolates: susceptible (S) (≤ 0.5 to 2 µg/mL); resistant (R) (64 to > 64 µg/mL).

Discussion

The spread of carbapenemases causing outbreaks in South African hospitals has been linked to horizontal gene transfer (HGT), and clonal expansion via diverse *K. pneumoniae* clonal lineages, of which few have been investigated individually[4–6]. This study described the genomic characteristics of *K. pneumoniae* MDR high-risk clones in Gauteng, South Africa.

In this study, ST307 isolates were clonally related, and possessed similar traits as the South African ST307 from clade VI [e.g. $bla_{OXA-181}$, IncX3, KL102 (*wzi173*), *parC* and *gyrA*][4,5]. This is evidence that similar ST307 strains are circulating in South Africa. The majority (80%) of isolates co-harbouring carbapenemases showed high carbapenem MICs (16 to > 32 µg/mL). Additionally, evolutionary development was observed among ST307 isolates: ST307 UP-KT-73CKP isolate co-harboured multiple carbapenemase genes (bla_{NDM-1}, bla_{OXA-48} and $bla_{OXA-181}$), similarly to a recent Chinese study, where a ST307 isolate co-harboured bla_{KPC-2}, bla_{NDM-1} and bla_{IMI-3}[12]. The

Profile	Sequence types detected								
	ST307	ST2497	ST17	ST147	ST152	ST2975	ST530	ST34	ST353
No of isolates (n = 22)	3	5	4	3	3	1	1	1	1
Isolates type									
Urine	2	2	0	1	2	1	0	0	1
Rectal carriage	1	3	4	2	1	0	1	1	0
Patient population									
Inpatients/urine	2	2	0	1	1	1	0	0	1
Outpatient	0	0	0	0	1	0	0	0	0
Inpatients/rectal screening	1	3	4	2	1	0	1	1	0
Ward type									
ICU	1	1	0	1	0	1	0	0	1
General	1	1	0	0	0	0	0	0	0
Rehabilitation	0	0	0	0	1	0	0	0	0
Outpatient	0	0	0	0	1	0	0	0	0
Unknown	1	3	4	2	1	0	1	1	0
Capsule (K) serotype									
KL25 (*wzi*141)	0	0	4	0	0	0	0	0	0
KL102 (*wzi*173)	3	0	0	1	0	1	0	0	0
KL64 (*wzi*64)	0	4	0	3	0	0	0	0	0
KL149 (*wzi*110)	0	0	0	0	3	0	0	0	0
KL54 (*wzi*115)	0	0	0	0	0	0	1	0	0
KL14 (*wzi*-unknown)	0	0	0	0	0	0	0	1	0
KL110 (*wzi*346)	0	0	0	0	0	0	0	0	1
KL111 (*wzi*113)	0	0	0	0	0	0	0	0	0
O serotype									
O1	0	5	0	0	0	0	1	0	0
O2a	0	0	0	2	0	0	0	0	0
o2afg	3	0	0	1	0	1	0	0	0
O3b	0	0	0	0	0	0	0	0	1
O4	0	0	0	0	3	0	0	0	0
O5	0	0	4	0	0	0	0	0	0
OL104	0	0	0	0	0	0	0	1	0
Antimicrobial resistance genes									
ESBLs									
CTX-M-15	2	5	4	3	3	1	1	0	1
TEM-	1	5	4	3	3	0	1	0	1
SHV-	3	5	4	3	3	1	1	1	1
Aminoglycosides									
aac(6')Ib-cr	1	4	3	2	3	1	1	1	1
aac-IIa	1	1	4	2	2	0	0	1	0
aadA	1	4	3	2	3	1	1	1	1
Fluoroquinolones									
qnrS1	2	1	4	3	1	1	1	0	0
qnrB-	1	5	0	2	3	1	0	0	0
Trimethoprim/Sulfamethoxazole									
dfrA-	3	5	3	3	3	1	1	0	1
sul1-2	3	5	3	3	3	1	1	1	1
Clonal group	307	10190	17	147	152	307	530	13050*	353
Sublineage	307	395	17	147	152	307	530	34	353

Table 2. Characteristics of the ST307 and non-ST307 *Klebsiella pneumoniae* clones in this study. *New clonal group.

