BMC Microbiology

RESEARCH

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Drug resistant *Klebsiella pneumoniae* from patients and hospital effluent: a correlation?

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Abstract

Background The application of wastewater-based epidemiology has gained traction as a cost effective tool in antimicrobial resistance (AMR) surveillance with studies showing a correlation between the presence of resistant bacteria from hospital sewage and patients. This study compared *Klebsiella pneumoniae* from patients and hospital effluent in terms of antibiotic resistance patterns, antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) and phylogenomic relationships.

Results Pooled effluent samples were collected from the final effluent point of a regional hospital and *K. pneumoniae* isolates were identified on selective media. Clinical isolates were also collected from the same hospital. Antimicrobial susceptibility testing (AST) was performed using the VITEK® 2 system. DNA was extracted prior to whole genome sequencing (WGS). The resistome, mobilome, and phylogenetic lineages of sequenced isolates were assessed using bioinformatics analysis. A total of 10 randomly selected presumptive and 10 clinical *K. pneumoniae* constituted the sample and were subjected to AST. Total resistance was observed in the clinical samples to cefuroxime, cefotaxime, piperacillin/tazobactam, gentamicin, tobramycin and trimethoprim/sulfamethoxazole. The effluent isolates exhibited total susceptibility to most antibiotics but showed resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam (100%), and tigecycline (10%). The effluent isolates did not exhibit a diverse resistome, while the clinical isolates harboured genes conferring resistance to aminoglycoside (*aph(6)-Id*, *aph(3")-Ib*, *aac(6')-Ib-cr*, *aadA16*), ß-lactam (bla_{SVH} group, bla_{OXA} group, bla_{TEM} group), and fluoroquinolone (*oqxA*, *oqxB*) antibiotics. Only class 1 integrons were identified. Phylogenetic analysis revealed that effluent isolates from this study were not closely related to the clinical isolates.

Conclusion This study showed no correlation between the resistance profiles of the clinical and effluent isolates. The relationship between AMR in hospital effluent and clinical resistance may depend on the antimicrobial agents and bacterial species studied.

Keywords *Klebsiella pneumoniae*, Antibiotic resistance, Hospital effluent, South Africa

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Background

Klebsiella pneumoniae is a Gram-negative opportunistic bacterium that forms part of the normal flora of the skin, mouth and gut. *K. pneumoniae* belongs to the order Enterobacterales, and is a major cause of hospital-acquired infections. It is mainly responsible for urinary, respiratory and bloodstream infections [1, 2]. It is part of the *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp, and *Escherichia coli* (ESKAPEE) group of pathogens that are increasingly associated with several multi-drug resistant nosocomial infections [3]. They carry antibiotic resistance genes (ARGs) on mobile genetic elements (MGEs) that encode resistance to the most commonly-used classes of antibiotics [4, 5]. These ARGs and MGEs are present in various environments, and can spread among different species of microorganisms, humans, animals, soil, and water [6]. Carbapenem-resistant and 3rd -generation cephalosporin-resistant Enterobacterales are on the World Health Organization's (WHO) bacterial priority pathogens list for research and development of new antibiotics [7].

The development and rapid spread of antibiotic resistance (AR) increases the risk of common infections becoming impossible to treat [8]. AR, primarily caused by use, misuse or overuse of antimicrobial agents, is a serious global health concern claiming above 700 000 lives each year [9]. AMR is an increasing threat to healthcare systems resulting in the reduced ability of antimicrobial therapies to effectively combat infections and leading to increased morbidity and mortality rates [10]. Drug-resistant bacteria are usually spread by direct contact of the infected patient with staff, other patients, and visitors. Clinical surfaces and hospital effluent are potential reservoirs of multi-drug-resistant (MDR) bacteria [11].

Hospital effluent serves as a channel for the disposal of waste materials, including, faecal matter, clinical and biological waste, and used clinical supplies that could potentially be contaminated with pathogenic bacteria [11, 12]. Such effluent contains large quantities of diverse ARGs and their associated MGEs [4]. This makes them reservoirs and hotspots for the prevalence and dissemination of drug-resistant strains via effluent [12, 13]. Hospital effluents are an ideal environment for the exchange of resistance genes between clinical and environmental pathogens, given the mixed population of various bacteria, antimicrobial agents, and nutrients in the sewage environment [14, 15].

This study delineates the prevalence and antibiotic resistance profiles of *Klebsiella pneumoniae* from hospital effluent in comparison with clinical isolates by whole genome sequencing and bioinformatics to delineate their resistomes, mobilomes, and phylogeny in a regional hospital, KwaZulu-Natal.

Methods

Sample collection

Hospital waste water (HWW) samples were collected from the final effluent discharge point that receives effluent from all the hospital wards and buildings at a regional hospital which has a bed capacity of 897 beds. A composite sampling method was used, where water samples were manually grabbed over a period of 3 h (30 s intervals); and pooled to create one sample. Clinical isolates on agar plates were received weekly for the duration of the study from the National Health Laboratory Services (NHLS). Sampling was conducted during November 2023.

Bacterial isolation and identification

The membrane filtration technique was used to process the effluent samples where water samples were passed through a porous membrane (0.45 µm) (ISOLAB Laborgerāte GmbH, Eschau, Germany) using a filter funnel and vacuum system (ISOLAB Laborgerāte GmbH, Eschau, Germany). The membrane filter was applied onto agar plates containing selective media for *K. pneumoniae* identification, Simmons Citrate Agar (Merck, Darmstadt, Germany) supplemented with Myo-Inositol (SCAI) and adjusted to a pH of 7.2 was used. After 24 h at 37 °C, yellow, moist colonies were produced.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed using the VITEK® 2 System AST (bioMérieux, North Carolina, USA) card N-256 for Gram-Negative for the following panel of antibiotics: cefuroxime, ceftazidime, cefotaxime, cefepime, amoxicillin/clavulanic acid, piperacillin/tazobactam, imipenem, doripenem, meropenem, ertapenem, gentamicin, tobramycin, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole and tigecycline.

Whole genome sequencing and bioinformatics

Genomic deoxyribonucleic Acid (DNA) was extracted from selected isolates exhibiting phenotypic resistance to at least one antibiotic. DNA was extracted using the GenElute bacterial genomic DNA kit (Sigma Aldrich, St. Loius, MO, USA). The quality of the DNA was assessed using NanoDrop Lite Plus (Thermo Fisher Scientific, Wilmington, Delaware, USA) at 260/280 nm wavelength. WGS using the Illumina Miseq machine (Illumina, San Diego, California, USA) was performed at the National Institute for Communicable Diseases (NICD) Sequencing Core Facility. Raw reads were trimmed using Sickle (https://github.com/najoshi/sickle) for quality trimming and assembled using the SPAdes assembler. Resistance genes were identified using ResFinder (http://genepi.food.dtu.dk/resfinder) [16, 17] at a threshold ID of 90% and minimum length of 60%. PlasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/) was used to ide

ntify the plasmid replicons. The INTEGRALL database was used to identify and classify integrons and gene cassettes. NCBI's PGAP and ISFinder (https://isfinder.biotoul.fr/) were used to annotate transposons and insertion sequences. The Comprehensive Antimicrobial Resistance Database (CARD) was used to identify the efflux pump proteins (https://card.mcmaster.ca/) [18]. MLST 2.0 (https://cge.food.dtu.dk/services/MLST/) was used to identify the sequence types of the isolates. The sequences were submitted to GenBank and assigned accession numbers under BioProject PRJNA1143548 (Supplementary Table S1).

Phylogenomic analysis

Whole genome sequences of *K. pneumoniae* isolates from this study were compared to clinical and environmental isolates from South Africa and other African countries curated at the BV-BRC (https://www.bv-brc.org/) website. The downloaded sequences for each organism were used alongside the sequences from this study to construct a phylogenetic tree on BV-BRC. The constructed phylogenetic tree was annotated, viewed and edited using iTOL version 7 (https://itol.embl.de/).

Results

Prevalence of *Klebsiella pneumoniae* in clinical samples and hospital effluent

A total of 20 *Klebsiella pneumoniae* isolates from clinical (10) and hospital effluent (10) were subjected to antimicrobial susceptibility testing and WGS. Sixteen of the isolates were positively identified as *K. pneumoniae* by WGS. These consisted of 10 effluent and six clinical isolates. The non - *K. pneumoniae* isolates identified after WGS included *Klebsiella quasi-pneumoniae*, *Klebsiella variicola* and *Klebsiella aerogenes* (2). The results presented relate only to the positively identified *K. pneumoniae* isolates.

Antibiotic susceptibility profiles

The clinical *K. pneumoniae* isolates showed overall high resistance rates compared to the effluent isolates, which can be explained by use of these antibiotics in the clinical setting. All the clinical isolates were resistant to at least seven or more antibiotics belonging to three or more classes, while the effluent isolates were resistant to at least two antibiotics. High (100%) resistance rates were observed in the clinical *K. pneumoniae* against cefuroxime, cefotaxime, piperacillin/tazobactam, gentamicin, tobramycin and trimethoprim/sulfamethoxazole (Fig. 1).

The effluent isolates exhibited total susceptibility against all tested antibiotics except amoxicillin/clavulanic acid and piperacillin/tazobactam to which they were 100% resistant and tigecycline (10%). Only 33.33% of the clinical isolates were resistant to the carbapenem antibiotics and the same isolates showed resistance to amikacin (Fig. 1).

Genomic characteristics

The genomic features of the *Klebsiella pneumoniae* are presented in terms of genome size, GC content, sequence length, L50 and N50 values in the supplementary material (Table S1).

