BMC Genomics

RESEARCH Open Access

Whole-genome analysis of a multidrug-resistant *Klebsiella michiganensis* environmental isolate from an orthopedic ward in Mwanza, Tanzania reveals IncF-family plasmid replicon signatures associated with resistance determinants

Gerald Mboowa[1,2*], Benson R. Kidenya[3*], Ivan Sserwadda[1,2], Stephen Kanyerezi[1,2] and Jeremiah Seni[4]

Abstract

Background *Klebsiella michiganensis*, a member of the *Klebsiella oxytoca* species complex, is increasingly recognized in healthcare-associated infection and can persist in hospital environmental reservoirs. However, genome-resolved data on environmental *K. michiganensis* from Africa remain limited.

Methods We performed short-read whole-genome sequencing (WGS) of an isolate recovered from a wheelchair handle in the orthopedic ward of a tertiary hospital in Mwanza, Tanzania. The isolate was initially recorded during routine surveillance as an unidentified Gram-negative ESBL bacterium and was provisionally identified phenotypically as *K. oxytoca* using routine laboratory methods. Draft assemblies were analyzed using the rMAP-2.0 bioinformatics pipeline. Genome-based taxonomic assignment using GTDB-Tk and ANI reclassified the study isolate as *Klebsiella michiganensis*. We then contextualized this genome with publicly available African *K. michiganensis* genomes and generated a core-genome phylogeny visualized in iTOL.

Results GTDB-Tk reclassified the study isolate A55848, and the comparator public genomes included in the final revised phylogeny as *Klebsiella michiganensis*, with the closest GTDB reference genome being GCF_002925905.1 and an ANI of 98.97% for A55848. The Tanzanian environmental isolate carried IncFIB(K) and IncFII(K) plasmid replicons, together with Col440I/Col440II. ResFinder-based screening identified a multidrug resistance gene repertoire including *blaOXY* family alleles (e.g., *blaOXY-1-2*), *blaTEM-1B*, *oqxA/oqxB*, *aph(3')-Ia*, *aac(6')-Ib*, and *aadA2*, consistent with an MDR genotype. Phenotypic testing demonstrated an ESBL profile with reduced susceptibility to oximino-cephalosporins and piperacillin–tazobactam, while susceptibility to meropenem, amikacin, and ciprofloxacin was retained. MLST did

*Correspondence:
Gerald Mboowa
gmboowa@gmail.com
Benson R. Kidenya
benkidenya@gmail.com

Full list of author information is available at the end of the article

not assign a recognized sequence type. Comparative analysis showed that African genomes frequently harbored IncFIB(K)/IncFII(K) replicons and clinically important β-lactamases, including *blaCTX-M-15* and *blaOXA-1*, with one isolate carrying *blaNDM-1*. Core-genome phylogeny further indicated that related *K. michiganensis* lineages occur across multiple African countries and that MDR is not confined to a single lineage, supporting resistance acquisition within shared genomic backgrounds.

Conclusions Recovery of an MDR *Klebsiella michiganensis* from an orthopedic ward wheelchair handle, carrying IncF-family plasmid replicon signatures together with multiple antimicrobial resistance determinants, underscores the potential for hospital environmental surfaces to serve as reservoirs of resistance. These findings highlight the value of strengthening targeted environmental hygiene and integrating routine genome-informed surveillance to detect, monitor, and mitigate transmission risks in African hospital settings.

Keywords *Klebsiella michiganensis*, Antimicrobial resistance, Hospital environment, Orthopedic ward, Plasmids, IncFIB(K), IncFII(K), Whole-genome sequencing, Tanzania

Introduction

Klebsiella michiganensis is a member of the *Klebsiella oxytoca* species complex and is increasingly recognized as an opportunistic healthcare-associated pathogen [1, 2]. Because species within the complex can be difficult to distinguish using routine phenotypic methods, isolates may be misidentified or reported as *K. oxytoca* unless genome-based taxonomy is applied [2, 3]. Like other members of the complex, *K. michiganensis* can persist in hospital environments, including sinks, drainage systems, surfaces, and shared medical equipment, creating opportunities for transmission to patients and healthcare workers, particularly in high-contact clinical areas [4, 5]. Outbreak investigations have further shown that contaminated hospital infrastructure, including handwashing sinks and wastewater systems, can act as long-term reservoirs for multidrug-resistant strains within the *K. oxytoca* species complex [4, 6, 7].

The emergence of multidrug resistance (MDR) in *K. michiganensis* further complicates infection prevention and control. Resistance determinants are often associated with mobile genetic elements, particularly IncF-family plasmids such as IncFIB(K) and IncFII(K), which are known to carry extended-spectrum β-lactamase (ESBL) and carbapenemase genes and facilitate horizontal gene transfer across Enterobacterales. These plasmid backbones contribute to the rapid dissemination of antimicrobial resistance within and between healthcare settings [8–10].

Despite increasing recognition of *K. michiganensis* as a clinically relevant and environmentally persistent organism, genome-resolved data from African settings remain limited. There is a paucity of studies characterizing environmental isolates and their role in sustaining antimicrobial resistance within hospital ecosystems. In this study, we performed whole-genome sequencing of an environmental *K. michiganensis* isolate recovered from a wheelchair handle in an orthopedic ward in Mwanza, Tanzania. We further contextualized this isolate within a comparative set of publicly available African genomes to investigate its phylogenetic relationships, plasmid content, and antimicrobial resistance determinants, with implications for infection prevention and genomic surveillance.

Materials and methods

Study setting, sampling, and initial identification

On 16 January 2020, a surface swab was collected from the handle of a wheelchair in the orthopedic ward (E8) of a tertiary referral hospital in Mwanza, Tanzania (study code ORTHO-014E; isolate ID A55848). During routine surveillance, the culture-positive isolate was initially recorded on site as an unidentified Gram-negative organism with an ESBL-like phenotype on screening. Subsequent phenotypic species identification using the VITEK system, performed according to local standard operating procedures, classified the isolate as *Klebsiella oxytoca*.

DNA extraction, sequencing, and genomic read processing

Genomic DNA was extracted at the Makerere University Molecular Biology Laboratory using commercially available extraction kits following the manufacturer's instructions. Whole-genome sequencing was performed at the Earlham Institute (Norwich, United Kingdom) using the Low Input, Transposase Enabled (LITE) Illumina library preparation protocol and sequenced on the NovaSeq 6000 platform to generate 2 × 150 bp paired-end reads.

Raw FASTQ files were processed using a reproducible Workflow Description Language (WDL) [11]. Read-level quality assessment was performed with FastQC [12]. Adapter sequences and low-quality bases were removed using Trimmomatic [13] in paired-end mode, applying sliding-window quality trimming and minimum read-length filtering (Phred + 33 encoding), to generate high-quality paired-end reads for downstream analysis.