ST307 UP-KT-73CKP also harboured plasmids such as IncX3 with the highest number of bacteriophages, which might aid in its acquisition of new AMR traits, and increased virulence via HGT[38–40]. The ST307 UP-KT-139UKP

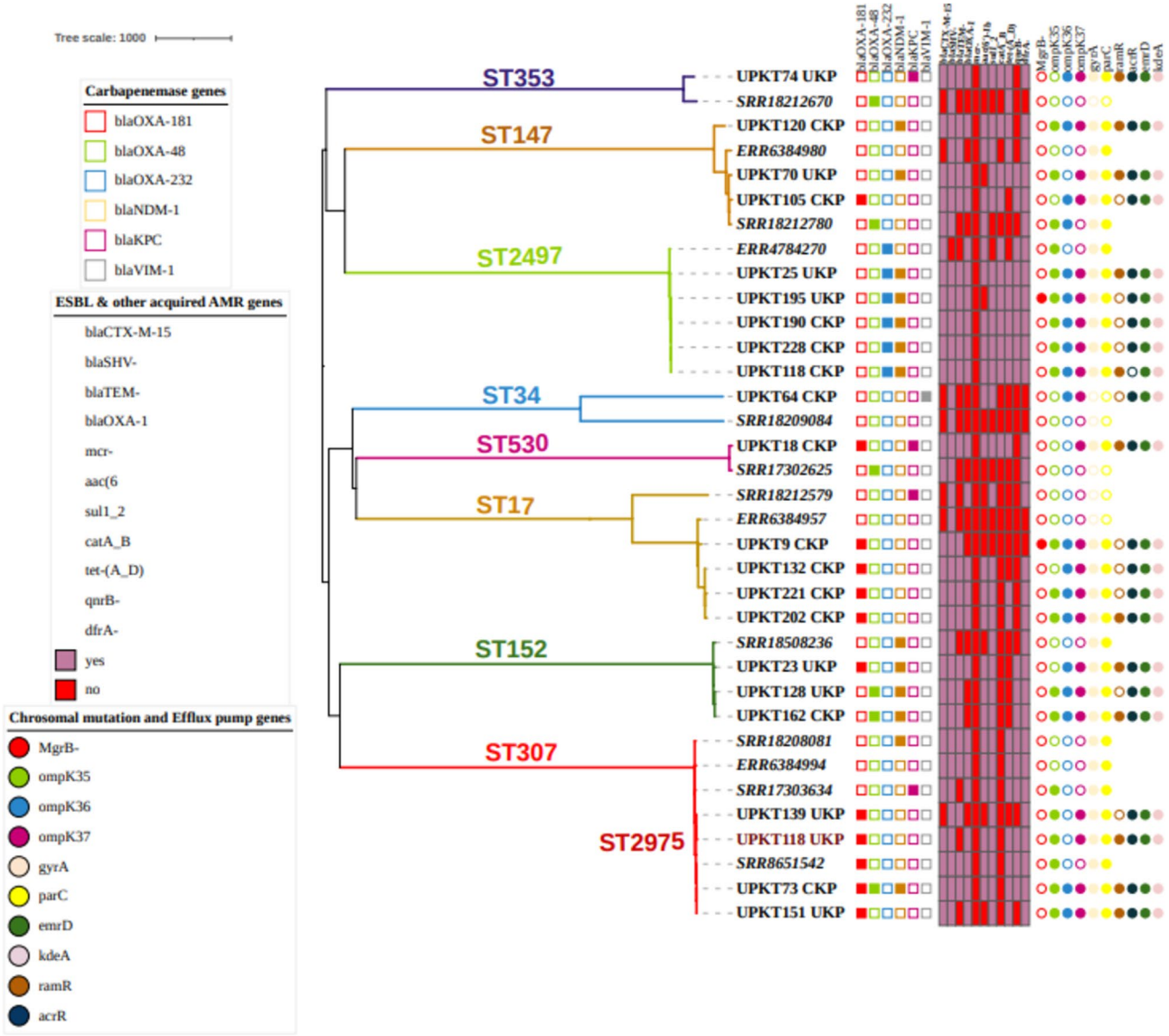

Fig. 2. Phylogenetic tree of the *Klebsiella pneumoniae* high-risk clones showing accessory genome. The 22 study isolates reconstructed with ROARY and aligned with MAFFET compared against reference genomes within Pathogenwatch via BLASTn. The tree branches (n = 35) indicate isolate names with respective STs (colours) and 13 K. *pneumoniae* reference genomes from Pathogenwatch database. The outgroup was not shown. Isolate branches tips are coloured by the metadata columns showing accessory genome (showing the presence of carbapenemase genes in filled squares), heatmap of ESBL and other acquire AMR genes in purple and red, chromosomal mutation and efflux pump genes in filled circles.

isolate lacked virulence determinants, but possess *iutA*, which is usually associated with hvKp. Similarly, a case study in India reported a clinical isolate exhibiting hypermucoviscosity phenotype, but lacked hypervirulence determinants [e.g. regulator of mucoid phenotype (*rmp*)-*A*][41]. A ST307 UP-KT-151UKP isolate acquired *ybt17* on ICEKp1 and colibactin genotoxin. These virulence traits have been associated with hvKp causing invasive and life-threatening infections such as sepsis[42,43]. Studies have shown the presence of colibactin among ST11 *K. pneumoniae* clone, but it has not been linked to ST307 until now[44,45]. Acquisition of virulence determinants can further enhance *K. pneumoniae* pathogenicity[45]. However, increased virulence of colibactin-carrying strains in the absence of a virulence plasmid has not been well established[31].

The non-ST307 *K. pneumoniae* high-risk clone ST2497 (n = 5) exhibited a high level of AMR harbouring double carbapenemase genes (bla_{NDM-1} and $bla_{OXA-232}$). The ST2497 strains were linked to other AMR mechanisms, and several MGEs, consistent with a case report in the US, in which the strain was resistant to numerous antimicrobials, except tigecycline and colistin[46]. The ST2497 in this study exhibited pandrug resistant (PDR) and extensively drug resistant (XDR) phenotypes. This is worrisome because three of these ST2497 strains were obtained from rectal carriage of patients, who might be colonised by CRKp. This may cause person-to-person transmission within the hospital and community settings or possible development of subsequent ST2497 infection[47].