Antibiotic resistance genes

The clinical *K. pneumoniae* isolates harboured more resistance genes compared to the effluent isolates. All the clinical isolates carried several ß-lactam resistance genes, with the most common genes being $bla_{CTX-M-15}$ and bla_{TEM-1B} found in all six isolates. In the effluent isolates, only 50% harboured the ß-lactam resistance gene bla_{SHV-1}, which is responsible for intrinsic resistance to ampicillin in *K. pneumoniae*, whereas in the clinical isolates, five different bla_{SHV} genes were identified (bla_{SHV-81}, bla_{SHV-94}, bla_{SHV-96}, $bla_{SHV-110}$, $bla_{SHV-172}$). $Bla_{SHV-187}$ was found only in one effluent isolate. The bla_{NDM-1} gene was only present in the two (33.33%) clinical isolates. The extended-spectrum β-lactamase (ESBL) bla_{SHV} ($bla_{SHV-172}$, bla_{SHV-96}, bla_{SHV-94}, $bla_{SHV-110}$ and bla_{SHV-81}), $bla_{CTX-M-15}$, and bla_{TEM} ($bla_{TEM-206}$, $bla_{TEM-141}$, bla_{TEM-1B} and $bla_{TEM-214}$) genes were co-carried in the clinical isolates (Table 1).

Aminoglycoside resistance genes (*aph(6)-Id*, *aph(3")-Ib*, *aac(6')-Ib-cr* and *aadA16*) were the most common among the clinical isolates. The effluent isolates did not harbour any aminoglycoside resistance genes, which was in line with their phenotypic resistance patterns (Table 1).

The clinical isolates also harboured the fluoroquinolone resistance genes *qnrB1*($n=1$), *qnrB6*($n=2$) and *qnrS1*($n=2$). *OqxA* and *oqxB* that encode the multidrug efflux pump *oqxAB* was found in five (83.33%) of the six clinical isolates. The effluent isolates, although not phenotypically resistant to a number of the tested antibiotics, also harboured the genes encoding the multidrug efflux pump *oqxAB*. Trimethoprim-sulfamethoxazole resistance genes *sul1*, *sul2*, and *dfrA27* were carried by five of the clinical isolates, while the other one harboured *sul2* with *dfrA14*.

No tetracycline resistance genes were carried by the clinical isolates that showed resistance to tigecycline, while the effluent *K. pneumoniae* showed the tetracycline resistance gene *tetD*, although only one showed phenotypic resistance to tigecycline. Five of the clinical isolates carried the *qacE* gene that encodes resistance to quaternary ammonium compounds.

Mobile genetic elements and their association with ARGs

The effluent *K. pneumoniae* isolates had only one plasmid replicon type present (IncFIB(K)), which was also

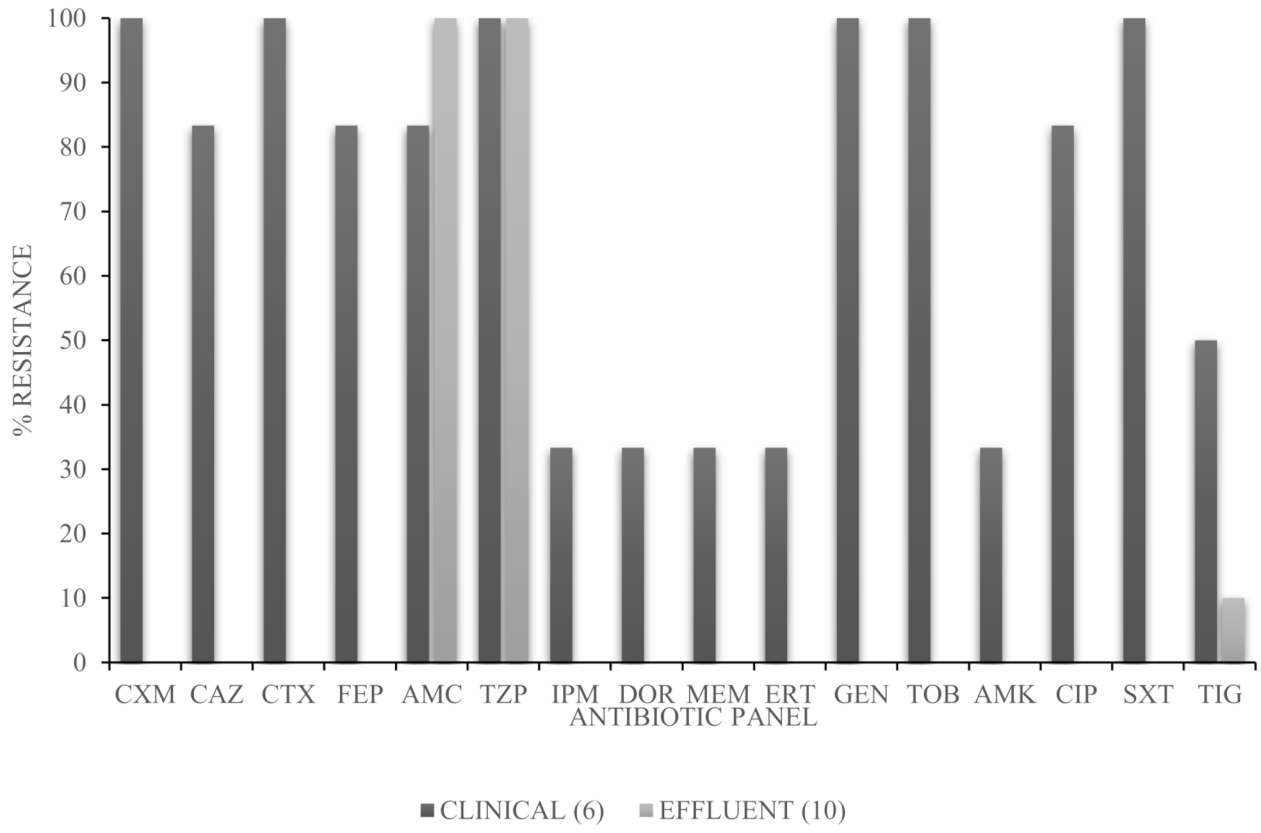

Fig. 1 Antibiotic resistance rates of the *K. pneumoniae* from human and hospital effluent
KEY: *CXM = cefuroxime, CAZ = ceftazidime, CXT = cefotaxime, FEP = cefepime, AMC = amoxicillin/clavulanic acid, TZP = piperacillin/tazobactam, IPM = imipenem, DOR = doripenem, MEM = meropenem, ERT = ertapenem, GEN = gentamicin, TOB = tobramycin, AMK = amikacin, CIP = ciprofloxacin, SXT = trimethoprim/sulfamethoxazole, TIG = tigecycline*

common in the clinical isolates (Table 2). Other plasmid replicon types in the clinical isolates included IncFIA(HI1), IncFII(K), IncHI1B(pNDM-MAR), repB(R1701), colKP3, IncR, and IncFIB(pKPHS1). Resistance genes were carried on insertion sequences and/or transposable elements, and just a few carried on *intI1* integron. Most ARGs on the effluent *K. pneumoniae* isolates were not associated with mobile genetic elements. Resistance gene *blaCTX-M-15* was carried on the transposon Tn3 on all the clinical isolates that harboured the gene and associated with other resistance genes (*aac(3)-IIa* and *blaTEM-1B*) and insertion sequences (*ISEcp1*, *ISKpn11*, *ISKpn12*, *IS3*) on three of the isolates (CKP2, CKP5 and CKP8). This combination of ARGs was also bracketed by insertion sequences *IS1*, *ISKpn26* on CKP and CKP8 and associated with *ISNCY* and *IS1* × *2* on CKP8 (Table 2).

In all the effluent isolates, the *catA1* gene was carried on TnAs3, and associated with insertion sequence *IS1B* in one isolate; while only one of the clinical isolates harboured the gene and insertion sequence *IS1*. Only one clinical isolate harboured bla_{OXA-48} that was carried on Tn3 transposon and associated with the *IS300* and *ISKpn19* insertion sequences. All the effluent isolates did not have the class 1 integron, while 4 out of 6 clinical isolates had the class 1 integrase gene *intI1* (Table 3). In1021, which contained the gene cassette *aacA4cr-arr3-dfrA27-aadA16* was common in 3 of the clinical *K. pneumoniae*, the other isolate harboured the *dfrA14* gene cassette.

MLST and phylogeny

MLST showed that all the effluent *K. pneumoniae* isolates had a common sequence type ST29. Four of the clinical isolates had a common sequence type ST17 while two had unique sequence types ST348 and ST152. Phylogenetic analysis revealed that clinical and effluent isolates from this study were not closely related and did not cluster together (Fig. 2). In contrast, the effluent isolates clustered together according to their sequence type and formed a clade with South African environmental isolates. CKP2, CKP5, CKP8 and CKP10 (ST17) all clustered together with South African isolates from human and environmental settings according to sequence types.