Genome assembly and quality assessment

Trimmed paired-end reads were assembled de novo using MEGAHIT [14] with default parameters and a minimum contig-length threshold of 50 bp. The resulting draft assemblies were exported in FASTA format for downstream analyses. Assembly quality metrics, including total assembly length, number of contigs, and N50, were calculated from the assembly outputs and used to assess contiguity and completeness. Quality control summaries for sequencing and assembly outputs were compiled within the reproducible workflow and reviewed using workflow-generated QC reports.

Comparative genome dataset selection

To contextualize the study, isolate within regional diversity, a comparative set of publicly available *Klebsiella michiganensis* genomes was assembled from the NCBI Sequence Read Archive (SRA). Datasets were included based on the following criteria: (i) assignment to *K. michiganensis* within the *Klebsiella oxytoca* species complex, (ii) African geographic origin, and (iii) availability of Illumina short-read sequencing data suitable for standardized downstream processing. Relevant SRA accession numbers were identified through targeted database searches and retrieved for analysis.

Genome-based species confirmation

Species identity was confirmed using a genome-based classification framework incorporating Average Nucleotide Identity (ANI), GTDB-Tk [15], and BLAST [16] analysis. ANI was calculated with FastANI [17] against the selected *Klebsiella michiganensis* reference genome to assess whole-genome similarity. Taxonomic assignment was further evaluated using GTDB-Tk, which places genomes within a standardized phylogenomic framework. In addition, BLAST analysis was performed to provide sequence-level confirmation of species identity based on similarity to publicly available reference genomes.

Read mapping and variant calling

Trimmed reads were mapped against the *Klebsiella michiganensis* reference genome GCF_002925905.1 using BWA-MEM [18]. Resulting alignments were processed with SAMtools [19] to generate sorted and indexed BAM files. Variant calling was then performed with bcftools [20] to identify single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels). To retain high-confidence variants for downstream analyses, calls were filtered using minimum thresholds for read depth, base quality, and mapping quality.

Core-genome SNP alignment and recombination filtering

Core-genome single SNPs shared across isolates were extracted using the Snippy [21] workflow to generate a multiple sequence alignment for comparative analysis. Sites containing missing data or ambiguous base calls were excluded to retain a high-confidence core SNP alignment. To reduce the confounding effects of homologous recombination, recombinant regions were identified and masked using Gubbins [22], and the resulting recombination-filtered alignment was used for downstream phylogenetic inference.

Phylogenetic analysis

Maximum-likelihood phylogenetic inference was performed using IQ-TREE [23] based on a recombination-filtered core-genome SNP alignment. Prior to tree construction, homologous recombination was identified and masked using Gubbins, and the resulting filtered alignment was used as input for phylogenetic analysis. The optimal nucleotide substitution model was selected automatically using the model selection function implemented in IQ-TREE. Branch support was assessed using bootstrap resampling to evaluate the robustness of the inferred phylogenetic relationships.

SNP distance analysis

Pairwise SNP distances between genomes were calculated using snp-dists based on the recombination-filtered core-genome SNP alignment. The resulting SNP distance matrix was used to quantify genomic relatedness among isolates and to support interpretation of phylogenetic clustering patterns.

Phylogenetic visualization and annotation

The resulting phylogenetic tree was visualized using the Interactive Tree of Life (iTOL) platform [24]. Isolate-associated metadata, including country of origin, isolation source, and multilocus sequence type (MLST), were integrated and displayed as annotation tracks to facilitate epidemiological interpretation of clustering patterns within the tree.

Resistome and plasmid analysis

Antimicrobial resistance determinants were identified using ResFinder [25] based on assembled genome sequences. Putative plasmid sequences were reconstructed using MOB-suite [26], which was also used to assign contigs to predicted plasmid bins and infer plasmid-associated features. Plasmid replicon typing was performed to identify major incompatibility groups and to evaluate the association of resistance genes with specific plasmid backbones.

Virulence gene analysis

Putative virulence-associated genes were identified using curated virulence databases, including the Comprehensive Antibiotic Resistance Database (CARD) [27]. Genome assemblies were screened against these databases to detect genes associated with colonization, fitness, and pathogenicity, and to characterize the distribution of virulence-related features across the analyzed genomes.

Genome visualization

Genome architecture was visualized using Proksee (CGView) [28]. Draft genome assemblies were annotated and rendered as circular maps showing genomic features across both strands. GC content and GC skew were calculated and displayed as inner rings, while annotation tracks were used to visualize coding sequences, RNA features, antimicrobial resistance loci, and mobile genetic element–associated functions.

Workflow and reproducibility

Analyses were conducted using the rMAP-2.0 [11] pipeline, a modular and reproducible workflow implemented in Workflow Description Language (WDL) and executed with containerized environments. Software dependencies and computational steps were managed using Docker, ensuring reproducibility and portability of the analysis across computing platforms.

Results

Genome assembly quality metrics and variant summary for African *Klebsiella michiganensis* isolates

Genome assembly and whole-genome sequencing (WGS) quality assessment showed that the African *K. michiganensis* dataset had a median assembly length of 6.21 Mb, with most genomes ranging from approximately 6.0 to 6.8 Mb, consistent with expected genome sizes for members of the *Klebsiella oxytoca* species complex. One isolate had a markedly reduced assembly size, suggesting incomplete genome recovery and aligning with its other low-quality assembly metrics.

GC content was tightly conserved across isolates (53.59–55.98%; mean 55.19%), supporting species-level concordance. Assembly contiguity varied substantially: most genomes showed moderate assembly quality, with N50 values ranging from 48.2 kb to 134.4 kb (median 91.2 kb) and relatively low contig counts. In contrast, one isolate (SRR33931756) was highly fragmented, with a very low N50 (~1.1 kb) and an elevated contig count, indicating poor assembly quality and warranting caution in downstream analyses.

Overall, most assemblies were of sufficient quality to support comparative genomic analyses, including resistome and virulome profiling and phylogenetic reconstruction. However, the observed heterogeneity in assembly completeness and contiguity should be considered when interpreting isolate-level differences, particularly in gene-content comparisons.