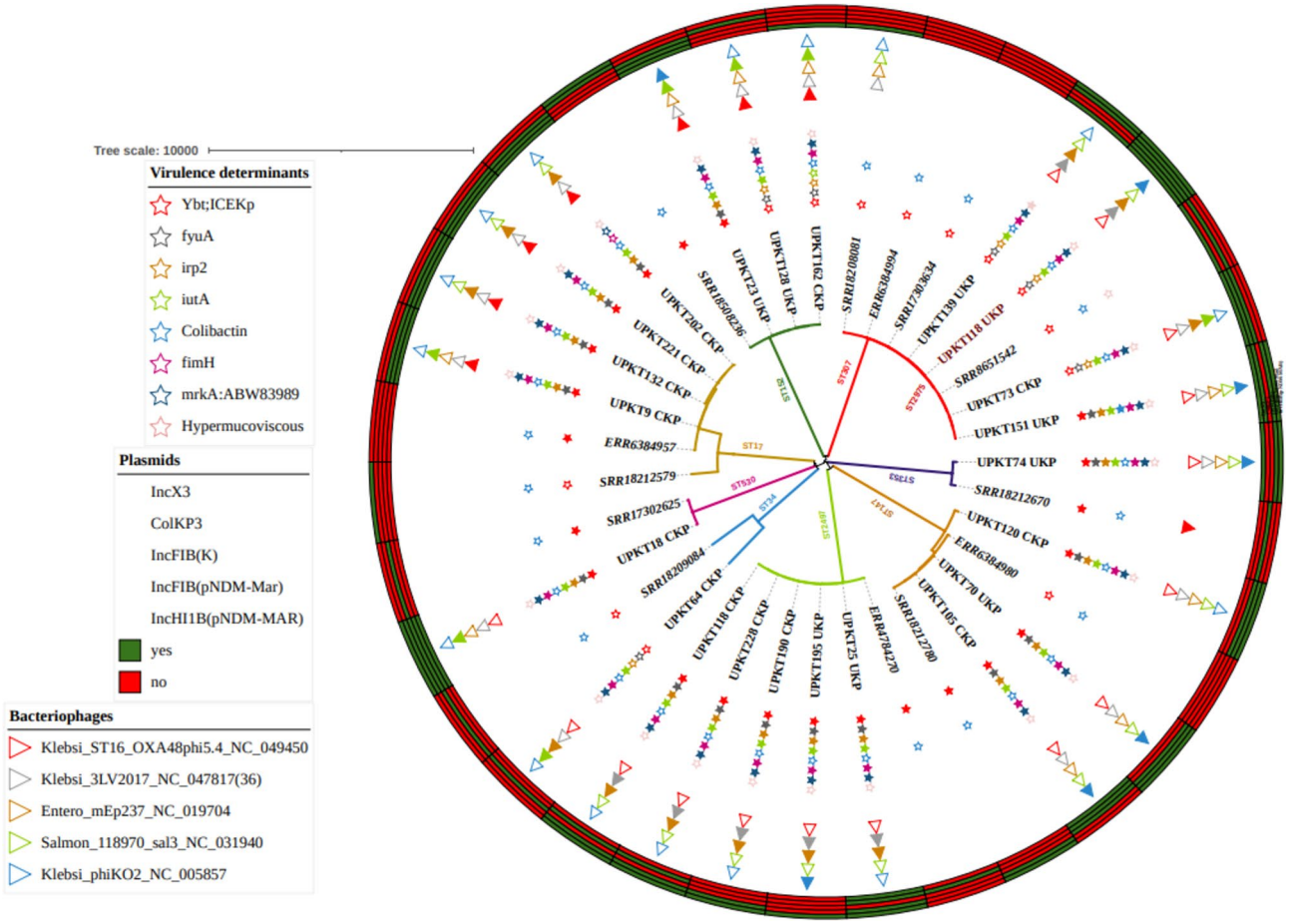

Fig. 3. Neighbour-joining phylogenetic tree displaying the relationship of the *Klebsiella pneumoniae* high-risk clones based on virulence, bacteriophages and plasmids. Relationship among isolates sequenced in this study and similar strains obtained from the Pathogenwatch database (details in Fig. 1). Public *K. pneumoniae* genomes were obtained from Australia, India, South Africa, United Kingdom and the US. Clonality of the isolates were confirmed using cgMLST and cgLIN code information from Pathogenwatch. Isolate branch tips are coloured by metadata columns showing the presence of virulence determinants in filled star, the commonly detected bacteriophages in filled triangle and heatmap of plasmids in green and red filled squares.

Klebsiella pneumoniae co-harbouring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-232}}$ was first reported in the US in 2014 in ST14[48]. It was later reported in other STs in Italy, Malaysia, South Korea and recently in France in ST2497[49–52]. Resistance of *K. pneumoniae* strains harbouring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-232}}$ to the last-resort antimicrobials including the combination therapy like ceftazidime/avibactam have been reported[46,52]. The reported resistance might be because ceftazidime/avibactam is highly active against OXA-48-like carbapenemases but lacks activity against strains producing NDM and other metallo-β-lactamases[53,54]. There is a need for antimicrobials active against metallo-β-lactamases-producing strains such as cefiderocol or aztreonam with ceftazidime/avibactam combinations[53,54].