Table 1 Antibiotic resistance genes, resistance patterns, efflux resistance mechanisms and plasmids found in the *Klebsiella pneumoniae* pathogens from clinical and hospital effluent samples

Isolate ID	Source of isolation	Resistance pattern	Resistance genes	Plasmid replicons	Efflux mechanism of resistance
CKP1	Clinical	CXM-CTX-TZP-TOB-CIP-SXT-TIG	*$bla_{TEM-206}$*, *$bla_{TEM-141}$*, *bla_{TEM-1B}*, *bla_{OXA-1}*, *$bla_{SHV-110}$*, *bla_{SHV-81}*, *$bla_{CTX-M-15}$*, *$bla_{TEM-214}$*, *aph(6)-Id*, *aph(3")-Ib*, *aac(6')-Ib-cr*, *sul2*, *dfrA14*, *qnrB1*	IncFIB(K), IncFIB(pKPHS1), IncFII(K)	*Klebsiella pneumoniae* KpnF, LptD, H-NS, emrR, *Klebsiella pneumoniae* KpnG, *Klebsiella pneumoniae* KpnH, marA, *Klebsiella pneumoniae* KpnE
CKP2	Clinical	CXM-CAZ-CTX-FEP-AMC-TZP-IPM-DOR-MEM-ERT-GEN-TOB-AMK-CIP-SXT	*$bla_{SHV-172}$*, *bla_{SHV-96}*, *bla_{SHV-94}*, *$bla_{CTX-M-15}$*, *bla_{TEM-1B}*, *$bla_{OXA-181}$*, *bla_{NDM-1}*„ *aac(3)-IIa*, *aph(6)-Id*, *aac(6')-Ib-cr*, *aph(3")-Ib*, *aadA16*, *armA*, *aadA1*, *sul1*, *sul2*, *dfrA27*, *qnrS1*, *OqxB*, *OqxA*, *qacE*	ColKP3, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncHI1B(pNDM-MAR), IncR, IncX3	*Klebsiella pneumoniae* KpnE, *Klebsiella pneumoniae* KpnF, oqxA, cmlA5, qacEΔ1, AcrAB-TolC
CKP4	Clinical	CXM-CAZ-CTX-FEP-AMC-TZP-GEN-TOB-CIP-SXT-TIG	*$bla_{CTX-M-15}$*, *bla_{OXA-1}*, *bla_{TEM-1B}*, *aph(6)-Id*, *aph(3")-Ib*, *aac(3)-Iia*, *aadA16*, *aac(6')-Ib-cr*, *sul2*, *sul1*, *dfrA27*, *OqxB*, *OqxA*, *qnrB6*, *tet(D)*, *qacE*	ColRNAI, IncFIB(K), repB(R1701)	*Klebsiella pneumoniae* KpnF, LptD, oqxA, tet(D), qacEΔ1, adeF, H-NS, AcrAB-TolC, CRP
CKP5	Clinical	CXM-CAZ-CTX-FEP-AMC-TZP-GEN-TOB-SXT	*$bla_{CTX-M-15}$*, *bla_{TEM-1B}*, *$bla_{SHV-172}$*, *bla_{SHV-96}*, *bla_{SHV-94}*,*bla_{SCO-1}*„ *aadA16*, *aac(6')-Ib-cr*, *aac(3)-Iia*, *aph(6)-Id*,, *aph(3")-Ib*, *sul1*, *sul2*, *dfrA27*, *OqxB*, *OqxA*, *qacE*	IncFIA(HI1), IncFIB(K), IncFII(K), IncR	*Klebsiella pneumoniae* KpnE, *Klebsiella pneumoniae* KpnF, oqxA, LptD, qacEΔ1, adeF, *Klebsiella pneumoniae* KpnG
CKP8	Clinical	CXM-CAZ-CTX-FEP-AMC-TZP-IPM-DOR-MEM-ERT-GEN-TOB-AMK-CIP-SXT	*$bla_{CTX-M-15}$*, *bla_{TEM-1B}*, *$bla_{SHV-172}$*, *bla_{SHV-96}*, *bla_{SHV-94}*, *$bla_{OXA-181}$*, *bla_{NDM-1}*, *aac(3)-Iia*„ *aph(6)-Id*, *aph(3")-Ib*, *armA*, *aadA16*, *aac(6')-Ib-cr*, *sul1*, *sul2*, *dfrA27*, *aadA1*, *qnrS1*, *OqxB*, *OqxA*, *qacE*	ColKP3, IncFIA(HI1), IncFIB(K), IncFIB(pNDM-Mar), IncFII(K), IncHI1B(pNDM-MAR), IncR, IncX3	*Klebsiella pneumoniae* KpnE, *Klebsiella pneumoniae* KpnF, oqxA, cmlA5, qacEΔ1
CKP10	Clinical	CXM-CAZ-CTX-FEP-AMC-TZP-GEN-TOB-CIP-SXT-TIG	*$bla_{CTX-M-15}$*, *bla_{TEM-1B}*, *$bla_{SHV-172}$*, *bla_{SHV-96}*, *bla_{SHV-94}*,*aac(3)-Iia*, *aph(6)-Id*, *aph(3")-Ib*, *aadA16*, *aac(6')-Ib-cr*, *sul2*, *sul1*, *dfrA27*, *OqxB*, *OqxA*, *qnrB6*, *qacE*	IncFIA(HI1), IncFIB(K), IncFII(K), IncR	OqxA, *Klebsiella pneumoniae* KpnE, *Klebsiella pneumoniae* KpnF, qacEΔ1,
SEB1	Effluent	AMC-TZP	*blaSHV-1*, *OqxB*, *OqxA*, *tet(D)*	IncFIB(K)	MdtQ, oqxA, marA, KpnEF, H-NS, adeF, KpnH
SEB2	Effluent	AMC-CTX-TZP	*blaSHV-1*, *OqxB*, *OqxA*, *tet(D)*	IncFIB(K)	MdtQ, oqxAB, marA, KpnEF, H-NS, adeF, KpnH
SEB3	Effluent	AMC-TZP-TIG	*OqxB*, *OqxA*, *tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF, LptD, marA
SEB4	Effluent	AMC-TZP	*bla_{SHV-1}*, *OqxB*, *OqxA*, *tet(D)*	IncFIB(K)	MdtQ, marA, tet(D), oqxA, KpnG, KpnEF
SEB5	Effluent	AMC-TZP	*OqxB*, *OqxA*, *tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF, LptD, marA
SEB6	Effluent	AMC-TZP	*bla_{SHV-1}*, *$bla_{SHV-187}$*, *OqxB*, *OqxA*, *tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF,

Table 1 (continued)

Isolate ID	Source of isolation	Resistance pattern	Resistance genes	Plasmid replicons	Efflux mechanism of resistance
SEB7	Effluent	AMC-TZP	*OqxB, OqxA, tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF, LptD, marA
SEB8	Effluent	AMC-TZP	*OqxB, OqxA, tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF, LptD, marA
SEB9	Effluent	AMC-TZP	*OqxB, OqxA, tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF, LptD, marA
SEB10	Effluent	AMC-TZP	bla_{SHV-1}, *OqxB, OqxA, tet(D)*	IncFIB(K)	MdtQ, oqxA, tet(D), H-NS, adeF, emrR, qacG, KpnEF, LptD

Clinical CKP1 formed a separate clade with 2 clinical isolates and an environmental isolate from South Africa. Clinical isolate CKP4 was also closely related and clustered with South African clinical and environmental isolates according to sequence type and formed a separate clade with an isolate from a water source in Tunisia (Fig. 2).

Discussion

This point-prevalence study investigated the phenotypic and genomic antibiotic resistance profiles of *Klebsiella pneumoniae* isolates from patients and hospital effluent. The frequency and characteristics of antibiotic resistant bacteria in hospital effluent is a simple technique to monitor the spread of resistance from the clinical setting into the environment [19] and there are a number of studies that report that hospital sewage is increasingly associated with multidrug resistant bacteria [20–22]. While no such similarities were observed in our study, we report on the complexity and diversity of resistance observed in clinical isolates compared with isolates from hospital effluent that did not harbour major antibiotic resistance, nor resistance genes.

This study did not reveal any relationship in the resistance of *K. pneumoniae* from the hospital sewage and clinical samples (Fig. 1). Higher resistances across all tested antibiotics were observed in the clinical isolates from patients at the hospital. The low levels of phenotypic antibiotic resistance in the *K. pneumoniae* recovered from hospital effluent in this study contradicts some studies that discovered above 40% resistance rates against the tested antibiotics on *K. pneumoniae* from hospital effluent [23, 24].

The antibiogram of the clinical isolates showed multidrug resistance to commonly used antibiotics, with higher resistance rates to cefuroxime, cefotaxime, piperacillin/tazobactam, gentamicin and trimethoprim/sulfamethoxazole (100%) compared with others (Fig. 1). Resistance rates above 70% were observed against ceftazidime, cefepime, amoxicillin/clavulanic acid and ciprofloxacin. Similar resistance rates have been described in other studies. A study by [25] reporting on virulent and multidrug-resistant *K. pneumoniae* from clinical samples in Pakistan showed similar high resistance rates in cefotaxime and amoxicillin/clavulanic acid (100%), and gentamicin (76.3%); while [26], investigating multidrug-resistant *K. pneumoniae* in patients admitted in Brazilian hospitals also reported higher resistance rates of 85.7% and 90.4% against trimethoprim/sulfamethoxazole and cefuroxime, respectively. However [25], also reported lower resistance against piperacillin/tazobactam and [27] evaluating antibiotic sensitivity patterns of *K. pneumoniae* and *K. oxytoca* from clinical samples reported lower resistance against ciprofloxacin (37.5%)

Table 2 Mobile genetic elements associated with antibiotic resistance genes in *K. pneumoniae* isolates