Genome-based species confirmation (BLAST, ANI and GTDB-Tk)

Genome-based analyses consistently reclassified isolate A55848 and the five publicly available comparator genomes as *Klebsiella michiganensis* within the *Klebsiella oxytoca* species complex (Table 1). For A55848, ANI [17] analysis showed the highest similarity to the *K. michiganensis* reference genome GCF_002925905.1, with an ANI of 98.97% and an alignment fraction of 0.868. Across all six genomes, ANI values ranged from 98.46% to 99.14%, and GTDB-Tk assigned each genome to *K. michiganensis*, supporting consistent species-level classification (Table 1). BLAST analysis provided additional confirmation, with top matches corresponding predominantly to *K. michiganensis* genomes, including FDAARGOS_647 and GYRY158, and nucleotide identities reaching 99.997%(https://gmboowa.github.io/rMA

Table 1 GTDB-Tk taxonomic classification of assembled genomes

Sample ID	Species	Genus	Closest Reference	ANI (%)	Alignment Fraction	Classification Method
A55848	*Klebsiella michiganensis*	Klebsiella	GCF_002925905.1	98.97	0.868	Taxonomic classification defined by topology and ANI
SRR19859809	*Klebsiella michiganensis*	Klebsiella	GCF_002925905.1	98.77	0.885	Taxonomic classification defined by topology and ANI
SRR2244281	*Klebsiella michiganensis*	Klebsiella	GCF_002925905.1	98.95	0.863	Taxonomic classification defined by topology and ANI
SRR33931756	*Klebsiella michiganensis*	Klebsiella	GCF_002925905.1	99.14	0.806	Taxonomic classification defined by topology and ANI
SRR33931804	*Klebsiella michiganensis*	Klebsiella	GCF_002925905.1	98.46	0.889	Taxonomic classification defined by topology and ANI
SRR33931892	*Klebsiella michiganensis*	Klebsiella	GCF_002925905.1	98.90	0.867	Taxonomic classification defined by topology and ANI

Species assignments were supported by phylogenetic placement and average nucleotide identity (ANI) to the closest GTDB reference genome

P-2.0/non-eskapee/assets/sections/A55848_blast.html), while core-gene phylogenetic analysis against *Klebsiella oxytoca* isolates showed the study genomes forming a distinct separate cluster (https://gmboowa.github.io/rMAP-2.0/non-eskapee/). Collectively, these concordant results support the final classification of the study isolate and all comparator genomes as *K. michiganensis*.

Comparative genome collection

To contextualize the Tanzanian environmental isolate within regional *Klebsiella michiganensis* diversity, we compiled a comparative set of publicly available African *K. michiganensis* genomes from the NCBI Sequence Read Archive (SRA) (Table 2). In addition to the Tanzanian study isolate A55848, the final dataset included five publicly available genomes from South Africa and Nigeria, representing both environmental and clinical contexts where metadata were available. Genomes were included if they met three criteria: (i) assignment to *K. michiganensis*, (ii) African geographic origin, and (iii) availability of Illumina short-read sequencing data suitable for standardized downstream comparative analyses. Relevant accession numbers and associated metadata for all included genomes are summarized in Table 2 and provided in full in the Data Availability statement.

Population structure and MLST profiles

Multilocus sequence typing (MLST) analysis of the African *Klebsiella michiganensis* genome set revealed a largely unresolved sequence-type structure (Table 2). Among the six genomes analyzed, only one isolate, SRR2244281 from South Africa (Durban), was assigned to a defined sequence type (ST170), while the Tanzanian environmental isolate A55848 (SRR36571001) and the remaining comparator genomes from South Africa and Nigeria lacked assigned sequence types.

The predominance of unassigned sequence types suggests that many African *K. michiganensis* isolates fall within genomic backgrounds that are not well represented in current MLST reference schemes for the *Klebsiella oxytoca* species complex. This limited resolution constrains lineage-level interpretation based on MLST alone.

MDR was observed across both typed and untyped genomes. The Tanzanian isolate A55848 and other untyped comparator genomes were classified as MDR, while the only typed isolate (ST170) also exhibited an MDR profile. In contrast, one untyped genome (SRR33931756) was classified as non-MDR. These findings indicate that MDR is not restricted to a single sequence type and occurs across multiple genomic backgrounds.

Overall, the MLST results support a genetically heterogeneous *K. michiganensis* population within the currently available African dataset, with no evidence of a dominant lineage. The high proportion of untyped isolates highlights limitations of existing MLST frameworks for this species complex and underscores the need for expanded genomic sampling and improved typing schemes to better resolve population structure and resistance dynamics.

Recombination-filtered core-genome SNP phylogeny

Maximum-likelihood phylogenetic analysis based on the recombination-filtered core-genome SNP alignment revealed a genetically diverse population of *Klebsiella michiganensis* across the African isolates included in this study (Fig. 1). The phylogeny showed that isolates did not cluster strictly by geographic origin, with genomes from Tanzania, South Africa, and Nigeria interspersed across multiple branches.

The Tanzanian environmental isolate A55848 grouped within a cluster of South African isolates, indicating shared genomic backgrounds across geographically distinct settings. However, the overall tree topology did not support the presence of a single dominant lineage, consistent with a heterogeneous population structure.

Table 2 Geographic origin, sample collection date, MLST sequence type (ST), and source metadata for *Klebsiella michiganensis* isolates analyzed in this study

SRR / Sample ID	Country (location)	Genome source	Collection date	MLST ST	Isolate / sample source	MDR status
SRR36571001 (A55848)	Tanzania (Mwanza)	Our study	2020-1-16	-	Hospital environment (orthopedic ward); wheelchair handle	MDR
SRR33931892	South Africa	Public database (NCBI SRA)	2021-05-09	-	Not reported	MDR
SRR33931804	Nigeria	Public database (NCBI SRA)	2021-10-01	-	Not reported	MDR
SRR33931756	South Africa	Public database (NCBI SRA)	2021-08-18	-	Not reported	Non-MDR
SRR2244281	South Africa (Durban)	Public database (NCBI SRA)	Not reported	170	Not reported	MDR
SRR19859809	South Africa (Stellenbosch)	Public database (NCBI SRA)	2018-11-17	-	Not reported	MDR

MDR status was assigned based on detected antimicrobial resistance determinants identified using ResFinder spanning ≥ 3 antimicrobial classes (excluding generic efflux-only hits). "–" indicates that an MLST sequence type (ST) could not be assigned using the current MLST tool/database (i.e., no confident match to an existing ST in the *K. oxytoca* PubMLST scheme)

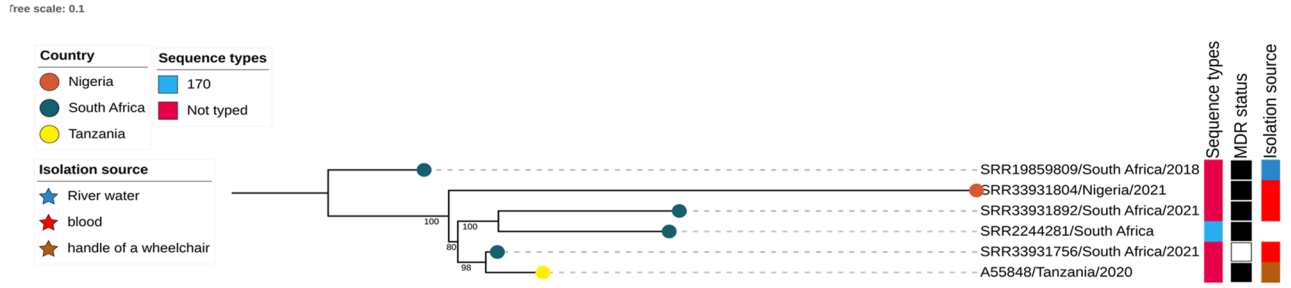

Fig. 1 Core-genome phylogeny and metadata of African *Klebsiella michiganensis* isolates. Maximum-likelihood phylogeny inferred from a core-genome SNP alignment of Klebsiella michiganensis isolates, including the study isolate A55848 (Tanzania). The tree illustrates the genetic relationships among isolates from multiple African countries. Tips are annotated by country of origin (Nigeria, South Africa, Tanzania), isolation source (river water, blood, wheelchair handle), and MLST, where available. The phylogeny shows that closely related isolates occur across different countries rather than forming strictly geographic clusters, with A55848 grouping with South African isolates. Sequence type information is limited, with some isolates untyped due to incomplete locus recovery. Together, the distribution of isolates across the tree supports a genetically diverse population structure and suggests that antimicrobial resistance is not restricted to a single lineage but may be shaped by horizontal acquisition of resistance determinants across related genomic backgrounds

MDR isolates were distributed across different branches of the phylogeny rather than forming a single clonal cluster. Both MDR and non-MDR genomes were observed in distinct phylogenetic positions, suggesting that antimicrobial resistance is not confined to a specific lineage but occurs across multiple genetic backgrounds.