The ST2497 associated plasmids IncHI1B(pNDM-MAR) and Col(pHAD28) have been linked to strains co-harbouring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-232}}$[55]. The ColE-type plasmid carrying $bla_{\text{OXA-232}}$ was isolated from patients returning from India, indicating travel-related transmission[56]. The $bla_{\text{OXA-232}}$ differs from $bla_{\text{OXA-181}}$ by one amino acid substitution, but both share similar genetic environments[57]. The presence of OXA-232 with NDM in a region like South Africa, where OXA-181 is highly prevalent might lead to a significant treatment challenge. The combination therapy available presently in South Africa are partly effective against this mechanism of resistance[58]. The IncHI1B-like plasmid carrying $bla_{\text{NDM-1}}$ was suggested to have been transferred from *Acinetobacter* spp. to *K. pneumoniae* via transpositional events[47,59]. The possible transfer of AMR genes by plasmids between diverse strains and species of bacteria are a threat to human health. This present study reported the occurrence of ST2497 co-harbouring $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-232}}$ in South Africa. Limited therapeutic options available against this clone might result in poor patient outcomes and high mortality rates. As evidenced in a recent outbreak of NDM-1 and OXA-181 CRKp among neonates resulting in a 64% (9/14) mortality rate in South Africa[60]. Continuous genomic surveillance of carbapenem-resistant *Enterobacterales* (CRE) is needed to monitor and control the spread into the community.