SAMPLE ID	MLST	CONTIGS	SYNTENY OF RESISTANCE GENES AND MGEs	PLASMID/CHROMOSOMAL SEQUENCE WITH CLOSEST NUCLEOTIDE HOMOLOGY (ACCESSION #)
CKP1	ST348	4	$bla_{SHV-110}$:bla_{SHV-11}::IS3	*Klebsiella pneumoniae* strain Kp_2016 – 319 chromosome (CP085101.1) (99.58%)
		41	*IS1*::*DfrA14*:*Intl1*	*Klebsiella pneumoniae* strain 1050 plasmid pKp1050-4 (CP023420.1) (100%)
		44	*ISEcp1*: *blaCTX-M-15*:Tn3	*Klebsiella pneumoniae* strainUHD-33_plasmid pESBL-PH-33 (CP121138.1) (100%)
		47	*QnrB1*:*IS3000*:Tn3	*Klebsiella pneumoniae* isolates 307 plasmid: P1 (OW48947.1) (100%)
		48	$bla_{TEM-206}$:$bla_{TEM-141}$:bla_{TEM-1B}:$bla_{TEM-214}$:*IS91*:*APH(6)-Id*:*APH(3")-Ib*:*sul2*	*Klebsiella pneumoniae* isolates 307 plasmid P:1 (OW48947.1) (100%)
CKP2	ST17	30	Tn3:$_{blaCTX-M-15}$:*ISEcp1*:bla_{TEM-1B}:*AAC(3)-IIa*:*ISE3*:*ISKpn11*:*ISKpn12*:*ISKpn26*:*IS1*:*ISNCY*	*Klebsiella pneumoniae* strain KpST17-2117 plasmid pKp2117_1 (CP075592.1)
		41	Tn3:*IS3000*:$bla_{OXA-181}$::*ISKpn19*:*QnrS1*	*Klebsiella pneumoniae* strain RIMV _C019688 plasmid pRIVM_C019688_3 (CP068958.1) (100%)
		42	*IS110*;*ISPa38*:*APH(6)-Id*;*APH(3")-Ib*:*Sul2*	*Klebsiella pneumoniae* strain 1,613,015,451 plasmid p3-1613015451 (CP083069.1) (99%)
		43	*ISEc33*:bla_{NDM-1}:*IS91*	*Klebsiella pneumoniae* C105 plasmid pC105-NDM1-IncHI3 (MN240794.1) (99%)
		44	*Mph(E)*:*msr(E)*: *ISEc29*:*IS5*:*ISCR1*	*Klebsiella pneumoniae* strain BA10835 plasmid pvirulence_VBA10835 (CP053766.1) (99%)
		74	*Sul1*:QacEΔ1	*Klebsiella pneumoniae* E196 plasmid pE196_IMP6 DNA (AP019405.1) (100%)
CKP4	ST152	56	bla_{TEM-1B}:*IS91*:*APH(6)-Id*:*APH(3")-Ib*:*sul2*	*Klebsiella pneumoniae* isolate 392 plasmid: P1 (OW968235.1) (100%)
		59	*QnrB6*:*ISCR1*	*Klebsiella pneumoniae* strain KP32558 plasmid pKP32558-2-mcr8 (CP076032.1) (100%)
		60	Tn3:$bla_{CTX-M-15}$	*Klebsiella pneumoniae* isolate 307 plasmid: P1 (OW848947.1) (100%)
		61	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
		62	MphR(A)::*Mph(A)*	*Klebsiella pneumoniae* isolate 11 plasmid: P1 (OW969612.1) (100%)
		66	*IS3*:*AAC(3)-IIa*	*Klebsiella pneumoniae* isolate 307 plasmid: P1 (OW848947.1) (100%)
		72	*IS1*:*catA1*	*Klebsiella pneumoniae* isolate 11 plasmid: P1 (OW969612.1) (100%)
		79	*Sul1*:QacEΔ1	*Klebsiella pneumoniae* E196 plasmid pE196_IMP6 DNA (AP019405.1) (100%)
CKP5	ST17	27	IS1:::*Sul1*:QacE:*AadA16*:*DfrA27*:*Arr-3*:*AAC(6')-Ib-cr*:Intl1	*Klebsiella pneumoniae* isolate 307 plasmid: P2 (OW849061.1) (99.99%)
		28	ISKpn12:ISKpn11:IS3:*AAC(3)-IIa*:*blaTEM-1*:ISEcp1:*blaCTX-M-15*:Tn3	*Klebsiella pneumoniae* strain KpST17-2177 plasmid pKp2177_1 (CP075592.1) (100%)
CKP8	ST17	18	IS1:IS3:IS1 × 2:ISKpn25:ISL3:ISNCY: ISKpn26:ISKpn12:ISKpn11:*AAC(3)-IIa*:*blaTEM-1*:ISEcp1:*blaCTX-M-15*:Tn3	*Klebsiella pneumoniae* strain KpST17-2177 plasmid pKp2177_1 (CP075592.1) (100%)
		39	Tn3:IS3000:*blaOXA-48*:EreA: ISKpn19	*Klebsiella pneumoniae* strain RIVM_C019688 plasmid pRIVM_C019688_3 (CP068958.1) (100%)
		40	ISAba125:ISEc33:*blaNDM-1*:IS91	*Klebsiella pneumoniae* strain CDC 0106 plasmid unitig_1 (CP022612.1) (100%)
		41	ISpa38:*APH(6)-Id*:*APH(3")-Ib*:*Sul2*	*Klebsiella pneumoniae* strain MAKM-3381 plasmid (CP129540.1) (99.98%)
		42	*Mph(E)*:*Msr(E)*:ISEc29:IS5:ISCR1	*Klebsiella pneumoniae* strain BA10835 plasmid pvirulence_VBA10835 (CP053766.1) (99%)
		43	*AadA16*:*dfrA27*:*Arr-3*:*AAC(6')-Ib-cr*:Intl1:Tn3:IS4321	*Klebsiella pneumoniae* KP17 plasmid pKP17_NDM1 DNA (LC521852.1) (98.85%)
		72	*Sul1*:QacEΔ1	*Klebsiella pneumoniae* E196 plasmid pE196_IMP6 DNA (AP019405.1) (100%)
CKP10	ST17	26	*BlaCTX-M-5*:ISEcp1:*blaTEM-1*:*AAC(3)-IIe*:*IS3*:*ISKpn11*:*ISKpn12*:*ISKpn26*:*IS1*:*ISNCY*:	
		33	*ISpa38*:*APH(6)-Id*:*APH(3")-Ib*:*Sul2*	*Klebsiella pneumoniae* strain MAKM-3381 plasmid (CP129540.1) (99.98%)
		35	*IS1*:::*Sul1*:QacEΔ1	*Klebsiella pneumoniae* isolate 307 plasmid: P2 (OW849061.1) (100%)
		36	*AadA16*:*DfrA27*:*Arr-3*:*AAC(6')-Ib-cr*:*Intl1*	*Klebsiella pneumoniae* strain HUM7199 plasmid pHUM7199_B (CP093316.1) (100%)
		39	*QnrB6*:*ISCR1*	*Klebsiella pneumoniae* strain KP32558 plasmid pKP32558-2-mcr8 (CP076032.1) (100%)

Table 2 (continued)

SAMPLE ID	MLST	CONTIGS	SYNTENY OF RESISTANCE GENES AND MGEs	PLASMID/CHROMOSOMAL SEQUENCE WITH CLOSEST NUCLEOTIDE HOMOLOGY (ACCESSION #)
SEB1	ST29	40	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		42	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
		40	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
SEB2	ST29	5	Recombinase:*OqxB*:*OqxA*	*Klebsiella pneumoniae* isolate INF171-sc-2,280,028 chromosome:1 (LR890446.1) (99.93%)
		37	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		41	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB3	ST29	37	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		39	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB4	ST29	39	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		42	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
		39	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
SEB5	ST29	38	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		40	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB6	ST29	36	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		39	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB7	ST29	38	IS1B:*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		41	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB8	ST29	38	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		40	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB9	ST29	38	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 512 plasmid: P1 (OW967602.1) (100%)
		40	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)
SEB10	ST29	38	*CatA1*:TnAs3	*Klebsiella pneumoniae* isolate 307 plasmid: P1 (OW848947.1) (100%)
		39	TetR(D):*Tet(D)*	*Klebsiella pneumoniae* strain S166-1 plasmid pS166-1.1 (CP063946.1) (100%)

Table 3 Class 1 integrons and gene cassettes found in the clinical *K. pneumoniae* isolates

ISOLATE ID (MLST)	INTEGRON	Cassette arrays			
		GC1	CG2	GC3	GC4
CKP1 (ST348)	None	*dfrA14*	-	-	-
CKP5 (ST17)	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*
CKP8 (ST17)	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*
CKP10 (ST17)	In1021	*aacA4cr*	*arr-3*	*dfrA27*	*aadA16*

and gentamicin (31%) compared with our study. Another study [28], who investigated antibiotic resistance patterns in MDR *E.coli* and *K. pneumoniae* from patients in Iran also reported lower resistance rates against ciprofloxacin (23.3%) and gentamicin (35.3%) compared with our study. Another study analysing the genotypic and phenotypic antibiotic resistance patterns of *K. pneumoniae* from clinical samples in Iran [29], reported similar resistance rates against trimethoprim/sulfamethoxazole (36.7%) and gentamicin (26.7%) as observed by [27]. These results were even lower than observed in this study. Only 33.3% of the clinical isolates were resistant to amikacin, similarly reported by [29] at 32.3% and [28] at 31%. Resistance to the carbapenem antibiotics, imipenem, ertapenem,