Together, the recombination-filtered core-genome phylogeny supports a model of geographically dispersed and genetically diverse *K. michiganensis* populations in Africa, with resistance traits likely shaped by horizontal acquisition rather than clonal expansion alone.

Genomic relatedness and SNP distances

Pairwise SNP distance analysis based on the recombination-filtered core-genome alignment revealed substantial genomic diversity among the *Klebsiella michiganensis* isolates. SNP distances ranged from approximately 2,982 to 24,315 SNPs across the six genomes, indicating varying degrees of relatedness within the dataset.

The closest genetic relationships were observed among subsets of South African isolates, while more distant relationships were evident between isolates from different geographic origins, including the Tanzanian environmental isolate A55848. Notably, A55848 showed moderate genetic distance to its closest relatives, consistent with its placement within a broader, non-clonal phylogenetic cluster.

Overall, the wide range of SNP distances supports the phylogenetic observation of a genetically heterogeneous population and argues against recent clonal expansion. Instead, the data are consistent with the presence of multiple distinct *K. michiganensis* lineages circulating across different African settings.

Resistome and plasmid architecture

Genome-wide screening of antimicrobial resistance determinants revealed widespread MDR across the *Klebsiella michiganensis* dataset. Based on genotype (resistance determinants spanning ≥ 3 antimicrobial classes), five of the six genomes were classified as MDR, while one isolate (SRR33931756) exhibited a non-MDR profile characterized primarily by intrinsic resistance genes.

Table 3 Summary of antimicrobial classes represented by resistance genes detected in isolate A55848

Antimicrobial class	Resistance genes detected	Mechanism	Presence in isolate A55848
β-lactams	*bla*CTX-M-15, *bla*OXA-1	β-lactamase	Present
Aminoglycosides	*aac*(6')-Ib	Enzymatic modification	Present
Fluoroquinolones	*qnrB*	Target protection	Present
Sulfonamides	*sul1*	Drug target bypass	Present
Trimethoprim	*dfrA*	Drug target modification	Present

The Tanzanian environmental isolate A55848 harbored a diverse resistome, including ESBL-associated beta-lactamases (*blaCTX-M-15*, *blaOXA-1*, *blaTEM-1B*, and intrinsic *blaOXY* alleles), together with determinants associated with aminoglycosides (multiple *aph* genes), fluoroquinolones/quinolones (*aac(6')-Ib-cr*, *qnrB1*), sulfonamides (*sul2*), trimethoprim (*dfrA14*), and tetracycline (*tet(A)*). This profile is consistent with an MDR genotype and aligns with the observed ESBL-like phenotype (Table 3).

Across the comparative genome set, clinically important resistance genes were variably distributed, including ESBL-associated genes (*blaCTX-M* variants) and, in one case, a carbapenemase gene (*blaNDM-1*) identified in a South African isolate (SRR2244281). This isolate additionally carried resistance determinants spanning multiple antimicrobial classes, including aminoglycosides

(e.g., *rmtC*), quinolones, sulfonamides, trimethoprim, and rifampicin resistance (ARR-3), representing the most extensive resistance profile in the dataset. MDR isolates frequently carried multiple resistance determinants across different antimicrobial classes, whereas non-MDR genomes were largely limited to intrinsic *blaOXY* alleles with few or no additional acquired resistance genes. Full isolate-level resistance, virulence, and plasmid profiles are summarized in Supplementary Table 4.

Plasmid analysis revealed a strong association between MDR profiles and the presence of IncF-family plasmid replicons, particularly IncFIB(K) and IncFII(K). These replicons were recurrently detected among MDR isolates and frequently co-occurred with ESBL and other clinically relevant resistance determinants. Additional plasmid types (including IncHI1B, IncR, IncL/M, IncN, and IncU) were identified in some genomes, further supporting the role of diverse mobile genetic elements in resistance dissemination. To facilitate interpretation of cross-isolate genomic patterns, plasmid replicon groups and major antimicrobial resistance classes are summarized in a dot-matrix representation (Fig. 2), highlighting recurrent co-occurrence of IncF-family plasmids with ESBL and carbapenemase-associated resistance among MDR isolates.

To provide isolate-level resolution without over-interpreting plasmid reconstruction from short-read genomic data, IncF-family replicon markers were examined at the contig level for A55848. IncFIB(K) was detected on contig k77_1921 (98.93% identity; 100% coverage), while IncFII(K) replicon sequences were identified on contigs k77_1856 and k77_972 (95.95–98.65% identity; 100% coverage), supporting the presence of IncF-type plasmid backbones associated with resistance determinants.

Virulence gene profiling indicated that most genomes encoded core fitness and colonization factors, including iron acquisition systems and outer membrane-associated functions. A55848 carried enterobactin-associated genes (*entA*, *entB*), together with adhesion and outer membrane-associated loci (*ompA*, *yagZ*/*ecpA*), and additional factors such as mgtB and astA. Several comparator genomes encoded the yersiniabactin locus (including *ybtS*, *ybtX*, *ybtQ*, *ybtP*, *ybtA*, *ybtU*, *ybtT*, *ybtE*, together with *irp1*, *irp2*, and *fyuA*), indicating the presence of iron-scavenging systems that may enhance persistence and opportunistic pathogenicity even in the absence of classical hypervirulence signatures.