The ST2975 clone isolated from an inpatient in the ICU originated from ST307 via a SLV (mutation in *rpoB* allele). This fact highlights the potential expansion of ST307 as a persistent nosocomial pathogen[61]. This ST2975 may pose a risk of acquiring additional AMR traits, which might result in effective transmission of extensive AMR

Isolates	Sequence types	Plasmids	Transposon	Insertion sequences	ICE	AMR determinants	Virulence genes
UP-KT-9CKP	ST17	ColKP3; IncFIB(K)	–	ISKra*4*; IS*1*; IS*5*; IS*66*	–	OXA-181; CTX-M-15; TEM1B; *aac-Iia*; *qnrS1*	–
UP-KT-18CKP	ST530	IncHI1B; IncX3; IncFII	Tn*6196*	IS*481*; ISL*3*; IS*3*	–	KPC2; TEM-40; TEM-150; TEM-171; TEM-1A; TEM-1C	–
UP-KT-73CKP	ST307	ColKP3; IncFIB(K); IncX3	Tn*5403*	IS*110*; IS*5*; ISKra*4*; IS*5*; ISL*3*; IS*630*; IS*5*	–	OXA-181; *qnrS1*; TEM-1B; *sul2*	–
***UP-KT-105CKP**	**ST147**	**ColKP3**	**–**	**ISKra*4***	**ICEKp1**	**OXA181; *qnrS1***	***irp2*; *fyuA***
UP-KT-118CKP	ST2497	IncFIB(pQil)	Tn*6196*	IS*5*; IS*6*; ISL*3*	–	*sul1*; *dfrA14*	–
UP-KT-120CKP	ST147	–	–	ISL3	–	OXA-1; *sul1*; *dfrA12*	–
UP-KT-132CKP	ST17	ColKP3; IncFIB(K)	–	ISKra*4*; IS*1380*; IS*6*; IS*3*; IS*5*	–	OXA181; *qnrS1*; TEM-1C; CTX-M; SHV-172; SHV-94; SHV-96	–
UP-KT-162CKP	ST152	IncFIB(K); IncFIB(pB171)	–	IS*1380*; IS*48*; IS*3*	–	TEM-1B; CTX-M-15	–
UP-KT-190CKP	ST2497	IncHI1B; IncFIB(pQil)	Tn*6196*	IS*5*; IS*110*; ISL*3*	–	*aadA*(homolog); *sul1*; *cmlA1*	–
UP-KT-202CKP	ST17	ColKP3; IncX3	–	ISKra*4*; IS*1380* (truncated); ISL*3*	–	*qnrS1*; OXA-181; CTX-M-15	–
UP-KT-221CKP	ST17	ColKP3	–	IS*3*; ISKra4; IS*1380*	–	OXA-181; *qnrS1*; CTX-M-15	*fimH*; *mrkA*: *ABW83989*
UP-KT-228CKP	ST2497	IncFIB(pQil)	Tn*6196*	IS*630*; IS*5*; IS*6*; ISL*3*	–	NDM-1; *sul1*; *dfrA14*	–
UP-KT-25UKP	ST2497	–	Tn*6196*	IS*4*	–	*aadA1*; *cmlA1*; *sul1*	–
UP-KT-70UKP	ST147	IncFIB(K)	–	ISKra*4*; IS*91*; IS*1380*; IS*66*; IS*110*	–	CTX-M-15; NDM-1; *qnrS1*; *sul2*	–
UP-KT-74UKP	ST353	–	Tn*6196*	IS*6*; IS*3*	ICEKp1	OXA-10; *aadA1*; *cmlA1*; *sul1*; *dfrA23*; *dfrA14*	*irp2*; *fyuA*
UP-KT-118UKP	ST2795	ColKP3; IncFIB(pNDM-Mar); IncFIB(K)	–	ISKra*4*; IS*1380*; IS*630*; IS*481*; IS*5*; IS*110*	–	CTX-M-15, OXA-181; *qnrS1*; *sul1*; *aadA2*; *dfrA12*,	–
UP-KT-128UKP	ST152	IncFII	–	IS*1380*; ISL*3*	–	TEM-1B; CTX-M-15; *sul*2	–
UP-KT-139UKP	ST307	ColKP3; IncHI1B	Tn*5403*	ISKra*4*; IS*110*	–	OXA-181; *qnrS1*	–
UP-KT-151UKP	ST307	ColKP3	Tn*5403*; Tn*6196*	ISKra*4*	–	OXA-181; *qnrS1*; *aadA1*; *cmlA1*; *sul1*; *dfrA15*	–
UP-KT-195UKP	ST2497	IncFIB(pQil)	Tn*6196*	IS*5*; IS*6*; ISL*3*	–	*aadA1*; *cmlA1*; *sul1*; *dfrA14*	–