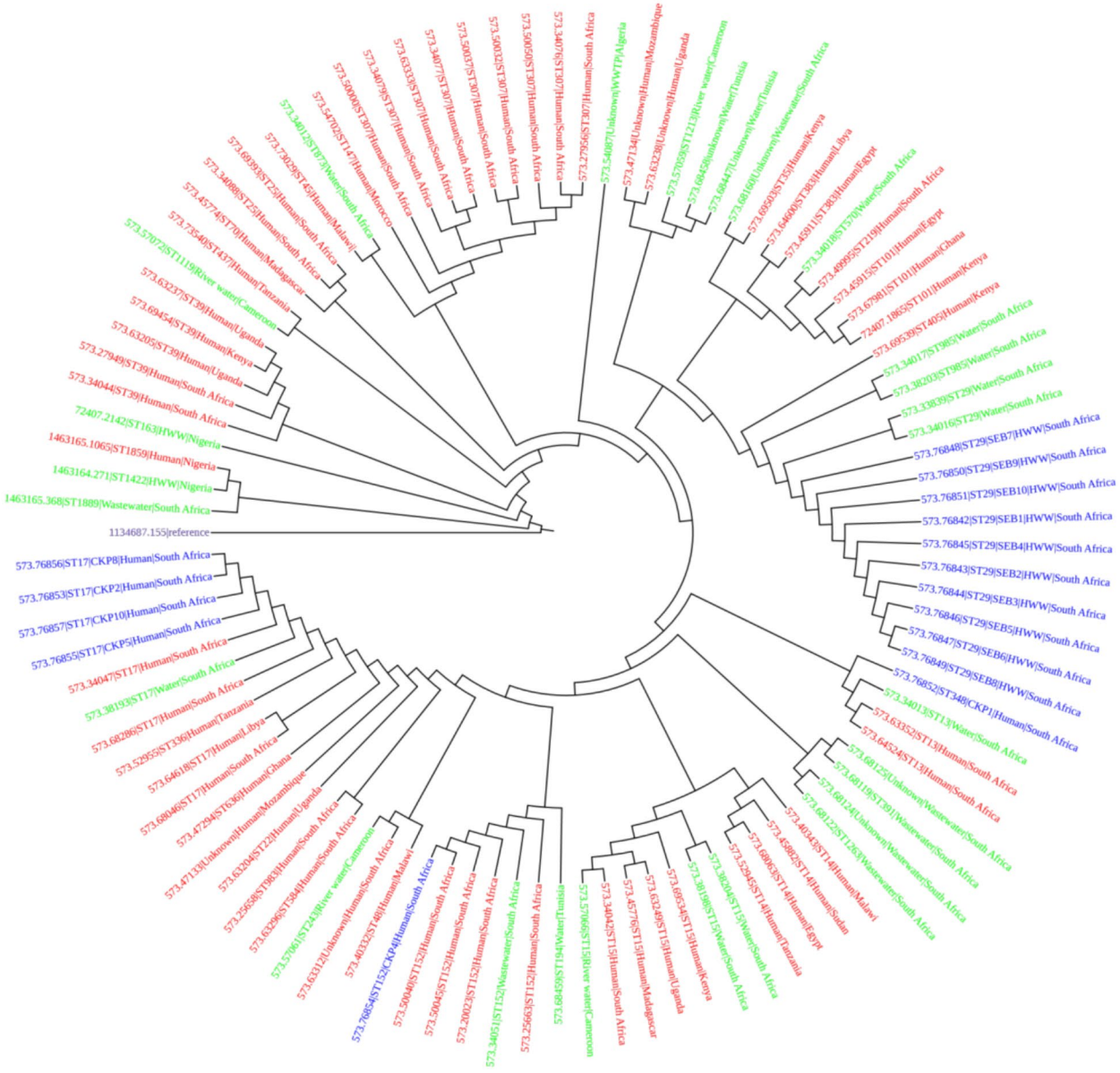

Fig. 2 Phylogenomic tree showing the relationship between clinical and effluent *K. pneumoniae* from this study (blue) with African isolates from human (red) and environmental (green) isolates

doripenem and meropenem was found to be 33.3% among the clinical isolates.

A total of 90% of the effluent isolates were susceptible to almost all tested antibiotics, and resistant against one class of antibiotics, the ß-lactams. High resistance rates were only observed against amoxicillin/clavulanic acid and piperacillin/tazobactam in all the effluent isolates. This is indicative of inhibitor resistance phenotype, which is characterized by resistance to ß-lactam-ß-lactamase inhibitor combinations and susceptibility to cephalosporins [30, 31]. Contrary to the high resistance against piperacillin/tazobactam in this study [23],, characterizing antibiotic resistance determinants in *K. pneumoniae* recovered from hospital effluent in the Eastern Cape province of South Africa; and [32], investigating antibiotic resistant *Klebsiella* species from the hospital, hospital effluent and effluent treatment plants in Kwazulu-Natal found high susceptibility in the effluent isolates against piperacillin/tazobactam. No resistance to the carbapenem antibiotics imipenem, meropenem, ertapenem and doripenem was observed in the effluent isolates in this study (Fig. 1). This is similar to what [32] reported in their study with 100% susceptibility against imipenem, ertapenem, meropenem, and less than 10% resistance to doripenem. Another study [23], also reported high susceptibility to imipenem (95%), ertapenem (88%) and meropenem (77%), while [33], who determined the occurrence of carbapenem-producing *Klebsiella* species in environmental niches in South Africa found similar lower resistance against meropenem (34%), ertapenem (35%), doripenem (35%) and imipenem (51%) in *K. pneumoniae* from hospital effluent. Another study by [34] who investigated AMR and detection of carbapenemases in *K. pneumoniae* isolates from raw hospital sewage reported that 79.2% of the isolates in their study showed resistance to carbapenems. Only one (10%) of the effluent isolates showed resistance to tigecycline. Low resistance in tigecycline (5.3%) was also reported by [34]. While the effluent isolates in this study were susceptible to a number of antibiotics, including the last resort antibiotics, the carbapenems, the clinical isolates showed extensive resistance to the commonly used antibiotics. This shows the limited efficacy of such antibiotics to effectively treat patients infected with *K. pneumoniae* [29].

Hospital effluent has been suggested as a hotspot for the prevalence and dissemination of resistance through horizontal gene transfer [13], and an important source of antibiotic resistant bacteria (ARB) and ARGs [35]. A systematic review by [19] reviewed studies that compared effluent surveillance of AMR and ARGs in parallel to clinical evidence; [36] assessed hospital effluent to determine if the resistome of the effluent differed from the general population, and [35] evaluated the diversity of AMR profiles in untreated hospital effluent to identify potential risks of dissemination of clinical resistant bacteria. These studies noted that the investigation of AMR in untreated hospital effluent is a helpful and effective tool that aides hospital-based infection control and our understanding of how clinical antibiotic resistance affects environmental ARB.

While effluent analysis can be a useful tool to provide data on AMR at a population level [37], it may not be a source of direct correlation and an ideal indicator of clinical AMR at the individual level [38]. Despite these limitations, researchers still argue that WBE can be a useful technique to provide broad understanding into clinical AMR trends [19, 37, 39, 40].

The phenotypic resistance characteristics correlated with known ARGs which were located on mobile genetic elements. Genes encoding multidrug efflux pumps were identified in most isolates (Table 1). Multidrug efflux pumps play an important role in mediating bacterial resistance to multiple antibiotic classes and heavy metals due to their ability to remove diverse molecules from inside the cell [41, 42]. There is increasing evidence that over-expression of multidrug efflux pumps is significantly associated with bacterial resistance to clinically relevant antibiotics [41, 43, 44].

Common efflux pumps found in both the clinical and effluent isolates included KpnEF and OqxA, while *tet(D)* was common among the effluent isolates, and AcrAB-TolC was identified in only two of the clinical isolates (CKP2 and CKP4) (Table 1). AcrAB-TolC, a common efflux pump reported in other studies, is a resistance-nodulation-division (RND) efflux pump found in Gram-negative bacteria that contributes to resistance to tetracyclines, fluoroquinolones, ß-lactams, macrolides and chloramphenicol [45–47]. OqxAB is also a member of the RND efflux pump family that has been shown to confer resistance to quinolones, nitrofurantoin, tigecycline, and chloramphenicol, detergents, and disinfectants [47–49]. Resistance to both tigecycline and quinolones was observed in three clinical (CKP1, CKP4, and CKP10) and one effluent isolate, (SEB3), the latter showing phenotypic resistance to tigecycline. A study in China reported on the AcrAB-TolC and OqxAB over-expression of efflux pumps that were associated with tigecycline resistance in *Klebsiella pneumoniae* [50]. However, the presence of *tetD* in all but one effluent isolate (SEB3) and the presence of *oqxA* and *oqxB* in all the effluent isolates did not show the commensurate phenotypic resistance and it was postulated that these isolates harboured these ARGs as silent or minimally expressed genes [51]. *K. pneumoniae* is known to harbour silent genes [52].

Several different ß-lactam resistance genes delineated in the clinical isolates correlated with the resistance observed, especially against cephalosporin antibiotics. For example, resistance to cefuroxime, cefotaxime and

ceftazidime was evident in isolates CKP2, CKP4, CKP5, CKP8 and CKP10 while CKP1 showed resistance against cefuroxime and cefotaxime only, with the ESBL ARGs, bla_{TEM}, bla_{CTXM-1}, and bla_{SHV} group (Table 1). Carbapenem resistance could be attributed to the bla_{NDM-1} and $bla_{OXA-181}$ ARGs in the two clinical isolates, CKP2 and CKP8. Characterizing carbapenemases, ESBLs plasmid mediated quinolone resistance (PMQR) in carbapenem resistant *K. pneumoniae* [53], reported the rate of bla_{NDM-1} to be 33.3% among the *K. pneumoniae* from patients. Other studies reported on low percentage of *K. pneumoniae* in hospital effluent harbouring carbapenem genes [23, 33]. The clinical isolates carried the bla_{TEM} group (CKP1, CKP2, CKP4, CKP5, CKP8 and CKP10), bla_{CTXM-1} (CKP1, CKP2, CKP4, CKP5, CKP8 and CKP10), and bla_{SHV} group (CKP1, CKP2, CKP5, CKP8 and CKP10) ESBL genes (Table 1). A study by [54] investigated antibiotic resistance profiles, pathogenicity and clonal relationships of *K. pneumoniae* isolated from patients and ICU settings reported on the abundance of the *K. pneumoniae* carrying ESBL encoding genes.

The prevalence of the aminoglycoside resistance genes was high in isolates CKP2, CKP4, CKP5, CKP8 and CKP10, that were resistant to at least two of the tested aminoglycoside antibiotics. These genes included the common ones observed among these isolates, *aph(6)-Id*, *aph(3")-Ib*, *aac(6')-Ib-cr*, *aadA16*, and *aac(3)-Iia*. ARGs *aadA1* and *armA* were only observed in the isolates that were resistant to amikacin (CKP2 and CKP8). Among the aminoglycoside resistant *K. pneumoniae* isolates from patients, *aadA16* was associated with gentamicin resistance in five (83.3%) of the isolates. The *aac(6')-Ib-cr* gene, which confers resistance to aminoglycosides and fluoroquinolones was present in all the clinical isolates. Fluoroquinolone resistance genes *qnrS* (2/6), *qnrB* (3/6), *oqxA* (6/6) and *oqxB* (5/6) were present among the clinical isolates. Isolates SEB1-SEB10 did not show phenotypic resistance to both aminoglycoside and fluoroquinolone antibiotics although they harboured the *oqxA* and *oqxB* genes.