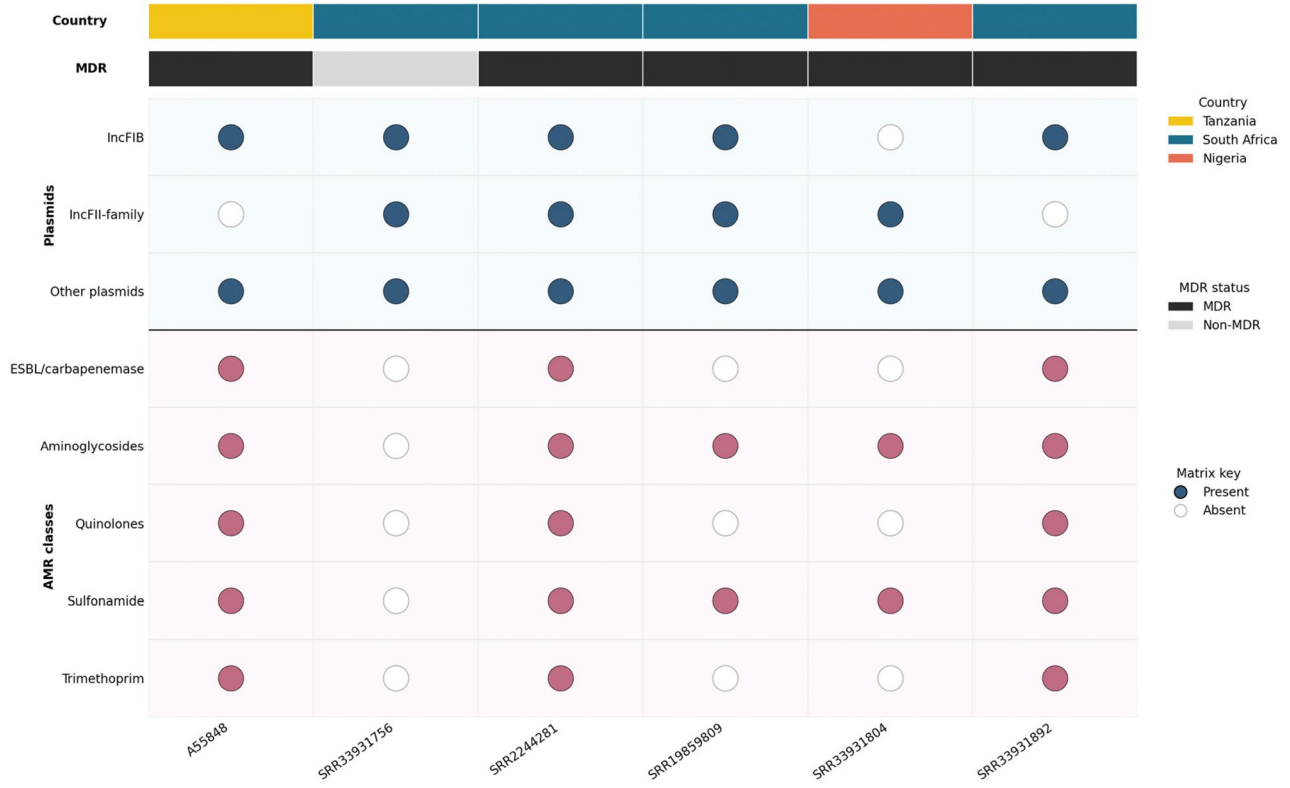

Fig. 2 Plasmid and antimicrobial resistance class distribution across African *Klebsiella michiganensis* isolates. Dot-matrix summary showing the presence or absence of key plasmid replicon groups and major antimicrobial resistance classes across six genomes. Top annotation bars indicate country of origin and multidrug resistance status. Recurrent co-occurrence of IncF-family plasmids with ESBL/carbapenemase-associated resistance is evident among MDR isolates

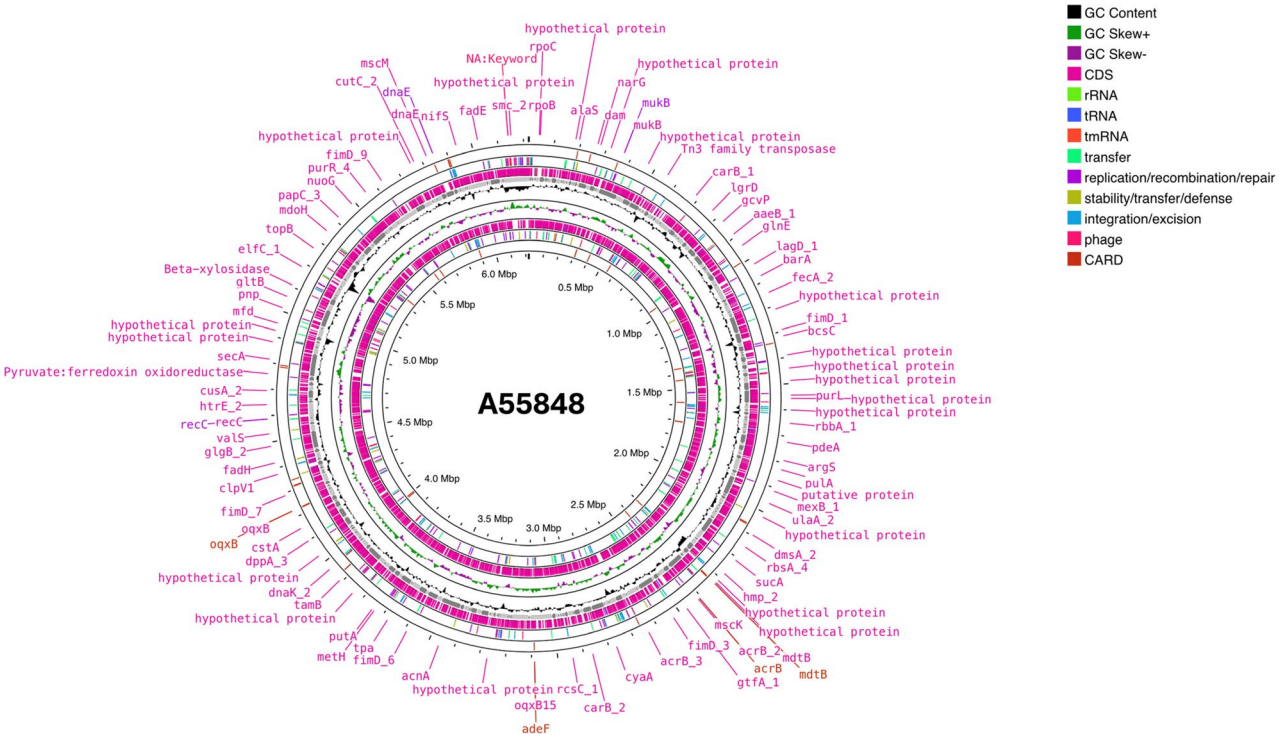

Fig. 3 The circular genome map of the study isolate A55848 (*Klebsiella michiganensis*) shows a typical chromosome organization with coding sequences distributed across both strands and conserved GC content and GC skew patterns. Antimicrobial resistance loci are dispersed throughout the genome, with multiple genes associated with β-lactam, aminoglycoside, and quinolone resistance. These loci frequently co-occur with features indicative of mobile genetic elements, including transposases and integration/excision functions, suggesting a role for horizontal gene transfer in shaping the resistance profile. Together, the genome architecture supports a multi-locus antimicrobial resistance phenotype consistent with the plasmid-associated ESBL determinants identified in this isolate

Overall, the co-occurrence of IncF-family plasmid backbones with ESBL genes and, in at least one case, carbapenemase genes, together with the distribution of resistance across multiple genomic backgrounds, supports a central role for plasmid-mediated horizontal gene transfer in shaping antimicrobial resistance in *Klebsiella michiganensis*. The detection of these features in a hospital environmental isolate further highlights the potential role of shared ward equipment and other fomites as reservoirs for MDR organisms and associated resistance elements.