Table 3. Mobile genetic elements associated with antimicrobial resistance and virulence genes among the *Klebsiella pneumoniae* high-risk clones. *Bold indicate the isolate harbouring both AMR genes on plasmid, and virulent determinants on ICE.

in hospitals, and thus community spread[61]. The ST2975 and a PDR ST147 UP-KT-105CKP *K. pneumoniae* high-risk clone were phenotypically resistant to colistin (MIC = 64 µg/mL) however, colistin resistance mechanisms were unknown. This is worrisome because colistin in combination with other antimicrobials is currently used as a last-resort treatment for severe CRE infections in South Africa. The available β-lactam/β-lactamase inhibitor combinations (e.g. ceftazidime/avibactam) in South African hospitals are expensive, and not easily accessible[62]. There is a need for further investigation into the colistin resistance mechanisms. It is concerning that within the same contig of the PDR ST147, AMR genes and virulence determinants were found on a ColKP3 plasmid associated with other MGEs. Similar to an outbreak in Italy linked to NDM-1-producing ST147, which harboured numerous virulence determinants[14]. This might be an indication of evolutionary development through mutation, which might result in the emergence of a convergence clone (MDR-hvKp)[13,63].

All the ST17 strains harboured $bla_{\text{OXA-181}}$, $bla_{\text{CTX-M-15}}$, several plasmids and capsular serotypes. The ST17 strains were exclusively obtained from rectal carriage, which might be related to colonisation. There has been an indication that ST17 infections occurs mostly by colonising strains, rather than causing outbreaks due to clonal expansion[64]. The divergence of ST17 to many sublineages with several AMR genes, virulence determinants, and plasmids has been attributed to the recombination of the K and O loci[64].

This current descriptive study was limited by a small sample size, so it cannot be concluded that the *K. pneumoniae* clones were dominant or emerging. It was inconclusive that the urine isolates were solely from patients who had UTIs due to lack of access to the clinical information. The patients screened for CRKp colonisation had no documented history of exposure to the healthcare facilities, so it cannot be concluded that the colonisation was exclusively from the community settings. Short-read sequencing did not provide full description of the plasmids as compared to long-read sequencing. However, this study provided a detailed view of the genomic and evolutionary relationship of the circulating *K. pneumoniae* high-risk clones in Gauteng, South Africa using short-read WGS.

Conclusion

This study revealed the importance of diverse multiple clonal lineages, and HGT through MGEs especially plasmids in the spread of carbapenemases and virulence determinants in South Africa. This study highlights the importance of local genomic surveillance systems to characterise *K. pneumoniae* MDR high-risk clones. The generated data will aid in designing infection and prevention measures for limiting the spread of *K. pneumoniae* with carbapenemases in Gauteng, South Africa.

Data availability

All short-reads and assemblies associated with this study are available at NCBI under Bio-Project number: PRJNA922902; with individual BioSamples details. The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher. Anyone interested in using the data for scientific purposes is free to request permission from the corresponding author.

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