The sulphonamide and trimethoprim resistance genes *sul1* (5/6, 83.3%) in CKP2, CKP4, CKP5, CKP8 and CKP10; *sul2* (6/6, 100%) in CKP1, CKP2, CKP4, CKP5, CKP8 and CKP10; *dfrA14* (1/6, 16.7%) in CKP1; and *dfrA27* (5/6, 83.3%) in CKP2, CKP4, CKP5, CKP8 and CKP10 were detected among the clinical isolates (Table 1). The susceptibility of the effluent isolates to trimethoprim-sulfamethoxazole is supported by the absence of these ARGs. Studies show the presence of genes encoding trimethoprim-sulfamethoxazole resistance in clinical and in effluent isolates [23, 55, 56]. Investigating the pathogenomics and evolutionary epidemiology of MDR clinical *K. pneumoniae* in South Africa [55], identified *sul1* (78%), *sul2* (86%), and *drfA27* in 19% of the isolates. Investigating antibiotic resistance determinants and plasmids in MDR *K. pneumoniae* from patients at a Malaysian medical centre [56], detected *sul1* (53.8%) as the most common gene encoding trimethoprim-sulfamethoxazole resistance, while [23] reported on the presence of the sulphonamide genes in effluent isolates to be high with the most common being *sul1* (68.4%).

Antibiotic resistance genes are carried on mobile genetic elements such as plasmids, integrons transposons, and can be transferred from organism to organism or between different species through conjugation, transformation and transduction [57]. ARGs were associated with a repertoire of MGEs in the clinical isolates but not in the effluent isolates (Table 2). The $bla_{OXA-181}$ gene was carried on plasmid replicon ColKp3 on isolates CKP2 and CKP8. This finding was supported by another study [58], analysing antibiotic resistance and MGEs in XDR *K. pneumoniae* from patients, reporting similar results. The most common plasmid type, IncFIB(K), was present in all the clinical and effluent isolates in this study. IncFII(K) plasmid types are associated with dissemination of ESBL and carbapenemases in *Klebsiella pneumoniae* clinical isolates [59, 60, 60]. reported on the molecular features of ESBL-producing *K. pneumoniae* from hospital and community acquired infections and found that the $bla_{CTX-M-15}$ gene was mostly carried on plasmid IncFII(K). We did not observe this in our study.

Only the class 1 integron integrase gene was harboured in 66% of the clinical isolates (Table 3). The *IntI1* was flanked by the insertion sequence *IS1* in two isolates (CKP1 and CKP5) (Table 2). Class 1 integrons are often associated with *sul1* and *qacEΔ1* genes [61], which was observed in isolate CKP5 that carried the *sul1*:*qac*EΔ1 gene cassette, on the same contig as *IntI1*; while the other isolates (CKP1, CKP8 and CKP10) carried this gene cassette on different contigs surrounding the *IntI1*. Transposable element Tn3 was the most common among the clinical isolates and was associated with $bla_{CTX-M-15}$ in CKP1, CKP2, CKP4 and CKP5 (Table 2). A study by [62], delineated molecular epidemiology of carbapenem resistant Enterobacterales (CRE) reported on Tn3 transposon commonly carrying the $bla_{CTX-M-15}$ or $bla_{CTX-M-3}$, and bla_{TEM1B}. Our findings were similar where $bla_{CTX-M-15}$ was mostly carried on Tn3 and flanked by ISEc9.

MLST revealed no common sequence types among the clinical and the effluent isolates. Moreover, the effluent isolates all clustered together and were related to other isolates from South Africa. Sequence type ST17 was more common among the clinical isolates (CKP2, CKP5, CKP8 and CKP10) while CKP4 (ST152) and CKP1 (ST348) were unique (Fig. 2). ST152 and ST17 have been reported in other South African studies [55, 62, 63]. Founou et al. (2019) characterised ESBL-producing *K. pneumoniae* from two hospitals and found ST152 in carriage

and clinical isolates while ST17 was only observed in carriage isolates. ST152 bla_{NDM-1} was an outbreak-related clone that has been reported by [64] who conducted an outbreak investigation of CRKP from bloodstream infections in a neonatal unit. This strain accounted for more than 60% of deaths. In this study, the NDM-1 and OXA-181 producing isolates, CKP2 and CKP8, were of the sequence type ST17.

Using WGS to reveal epidemiological relationships between clinical and environmental *K. pneumoniae* [65], reported that phylogeny analysis revealed some clinical and hospital sewage isolates in their study resided in the same clade, suggesting that they rise from a common ancestor. Another study by [66] investigating the transmission of MDR *K. pneumoniae* from the hospital to hospital effluent and its existence after chlorine treatment, showed 11 *K. pneumoniae* strains isolated from hospital effluent and the clinical sector were clonally related [67]. investigated the prevalence and phylogenetic relationships of MDR *K. pneumoniae* from patients, hospital effluent and surface waters. The phylogenetic analysis revealed a low similarity among the isolates in their study, even with those of the same sequence type [68]. investigated the prevalence of antibiotic-resistant bacteria in hospital effluent and receiving waters as a reflection of antibiotic prescription and infection cases. They generally highlighted a correlation between hospital infection cases and MDR in effluent. The genomic characteristics, supported by evolutionary representation of the isolates suggest that the clinical and the effluent isolates in this study were not closely related.

The main limitations of this study were the short study period, and the small sample size. The isolates from this study were from one region of the province, hence the results cannot be generalised to all South African hospitals.

Conclusion

K. pneumoniae clinical isolates showed higher rates of antibiotic resistance, including to the last resort antibiotics, compared with isolates from the hospital effluent. There was no evidence to show that the effluent reflects the clinical setting in the case of *K. pneumoniae* as there was no correlation between the resistome, mobilome and phylogenies of the clinical and effluent isolates. Results from this study suggest that the relationship between AMR in hospital effluent and clinical resistance may depend on the antimicrobial agent and bacterial species studied. While the effluent did not mirror AMR in the clinical setting, presence of ARB in the effluent cannot be overlooked. There is a need for continuous monitoring of the effluent to track the development of AMR and spread of resistant bacteria from the hospital to the environment.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03987-5.

Supplementary Material 1

Acknowledgements

We are grateful to the CEO of the hospital for permission to conduct sampling for this study. We are also grateful to the National Health Laboratory Service, Lancet Laboratories and National Institute for Communicable Diseases Sequencing Core Facility for technical assistance.

Author contributions

NSM, SYE, JM -Co-conceptualized the study: NSM, JM - Performed the experiments: NSM, JM, LAB - Analyzed the data: NSM - Wrote the paper: SYE, JM, LAB, AI - Supervision: SYE - Funding acquisition:; All authors undertook critical revision of the manuscript: All authors reviewed, edited, and approved the final manuscript.

Funding

This study was supported by the South African Research Chairs Initiative (SARChI) South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant No. 98342).

Data availability

Sequence data that supports the findings of this study have been deposited in the National Centre for Biotechnology Information (NCBI) with the BioProject number PRJNA1143548.

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) (Reference No.: BREC/00003640/2021) and the Provincial Health Research Ethics Committee (Reference No.:KZ_202203_023). Permission to collect sewage samples was granted by the Chief Executive Officer of a regional hospital in the uMgungundlovu District, KwaZulu-Natal Province. Clinical isolates were obtained with permission from the National Health Laboratory Services (NHLS), a South African public institute for laboratory service, research and training (reference: PR2225862). No patient data was collected.

Consent for publication

Not applicable.

Competing interests

SYE is the Chairperson of the Global Respiratory Infection Partnership and member of the Global Hygiene Council, both supported with unrestricted educational grants from Reckitt (Pty.), Ltd. UK.

Disclaimer

Any opinion, finding, and conclusion or recommendation expressed in this material is that of the authors, and neither the NRF nor the other funding bodies accept any liability in this regard.

Received: 25 January 2025 / Accepted: 22 April 2025
Published online: 11 May 2025

References

1. Abbas R, Chakkour M, Zein El Dine H, Obaseki EF, Obeid ST, Jezzini A, Ghssein G, Ezzeddine Z. General overview of Klebsiella pneumonia: epidemiology and the role of siderophores in its pathogenicity. Biology. 2024;13. https://doi.org/10.3390/biology13020078
2. Aires-de-sousa M, Manuel J, De O, Gonçalves ML, Pereira AL, Nordmann P, Poirel L. Epidemiology of carbapenemase- producing Klebsiella pneumoniae in a hospital, Portugal. Emerg Infect Dis. 2019;25:1632–8.