Plasmid context of ESBL determinants

To further investigate the genomic context of ESBL genes, plasmid reconstruction and resistance gene localization were performed for the study isolate A55848. Both *blaCTX-M-15* and *blaOXA-1* were assigned to a reconstructed plasmid bin carrying an IncFII-family replicon, suggesting a shared plasmid backbone associated with resistance dissemination. Although these genes were located on separate contigs in the draft assembly, their co-assignment to the same plasmid bin supports a plasmid-associated genomic context.

To avoid over-interpreting plasmid reconstruction from short-read genomic data, IncF-family replicon markers were further examined at the contig level. IncFIB(K) was detected on contig k77_1921 (98.93% identity; 100% coverage), while IncFII(K) replicon sequences were identified on contigs k77_1856 and k77_972 (95.95–98.65% identity; 100% coverage). These findings provide complementary evidence supporting the presence of IncF-type plasmid backbones associated with ESBL determinants in A55848.

Due to the limitations of short-read sequencing, the precise structural organization and physical adjacency of resistance genes within the plasmid cannot be fully resolved. Nevertheless, the combined evidence indicates that ESBL determinants in A55848 are likely co-localized within an IncF-associated plasmid backbone. The broader resistance profile of A55848, including genes representing multiple antimicrobial classes, is summarized in Table 3.

This observation is consistent with the wider dataset, in which IncF-family plasmids frequently co-occur with ESBL and, in at least one case, carbapenemase genes, supporting a central role for plasmid-mediated horizontal gene transfer in the dissemination of antimicrobial resistance.

Genome architecture and feature distribution

The circular genome map of isolate A55848 provides an overview of draft genome organization and the spatial distribution of key genomic features (Fig. 3). Predicted coding

sequences were broadly distributed across the assembly on both strands, with rRNA and tRNA loci detected at discrete positions. GC content and GC skew profiles showed the expected genome-wide compositional variation, with localized deviations consistent with regions of atypical sequence composition and possible horizontal gene transfer. Antimicrobial resistance loci detected by CARD-RGI were present at multiple positions across the genome rather than concentrated in a single region, supporting a multi-locus MDR genotype. In parallel, mobileOG-db annotations identified multiple mobile genetic element–associated features, including transposase and integration/excision-related functions, several of which co-localized with resistance loci. Together, these features indicate a genome architecture characterized by dispersed resistance determinants and mobile element activity, consistent with the observed MDR/ESBL-like phenotype.

Cross-isolate genomic patterns

Integration of phylogenetic, resistome, and plasmid analyses revealed consistent cross-isolate genomic patterns within the African *Klebsiella michiganensis* dataset. Notably, MDR was distributed across multiple branches of the phylogeny rather than being confined to a single lineage, indicating that resistance is not driven by clonal expansion alone.

A recurrent feature among MDR isolates was the presence of IncF-family plasmid replicons, particularly IncFIB(K) and IncFII(K), which frequently co-occurred with clinically relevant resistance determinants, including ESBL-associated genes. This pattern was observed across isolates from different geographic regions, supporting the role of shared plasmid backbones in disseminating antimicrobial resistance across diverse genomic backgrounds.

In contrast, non-MDR isolates were largely characterized by the presence of intrinsic *blaOXY* alleles with limited acquisition of additional resistance genes and lacked evidence of IncF-associated MDR profiles. This distinction further supports the role of mobile genetic elements, rather than lineage-specific traits, in shaping resistance phenotypes in *Klebsiella michiganensis*.

Taken together, these findings indicate that antimicrobial resistance in *K. michiganensis* within this dataset is primarily structured by horizontal gene transfer mediated by plasmids, rather than by expansion of a dominant resistant lineage. The observed convergence of resistance traits across genetically distinct isolates highlights the importance of plasmid dynamics in driving resistance dissemination in hospital and environmental settings.

Discussion

This study provides genome-resolved evidence that hospital environmental surfaces can act as reservoirs for *Klebsiella michiganensis*, a member of the *Klebsiella oxytoca* species complex that is increasingly recognized as an opportunistic healthcare-associated pathogen [2, 4]. We identified an MDR, ESBL-producing isolate (A55848), recovered from a wheelchair handle in an orthopedic ward in Mwanza, Tanzania, carrying IncF-family plasmid replicons and multiple AMR determinants. The recovery of this organism from a shared mobility aid highlights the potential role of high-touch equipment in the persistence and transmission of resistant Enterobacterales in clinical settings. This finding is consistent with prior infection prevention and control studies demonstrating contamination of shared medical equipment, including wheelchairs and bath chairs, with antibiotic-resistant organisms implicated in healthcare-associated transmission and outbreaks [4, 29, 30].

Placing A55848 in a broader African genomic context, core-genome phylogenetic analysis showed that genetically related *K. michiganensis* isolates are present across multiple countries rather than forming strictly country-specific clusters (Fig. 1). The Tanzanian isolate clustered most closely with isolates from South Africa, indicating the presence of related genomic backgrounds across geographically distinct settings. However, given the small sample size and limited geographic representation, these relationships should be interpreted cautiously and do not by themselves demonstrate direct transmission or epidemiological linkage. Instead, the observed pattern is most consistent with a broadly distributed and genetically heterogeneous population structure within the currently available African dataset.

MDR was observed across multiple branches of the phylogeny, including both typed and untyped isolates, indicating that resistance is not restricted to a single lineage. However, interpretation of lineage structure is constrained by the limited resolution of MLST in this dataset. Among the genomes analyzed, only a single isolate (SRR2244281) was assigned to a defined sequence type (ST170), while all other isolates, including A55848, remained untyped. This high proportion of unassigned sequence types likely reflects both incomplete MLST recovery from draft assemblies and the limited representation of *K. michiganensis* diversity in current typing schemes [31]. Consequently, the available data do not support conclusions about lineage-specific dissemination of resistance but instead suggest that MDR occurs across multiple, incompletely characterized genomic backgrounds.

Integration of plasmid reconstruction and resistance gene profiling provides a more robust explanation for the observed resistance patterns. In A55848, ESBL genes (*blaCTX-M-15* and *blaOXA-1*) were assigned to

a reconstructed IncFII-associated plasmid, supporting a plasmid-mediated genomic context for resistance. Across the dataset, IncF-family plasmid replicons were recurrently detected in MDR isolates and co-occurred with clinically relevant resistance determinants. Taken together, these findings support a model in which plasmid-mediated horizontal gene transfer, rather than clonal expansion alone, plays a central role in the dissemination of antimicrobial resistance in *K. michiganensis* within this setting.

Genome-wide analysis further supports the contribution of mobile genetic elements to resistance architecture. The circular genome map of A55848 shows a typical *Klebsiella* genome organization, with coding sequences distributed across both strands and conserved GC content and skew patterns. Antimicrobial resistance loci are dispersed throughout the genome and frequently co-localize with features associated with recombination, integration, and transposition, as annotated by mobileOG-db. These observations are consistent with previous reports demonstrating that AMR genes are often embedded within mobile genetic elements, facilitating their mobilization and persistence in hospital environments [32–35]. The presence of efflux-associated genes and stress-response mechanisms may further contribute to environmental survival on high-contact surfaces such as medical equipment [36–38].

From an IPC perspective, these findings have practical implications. The detection of MDR *K. michiganensis* on a wheelchair handle supports the need for targeted environmental cleaning and disinfection strategies focused on shared mobility aids and other high-touch surfaces [6]. In addition, hospital infrastructure such as sinks and drainage systems should be considered potential reservoirs, as previously demonstrated for Enterobacterales within the *K. oxytoca* species complex [7, 8, 10]. Importantly, the lack of clear lineage restriction of resistance in this dataset suggests that surveillance strategies should prioritize detection of AMR genes and plasmid content rather than relying solely on sequence type or phylogenetic clustering.

This study has several limitations. The use of short-read sequencing restricts complete reconstruction of plasmid structures and limits definitive assignment of AMR genes to specific plasmids or mobile elements. The comparative dataset is small and geographically limited, and conclusions regarding population structure and dissemination should therefore be interpreted with caution. In addition, the high proportion of untyped isolates limits the utility of MLST-based epidemiological inference. Future studies incorporating long-read sequencing, expanded regional sampling, and improved species-complex-aware typing frameworks will be important for resolving plasmid architecture, lineage structure, and transmission dynamics.

Despite these limitations, this study provides genome-level insight into the role of hospital environments as reservoirs of MDR *K. michiganensis* in Africa and highlights the importance of integrating environmental sampling with genomic surveillance to better understand and mitigate antimicrobial resistance transmission in healthcare settings.

Conclusion

This study provides genome-resolved evidence that hospital environmental surfaces can act as reservoirs of MDR *Klebsiella michiganensis* in African healthcare settings. The recovery of an MDR isolate from a wheelchair handle, carrying IncF-family plasmid replicons and clinically relevant antimicrobial resistance determinants, highlights the role of shared medical equipment in sustaining and potentially disseminating resistance within hospital environments.

Comparative genomic analysis demonstrated that antimicrobial resistance is distributed across genetically diverse lineages rather than confined to a single clone, with recurrent co-occurrence of IncF-family plasmids and ESBL-associated genes across isolates. These findings support a model in which plasmid-mediated horizontal gene transfer plays a central role in shaping resistance patterns in *K. michiganensis*.

Together, these results underscore the importance of integrating environmental surveillance with genome-informed approaches to better detect and control antimicrobial resistance reservoirs. Strengthening targeted infection prevention strategies focused on high-touch surfaces and shared equipment, alongside routine genomic monitoring of resistance determinants and mobile genetic elements, will be critical for mitigating transmission risks in resource-limited healthcare settings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-026-12837-1.

Supplementary Material 1.

Acknowledgements

We thank the hospital IPC and laboratory teams in Tanzania for facilitating environmental sampling and culture, and colleagues who provided feedback on the genomic analyses. We also acknowledge the teams who generated and deposited publicly available *K. michiganensis* genomes used as comparators in this study.

Authors' contributions

GM, BK, IS, SK and JS made substantial contributions to the conception, design, coordination, and execution of the study. IS, SK, and GM performed the genomic analyses and interpreted the data. GM and BK drafted the initial

version of the manuscript, which was critically revised by all authors. All authors reviewed and approved the final manuscript.

Funding
This study was part of the research project titled "Understanding transmission dynamics and acquisition of antimicrobial resistance at referral hospitals and community settings in East Africa," supported through the Grand Challenges Africa programme (grant numbers GCA/AMR/rnd2/058/002 and GCA/AMR/rnd2/058 – 01). Grand Challenges Africa is a programme of the African Academy of Sciences (AAS) implemented through the Alliance for Accelerating Excellence in Science in Africa (AESA), an initiative of the AAS and the African Union Development Agency (AUDA-NEPAD). The programme is supported by the Bill & Melinda Gates Foundation, the AAS, and partners. The project was implemented collaboratively by Makerere University College of Health Sciences (Kampala, Uganda) and the Catholic University of Health and Allied Sciences (Mwanza, Tanzania). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Research reported in this publication was supported by the Fogarty International Center of the National Institutes of Health under Award Numbers U2RTW012116 and D43TW013056. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data availability
Raw sequencing reads (FASTQ files) and the draft genome assembly for the Tanzanian environmental K. *michiganensis* isolate ORTHO-014E/A55848 have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1392631 (BioSample accession: SAMN54267858) and SRA run accession: SRR36571001.In addition to our Mwanza environmental isolate, we retrieved all publicly available African *Klebsiella michiganensis* Illumina short-read datasets available in the NCBI Sequence Read Archive (SRA) and analyzed them in parallel for comparative genomic context. The corresponding SRA run accessions are: SRR2244281, SRR19859809, SRR33931756, SRR33931804, and SRR33931892.All rMAP-2.0 workflow configurations and code used for the analyses are available at: [https://github.com/gmboowa/rMAP-2.0](https:/github.com/gmboowa/rMAP-2.0).

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the Declaration of Helsinki and relevant national regulations. It was approved by the Joint CUHAS/BMC Research and Ethics Committee (CREC/409/2019) and the National Health Research Ethics Review Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol. IX/3322) in Tanzania. Permission to conduct the study was obtained from the hospital administration, the Head of the Department of Orthopedics, and ward supervisors/in-charge nurses prior to sample and data collection.
Environmental sampling did not involve collection of identifiable patient information. In the parent study, written informed consent (or assent for children) was obtained from all participants where clinical samples and data were collected. Patients with suspected surgical site infections received appropriate clinical management, including culture and antimicrobial susceptibility testing to guide therapy.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]African Center of Excellence in Bioinformatics and Data-Intensive Sciences, Infectious Diseases Institute, College of Health Sciences, Makerere University, P.O Box 22418, Kampala, Uganda
[2]Department of Immunology and Molecular Biology, College of Health Sciences, School of Biomedical Sciences, Makerere University, P. O Box 7072, Kampala, Uganda
[3]Department of Biochemistry and Molecular Biology, Weill Bugando School of Medicine, Catholic University of Health and Allied Sciences, P. O. Box 1464, Mwanza, Tanzania
[4]Department of Microbiology and Immunology, Weill Bugando School of Medicine, Catholic University of Health and Allied Sciences, P. O. Box 1464, Mwanza, Tanzania

Received: 24 December 2025 / Accepted: 2 April 2026
Published online: 09 April 2026