3. Ruekit S, Srijan A, Serichantalergs O, Margulieux KR, Mc Gann P, Mills EG, Stribling WC, Pimsawat T, Kormanee R, Nakornchai S, Sakdinava C, Sukhchat P, Wojnarski M, Demons ST, Crawford JM, Lertsethtakarn P, Swierczewski BE. Molecular characterization of multidrug-resistant ESKAPEE pathogens from clinical samples in Chonburi, Thailand (2017–2018). BMC Infect Dis. 2022;22:1–12. https://doi.org/10.1186/s12879-022-07678-8
4. Hubeny J, Korzeniewska E, Ciesielski S, Płaza G, Harnisz M. The resistome of ESKAPEE pathogens in untreated and treated wastewater: a Polish case study. Biomolecules. 2022;12:1–16. https://doi.org/10.3390/biom12081160
5. Ragupathi NKD, Bakthavatchalam YD, Mathur P, Pragasam AK, Walia K, Ohri VC, Veeraraghavan B. Plasmid profiles among some ESKAPE pathogens in a tertiary care centre in South India. Indian J Med Res. 2019;149:222–31. https://doi.org/10.4103/ijmr.IJMR_2098_17
6. Endale H, Mathewos M, Abdeta D. Potential causes of spread of antimicrobial resistance and preventive measures in one health perspective-a review. Infect Drug Resist. 2023;16:7515–45. https://doi.org/10.2147/IDR.S428837
7. WHO. Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance. World Health Organization. 2024. https://www.who.int/publications/i/item/9789240093461 (accessed June 11, 2024).
8. WHO. Antimicrobial resistance: accelerating national and global responses. World Health Organization 2023. https://apps.who.int/gb/ebwha/pdf_files/EB154/B154_13-en (accessed June 6, 2024).
9. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. London: 2014.
10. Meybodi MME, Foroushani AR, Zolfaghari M, Abdollahi A, Alipour A, Mohammadnejad E, Mehrjardi EZ, Seifi A. Antimicrobial resistance pattern in healthcare-associated infections: investigation of in-hospital risk factors. Iran J Microbiol. 2021;13:178–82. https://doi.org/10.18502/ijm.v13i2.5978
11. Gashaw M, Gudina EK, Tadesse W, Froeschl G, Ali S, Seeholzer T, Kroidl A, Wieser A. Hospital wastes as potential sources for multi-drug-resistant ESBL-producing bacteria at a tertiary hospital in Ethiopia. Antibiotics. 2024;13:1–12. https://doi.org/10.3390/antibiotics13040374
12. Nakamura-Silva R, Dias LL, Sousa RC, Fujimoto RY, Pitondo-Silva A. Multidrug-resistant and potentially pathogenic enterobacteriaceae found in a tertiary hospital sewage in southeastern Brazil. Environ Monit Assess. 2022;194:1–7. https://doi.org/10.1007/s10661-022-10454-5
13. Karkman A, Do TT, Walsh F, Virta MPJ. Antibiotic-resistance genes in waste water. Trends Microbiol. 2018;26:220–8. https://doi.org/10.1016/j.tim.2017.09.005
14. Akya A, Chegenelorestani R, Shahvaisi-Zadeh J, Bozorgomid A. Antimicrobial resistance of Staphylococcus aureus isolated from hospital wastewater in Kermanshah, Iran. Risk Manage Healthc Policy. 2020;13:1035–42. https://doi.org/10.2147/RMHP.S261311
15. Kalaiselvi K, Mangayarkarasi V, Balakrishnan D, Chitraleka V. Survival of antibacterial resistance microbes in hospital-generated recycled wastewater. J Water Health. 2016;14:942–9. https://doi.org/10.2166/wh.2016.154
16. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:1–9. https://doi.org/10.1186/1471-2105-10-421
17. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020;75:3491–500. https://doi.org/10.1093/jac/dkaa345
18. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK, Baker SJC, Dave M, Mccarthy MC, Mukiri KM, Nasir JA, Golbon B, Imtiaz H, Jiang X, Kaur K, Kwong M, Liang ZC, Niu KC, Shan P, Yang JYJ, Gray KL, Hoad GR, Jia B, Bhando T, Carfrae LA, Farha MA, French S, Gordzevich R, Rachwalski K, Tu MM, Bordeleau E, Dooley D, Griffiths E, Zubyk HL, Brown ED, Maguire F, Beiko RG, Hsiao WWL, Brinkman FSL, Van Domselaar G, Mcarthur AG. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. Nucleic Acids Res. 2023;51:D690–9. https://doi.org/10.1093/nar/gkac920
19. Tiwari A, Kurittu P, Al-Mustapha AI, Heljanko V, Johansson V, Thakali O, Mishra SK, Lehto KM, Lipponen A, Oikarinen S, Pitkänen T, Heikinheimo A. Wastewater surveillance of antibiotic-resistant bacterial pathogens: a systematic review. Front Microbiol. 2022;13:1–19. https://doi.org/10.3389/fmicb.2022.977106
20. Cai L, Sun J, Yao F, Yuan Y, Zeng M, Zhang Q, Xie Q, Wang S, Wang Z, Jiao X. Antimicrobial resistance bacteria and genes detected in hospital sewage provide valuable information in predicting clinical antimicrobial resistance. Sci Total Environ. 2021;795:148815. https://doi.org/10.1016/j.scitotenv.2021.148815
21. Mustafa SS, Batool R, Kamran M, Javed H, Jamil N. Evaluating the role of wastewaters as reservoirs of antibiotic-resistant ESKAPEE bacteria using phenotypic and molecular methods. Infect Drug Resist. 2022;15:5715–28. https://doi.org/10.2147/IDR.S368886
22. Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J Hosp Infect. 2016;93:395–402. https://doi.org/10.1016/j.jhin.2016.01.010
23. Okafor JU, Nwodo UU. Molecular characterization of antibiotic resistance determinants in Klebsiella pneumoniae isolates recovered from hospital effluents in the Eastern cape Province, South Africa. Antibiotics. 2023;12:1139. https://doi.org/10.3390/antibiotics12071139
24. Seguni NZ, Kimera ZI, Msafiri F, Mgaya FX, Joachim A. Multidrug-resistant Escherichia coli and Klebsiella pneumoniae isolated from hospital sewage flowing through community sewage system and discharging into the Indian ocean. Bulletin of the National Research Centre; 2023. https://doi.org/10.1186/s42269-023-01039-4
25. Fatima S, Liaqat F, Akbar A, Sahfee M, Samad A, Anwar M, Iqbal S, Khan SA, Sadia H, Makai G, Bahadur A, Naeem W, Khan A. Virulent and multidrug-resistant Klebsiella pneumoniae from clinical samples in Balochistan. Int Wound J. 2021;18:510–8. https://doi.org/10.1111/iwj.13550
26. Nakamura-Silva R, Cerdeira L, Oliveira-Silva M, da Costa KRC, Sano E, Fuga B, Moura Q, Esposito F, Lincopan N, Wyres K, Pitondo-Silva A. Multidrug-resistant Klebsiella pneumoniae: a retrospective study in Manaus, Brazil. Arch Microbiol. 2022;204:1–8. https://doi.org/10.1007/s00203-022-02813-0
27. Chakraborty S, Mohsina K, Sarker PK, Zahangir Alam M, Abdul Karim MI, Abu Sayem SM. Prevalence, antibiotic susceptibility profiles and ESBL production in Klebsiella pneumoniae and Klebsiella oxytoca among hospitalized patients. Periodicum Biologorum. 2016;118:53–8. https://doi.org/10.18054/pb.2016.118.1.3160
28. Moini AS, Soltani B, Ardakani AT, Moravveji A, Erami M, Rezaei MH, Namazi M. Multidrug-resistant Escherichia coli and Klebsiella pneumoniae isolated from patients in Kashan, Iran. Jundishapur J Microbiol. 2015;8. https://doi.org/10.5812/jjm.27517
29. Khamesipour F, Tajbakhsh E. Analyzed the genotypic and phenotypic antibiotic resistance patterns of Klebsiella pneumoniae isolated from clinical samples in Iran. Biomedical Res (India). 2016;27:1017–26.
30. Russ D, Glaser F, Shaer Tamar E, Yelin I, Baym M, Kelsic ED, Zampaloni C, Haldimann A, Kishony R. Escape mutations circumvent a tradeoff between resistance to a beta-lactam and resistance to a beta-lactamase inhibitor. Nat Commun. 2020;11:1–9. https://doi.org/10.1038/s41467-020-15666-2
31. Chaïbi EB, Sirot D, Paul G, Labia R. Inhibitor-resistant TEM β-lactamases: phenotypic, genetic and biochemical characteristics. J Antimicrob Chemother. 1999;43:447–58. https://doi.org/10.1093/jac/43.4.447
32. King TLB, Schmidt S, Essack SY. Antibiotic resistant Klebsiella spp. From a hospital, hospital effluents and wastewater treatment plants in the uMgungundlovu district, KwaZulu-Natal, South Africa. Sci Total Environ. 2020;712:135550. https://doi.org/10.1016/j.scitotenv.2019.135550
33. Ebomah KE, Okoh AI. Detection of carbapenem-resistance genes in Klebsiella species recovered from selected environmental niches in the Eastern cape Province, South Africa. Antibiotics. 2020;9:1–12. https://doi.org/10.3390/antibiotics9070425
34. Sakkas H, Bozidis P, Ilia A, Mpekoulis G, Papadopoulou C. Antimicrobial resistance in bacterial pathogens and detection of carbapenemases in Klebsiella pneumoniae isolates from hospital wastewater. Antibiotics. 2019;8:85. https://doi.org/10.3390/antibiotics8030085
35. Wang Q, Wang P, Yang Q. Occurrence and diversity of antibiotic resistance in untreated hospital wastewater. Sci Total Environ. 2018;621:990–9. https://doi.org/10.1016/j.scitotenv.2017.10.128
36. Acosta N, Lee J, Bautista MA, Bhatnagar S, Waddell BJ, Au E, Pradhan P, Clark RG, Meddings J, Ruecker N, Achari G, Pitout JD, Conly J, Frankowski K, Hubert CRJ, Parkins MD. Metagenomic analysis after selective culture enrichment of wastewater demonstrates increased burden of antibiotic resistant genes in hospitals relative to the community. 2023. https://doi.org/10.1101/2023.03.07.23286790