References

1. Cosic A, Leitner E, Petternel C et al. Variation in Accessory Genes Within the Klebsiella oxytoca Species Complex Delineates Monophyletic Members and Simplifies Coherent Genotyping. *Front Microbiol*; 12. Epub ahead of print 2 July 2021. https://doi.org/10.3389/fmicb.2021.692453.
2. Yang J, Long H, Hu Y, et al. Klebsiella oxytoca Complex: Update on Taxonomy, Antimicrobial Resistance, and Virulence. Clin Microbiol Rev. 2022;35:e0000621.
3. Chapman P, Forde BM, Roberts LW, et al. Genomic Investigation Reveals Contaminated Detergent as the Source of an Extended-Spectrum-β-Lactamase-Producing Klebsiella michiganensis Outbreak in a Neonatal Unit. J Clin Microbiol. 2020;58:e01980–19.
4. Larsen AL, Pedersen T, Sundsfjord A, et al. Hospital toilets and drainage systems as a reservoir for a long-term polyclonal outbreak of clinical infections with multidrug-resistant *Klebsiella oxytoca* species complex. Infect Prev Pract. 2025;7:100430.
5. Li Y, Wu Y, Li D, et al. Multicenter comparative genomic study of Klebsiella oxytoca complex reveals a highly antibiotic-resistant subspecies of Klebsiella michiganensis. J Microbiol Immunol Infect. 2024;57:138–47.
6. Lowe C, Willey B, O'Shaughnessy A et al. Outbreak of Extended-Spectrum β-Lactamase–producing Klebsiella oxytoca Infections Associated with Contaminated Handwashing Sinks - 18, Number 8—August 2012 - Emerging Infectious Diseases journal - CDC. https://doi.org/10.3201/eid1808.111268.
7. Leitner E, Zarfel G, Luxner J, et al. Contaminated Handwashing Sinks as the Source of a Clonal Outbreak of KPC-2-Producing Klebsiella oxytoca on a Hematology Ward. Antimicrob Agents Chemother. 2014;59:714–6.
8. Kuzina ES, Kislichkina AA, Sizova AA et al. High-Molecular-Weight Plasmids Carrying Carbapenemase Genes blaNDM-1, blaKPC-2, and blaOXA-48 Coexisting in Clinical Klebsiella pneumoniae Strains of ST39. *Microorganisms* 2023; 11: 459.
9. Stohr JJJM, Kluytmans-van den Bergh MFQ, Weterings VATC, et al. Distinguishing blaKPC Gene-Containing IncF Plasmids from Epidemiologically Related and Unrelated Enterobacteriaceae Based on Short- and Long-Read Sequence Data. Antimicrob Agents Chemother. 2021;65. https://doi.org/10.1128/aac.00147-21.
10. Yao Y, Imirzalioglu C, Falgenhauer L, et al. Plasmid-Mediated Spread of Carbapenem Resistance in Enterobacterales: A Three-Year Genome-Based Survey. Antibiotics. 2024;13:682.
11. Mboowa G, Sserwadda I, Kanyerezi S. rMAP 2.0: a modular, reproducible, and scalable WDL–Cromwell–Docker workflow for genomic analysis of ESKAPEE pathogens. Bioinf Adv. 2026;6:vbag046.
12. Babraham Bioinformatics -. FastQC A Quality Control tool for High Throughput Sequence Data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed 26 March 2026).
13. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
14. Li D, Liu C-M, Luo R, et al. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015;31:1674–6.
15. Chaumeil P-A, Mussig AJ, Hugenholtz P, et al. GTDB-Tk v2: memory friendly classification with the genome taxonomy database. Bioinformatics. 2022;38:5315–6.
16. Camacho C, Coulouris G, Avagyan V, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:421.
17. Jain C, Rodriguez-R LM, Phillippy AM, et al. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018;9:5114.
18. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60.

19. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9.
20. Danecek P, McCarthy SA. BCFtools/csq: haplotype-aware variant consequences. Bioinformatics. 2017;33:2037–9.
21. Seemann T. tseemann/snippy, https://github.com/tseemann/snippy (2026, accessed 29 March 2026).
22. Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 2015;43:e15.
23. Nguyen L-T, Schmidt HA, von Haeseler A, et al. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol. 2015;32:268–74.
24. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 2021;49:W293–6.
25. Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother. 2020;75:3491–500.
26. Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb Genom. 2018;4:e000206.
27. McArthur AG, Waglechner N, Nizam F, et al. The Comprehensive Antibiotic Resistance Database. Antimicrob Agents Chemother. 2013;57:3348–57.
28. Grant JR, Enns E, Marinier E, et al. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Res. 2023;51:W484–92.
29. Peretz A, Koiefman A, Dinisman E, et al. Do wheelchairs spread pathogenic bacteria within hospital walls? World J Microbiol Biotechnol. 2014;30:385–7.
30. Okada N, Takahashi M, Yano Y, et al. Hospital outbreak of extended-spectrum beta-lactamase-producing Escherichia coli potentially caused by toilet and bath chair use. Infect Prev Pract. 2022;4:100239.
31. Maghembe RS, Magulye MAK, Makaranga A, et al. Comprehensive genomics reveals novel sequence types of multidrug resistant *Klebsiella oxytoca* with uncharacterized capsular polysaccharide K- and lipopolysaccharide O-antigen loci from the National Hospital of Uganda. Infect Genet Evol. 2024;123:105640.
32. Johansson MHK, Bortolaia V, Tansirichaiya S, et al. Detection of mobile genetic elements associated with antibiotic resistance in Salmonella enterica using a newly developed web tool: MobileElementFinder. J Antimicrob Chemother. 2021;76:101–9.
33. Shropshire WC, Konovalova A, McDaneld, et al. Systematic Analysis of Mobile Genetic Elements Mediating β-Lactamase Gene Amplification in Noncarbapenemase-Producing Carbapenem-Resistant Enterobacterales Bloodstream Infections. mSystems. 2022;7:e0047622.
34. Zhang R, Ou H-Y, Gao F, et al. Identification of Horizontally-transferred Genomic Islands and Genome Segmentation Points by Using the GC Profile Method. Curr Genomics. 2014;15:113–21.
35. Partridge SR, Kwong SM, Firth N, et al. Mobile Genetic Elements Associated with Antimicrobial Resistance. Clin Microbiol Rev. 2018;31:e00088–17.
36. Wales AD, Davies RH. Co-Selection of Resistance to Antibiotics, Biocides and Heavy Metals, and Its Relevance to Foodborne Pathogens. Antibiot (Basel). 2015;4:567–604.
37. Wand ME, Darby EM, Blair JMA, et al. Contribution of the efflux pump AcrAB-TolC to the tolerance of chlorhexidine and other biocides in Klebsiella spp. J Med Microbiol. 2022;71:001496.
38. Bf G, Nv C. Unravelling the mechanisms of antibiotic and heavy metal resistance co-selection in environmental bacteria. *FEMS microbiology reviews*; 48. Epub ahead of print 20 June 2024. https://doi.org/10.1093/femsre/fuae017.

Publisher's note