37. Munk P, Brinch C, Møller FD, Petersen TN, Hendriksen RS, Seyfarth AM, Kjeldgaard JS, Svendsen CA, Bunnik B, Van, Berglund F, Sewage G, Consortium S, Larsson DGJ, Koopmans M, Woolhouse M, Aarestrup FM. Genomic analysis of sewage from 101 countries reveals global landscape of antimicrobial resistance. 2022:1–16. https://doi.org/10.1038/s41467-022-34312-7
38. Perry MR, Lepper HC, McNally L, Wee BA, Munk P, Warr A, Moore B, Kalima P, Philip C, de Roda Husman AM, Aarestrup FM, Woolhouse MEJ, van Bunnik BAD. Secrets of the hospital underbelly: patterns of abundance of antimicrobial resistance genes in hospital wastewater vary by specific antimicrobial and bacterial family. Front Microbiol. 2021;12:1–10. https://doi.org/10.3389/fmicb.2021.703560
39. Clarke LM, Brien JWO, Murray AK, Thomas KV. A review of wastewater-based epidemiology for antimicrobial resistance surveillance. 2024. https://doi.org/10.20517/jeea.2023.29
40. Karkman A, Berglund F, Flach CF, Kristiansson E, Larsson DGJ. Predicting clinical resistance prevalence using sewage metagenomic data. Commun Biology. 2020;3. https://doi.org/10.1038/s42003-020-01439-6
41. Gaurav A, Bakht P, Saini M, Pandey S, Pathania R. Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. Microbiol (United Kingdom). 2023;169:1–13. https://doi.org/10.1099/mic.0.001333
42. Kumawat M, Nabi B, Daswani M, Viquar I, Pal N, Sharma P, Tiwari S, Sarma DK, Shubham S, Kumar M. Role of bacterial efflux pump proteins in antibiotic resistance across microbial species. Microb Pathog. 2023;181:106182. https://doi.org/10.1016/j.micpath.2023.106182
43. Sun J, Deng Z, Yan A. Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. Biochem Biophys Res Commun. 2014;453:254–67. https://doi.org/10.1016/j.bbrc.2014.05.090
44. Soto SM. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Landes Bioscience. 2013;Virulence:223–9.
45. Bialek-Davenet S, Lavigne JP, Guyot K, Mayer N, Tournebize R, Brisse S, Leflon-Guibout V, Nicolas-Chanoine MH. Differential contribution of acrab and OqxAB efflux pumps to multidrug resistance and virulence in Klebsiella pneumoniae. J Antimicrob Chemother. 2015;70:81–8. https://doi.org/10.1093/jac/dku340
46. Jang S. AcrAB–TolC, a major efflux pump in gram negative bacteria: toward Understanding its operation mechanism. BMB Rep. 2023;56:326–34. https://doi.org/10.5483/BMBRep.2023-0070
47. Ni RT, Onishi M, Mizusawa M, Kitagawa R, Kishino T, Matsubara F, Tsuchiya T, Kuroda T, Ogawa W. The role of RND-type efflux pumps in multidrug-resistant mutants of Klebsiella pneumoniae. Sci Rep. 2020;10:1–10. https://doi.org/10.1038/s41598-020-67820-x
48. Bharatham N, Bhowmik P, Aoki M, Okada U, Sharma S, Yamashita E, Shanbhag AP, Rajagopal S, Thomas T, Sarma M, Narjari R, Nagaraj S, Ramachandran V, Katagihallimath N, Datta S, Murakami S. Structure and function relationship of OqxB efflux pump from Klebsiella pneumoniae. Nat Commun. 2021;12. https://doi.org/10.1038/s41467-021-25679-0
49. Li J, Zhang H, Ning J, Sajid A, Cheng G, Yuan Z, Hao H. The nature and epidemiology of OqxAB, a multidrug efflux pump. Antimicrob Resist Infect Control. 2019;8:1–13. https://doi.org/10.1186/s13756-019-0489-3
50. Zhong X, Xu H, Chen D, Zhou H, Hu X, Cheng G. First emergence of acrab and OqxAB mediated tigecycline resistance in clinical isolates of Klebsiella pneumoniae pre-dating the use of tigecycline in a Chinese hospital. PLoS ONE. 2014;9:1–11. https://doi.org/10.1371/journal.pone.0115185
51. Deekshit VK, Srikumar S. To be, or not to be'—The dilemma of 'silent' antimicrobial resistance genes in bacteria. J Appl Microbiol. 2022;133:2902–14. https://doi.org/10.1111/jam.15738
52. Stasiak M, Mackiw E, Kowalska J, Kucharek K, Postupolski J. Silent genes: antimicrobial resistance and antibiotic production. Pol J Microbiol. 2021;70:421–9. https://doi.org/10.33073/pjm-2021-040
53. Al-Agamy MH, Aljallal A, Radwan HH, Shibl AM. Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in carbapenem-insensitive Escherichia coli and Klebsiella pneumoniae in Riyadh hospitals. J Infect Public Health. 2018;11:64–8. https://doi.org/10.1016/j.jiph.2017.03.010
54. Ferreira RL, Da Silva BCM, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MCA, Da Silva EML, De Melo Freire CC, Da Cunha AF, Da Silva Pranchevicius MC. High prevalence of multidrug-resistant Klebsiella pneumoniae harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. Front Microbiol. 2019;10. https://doi.org/10.3389/fmicb.2018.03198
55. Mbelle NM, Feldman C, Sekyere JO, Maningi NE, Modipane L, Essack SY. Pathogenomics and evolutionary epidemiology of multi-drug resistant clinical Klebsiella pneumoniae isolated from pretoria, South Africa. Sci Rep. 2020;10:1–17. https://doi.org/10.1038/s41598-020-58012-8
56. Al-Marzooq F, Mohd Yusof MY, Tay ST. Molecular analysis of antibiotic resistance determinants and plasmids in Malaysian isolates of multidrug resistant Klebsiella pneumoniae. PLoS ONE. 2015;10:1–21. https://doi.org/10.1371/journal.pone.0133654
57. Tang SS, Apisarnthanarak A, Hsu LY. Mechanisms of β-lactam antimicrobial resistance and epidemiology of major community- and healthcare-associated multidrug-resistant bacteria. Adv Drug Deliv Rev. 2014;78:3–13. https://doi.org/10.1016/j.addr.2014.08.003
58. Xanthopoulou K, Carattoli A, Wille J, Biehl LM, Rohde H, Farowski F, Krut O, Villa L, Feudi C, Seifert H, Higgins PG. Antibiotic resistance and mobile genetic elements in extensively drug-resistant Klebsiella pneumoniae sequence type 147 recovered from Germany. Antibiotics. 2020;9:1–13. https://doi.org/10.3390/antibiotics9100675
59. Dolejska M, Brhelova E, Dobiasova H, Krivdova J, Jurankova J, Sevcikova A, Dubska L, Literak I, Cizek A, Vavrina M, Kutnikova L, Sterba J. Dissemination of IncFIIK-type plasmids in multiresistant CTX-M-15-producing enterobacteriaceae isolates from children in hospital paediatric oncology wards. Int J Antimicrob Agents. 2012;40:510–5. https://doi.org/10.1016/j.ijantimicag.2012.07.016
60. Mansour W, Grami R, Ben Haj Khalifa A, Dahmen S, Châtre P, Haenni M, Aouni M, Madec JY. Dissemination of multidrug-resistant blaCTX-M-15/IncFIIk plasmids in Klebsiella pneumoniae isolates from hospital- and community-acquired human infections in Tunisia. Diagn Microbiol Infect Dis. 2015;83:298–304. https://doi.org/10.1016/j.diagmicrobio.2015.07.023
61. Nowrotek M, Kotlarska E, Aneta Ł, Felis E. The treatment of wastewater containing pharmaceuticals in microcosm constructed wetlands: the occurrence of integrons (int 1–2) and associated resistance genes (sul 1–3, Qac E Δ 1). 2017:15055–66. https://doi.org/10.1007/s11356-017-9079-1
62. Ramsamy Y, Mlisana KP, Amoako DG, Abia ALK, Ismail A, Allam M, Mbanga J, Singh R, Essack SY. Mobile genetic elements-mediated enterobacterales-associated carbapenemase antibiotic resistance genes propagation between the environment and humans: a one health South African study. Sci Total Environ. 2022;806:150641. https://doi.org/10.1016/j.scitotenv.2021.150641
63. Founou RC, Founou LL, Allam M, Ismail A, Essack SY. Whole genome sequencing of extended spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae isolated from hospitalized patients in KwaZulu-Natal, South Africa. Sci Rep. 2019;9:1–11. https://doi.org/10.1038/s41598-019-42672-2
64. Magobo RE, Ismail H, Lowe M, Strasheim W, Mogokotleng R, Perovic O, Kwenda S, Ismail A, Makua M, Bore A, Phayane R, Naidoo H, Dennis T, Ngobese M, Wijnant W, Govender NP. Outbreak of NDM-1- and OXA-181-producing Klebsiella pneumoniae bloodstream infections in a neonatal unit, South Africa. Emerg Infect Dis. 2023;29:1531–9. https://doi.org/10.3201/eid2908.230484
65. Runcharoen C, Moradigaravand D, Blane B, Paksanont S, Thammachote J, Anun S, Parkhill J, Chantratita N, Peacock SJ. Whole genome sequencing reveals high-resolution epidemiological links between clinical and environmental Klebsiella pneumoniae. Genome Med. 2017;9:1–10. https://doi.org/10.1186/s13073-017-0397-1
66. Popa LI, Gheorghe I, Barbu IC, Surleac M, Paraschiv S, Măruţescu L, Popa M, Pîrcălăbioru GG, Talapan D, Niţă M, Streinu-Cercel A, Streinu-Cercel A, Oţelea D, Chifiriuc MC. Multidrug resistant Klebsiella pneumoniae ST101 clone survival chain from inpatients to hospital effluent after chlorine treatment. Front Microbiol. 2021;11. https://doi.org/10.3389/fmicb.2020.610296
67. Davidova-Gerzova L, Lausova J, Sukkar I, Nechutna L, Kubackova P, Krutova M, Bezdicek M, Dolejska M. Multidrug-resistant ESBL-producing Klebsiella pneumoniae complex in Czech hospitals, wastewaters and surface waters. Antimicrob Resist Infect Control. 2024;13. https://doi.org/10.1186/s13756-024-01496-0
68. Siri Y, Bumyut A, Precha N, Sirikanchana K, Haramoto E, Makkaew P. Multidrug antibiotic resistance in hospital wastewater as a reflection of antibiotic prescription and infection cases. Sci Total Environ. 2024;908:168453. https://doi.org/10.1016/j.scitotenv.2023.168453

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