

Molecular Characterisation of Carbapenemase-Producing Multi-Drug Resistant *Klebsiella pneumoniae* from Human, Animal, and Environmental Samples from Ekiti and Ondo State, Nigeria

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Abstract

Background

The escalating global threat of human infections caused by multi-drug-resistant (MDR) Enterobacteriaceae, particularly *Klebsiella pneumoniae*, poses a serious challenge to public health. The study investigated the prevalence and molecular characteristics of carbapenemase-producing *Klebsiella pneumoniae* in human, animal, and environmental samples from Ekiti and Ondo States, Nigeria.

Methods

A total of 1329 samples comprising 399 clinical human samples, 404 non-clinical human samples, 393 animal samples, and 133 environmental samples were examined. The samples were processed using standard microbiological techniques, and antibiotic susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic resistance genes (*OXA-48*, *NDM-1*, *VIM*, and *KPC*) were determined in all *K. pneumoniae* isolates that were phenotypically positive for carbapenemase production. Whole-genome sequencing (WGS) was performed on two *K. pneumoniae* isolates from the same household, which exhibited similar resistance patterns. Data analysis was performed in Excel and the Statistical Package for Social Sciences (SPSS) version 23.

Results

The overall prevalence of *K. pneumoniae* was 105 (7.9%), with higher rates in animal samples 38 (9.4%) than in human samples 59 (7.3%) and in the environmental sample 8 (6.0%). Antibiotic resistance was significantly higher in human isolates than in animal and environmental isolates ($p < 0.05$). All isolates were susceptible to colistin and amikacin. Multidrug resistance was observed in 65 (61.9%) isolates, which was significantly more common in human samples ($p < 0.05$). Carbapenemase production was detected in 9.2% of MDR isolates, predominantly in human samples. The *OXA-48*, *VIM*, and *KPC* genes were identified in Carbapenemase-producing *K. pneumoniae*. Whole-genome sequencing of *K. pneumoniae* isolates NGEK23 and NGEK25 revealed distinct resistomes and mobile genetic elements between the two isolates, suggesting distinct origins and differing diversity of resistance genes.

Conclusion

The high frequency of multidrug resistance and the presence of carbapenemase-producing *K. pneumoniae* across human, animal, and environmental sources in the study samples highlight an urgent need for robust infection control and antibiotic stewardship programmes.

Introduction

Antimicrobial resistance (AMR) is a significant public health challenge [1], of concern is the rise in multidrug-resistant bacteria, particularly Carbapenem-Resistant Bacteria (CRB) [2]. Carbapenem-resistant Enterobacterales (CRE) and other carbapenem-resistant Gram-negatives are now reported on every continent, with CRE accounting for a substantial share of the global AMR burden that contributed to an estimated 1.27 million deaths directly attributable to AMR and 4.95 million associated deaths in 2019 [3, 4]. Numerous systematic reviews, meta-analyses, and surveillance studies conducted across regions have reported varying estimates of the prevalence of carbapenem-resistant *Klebsiella pneumoniae*. For instance, pooled data from parts of West Africa indicate a notably high burden of carbapenem-resistant strains *K. pneumoniae* and *E. coli* with dominant *blaNDM*, *blaVIM* and *blaOXA-48* carbapenemases [5]; in Latin American paediatric cohorts, carbapenem-resistant *K. pneumoniae* infections have reported mortality as high as 52.6% [6]; and in US surveillance, carbapenemase-producing CRE clinical cultures rose by about 18% between 2019 and 2023, with a roughly fivefold increase in NDM-producing CRE [7]. Hospital-based series from Asia and Africa often report carbapenem resistance rates in Enterobacterales and other major Gram-negatives in the 20–50% range, while colonisation surveys in African hospitals have found CRE carriage prevalences around 12%, indicating large unrecognised reservoirs that fuel ongoing transmission [8, 9]. There has been a global increase in the reporting of carbapenem-resistant bacteria. However, studies exploring the transmission dynamics of carbapenem resistance genotypes across one-health are limited and significantly low, especially in Africa [3, 10–12]. The global rise in human infections caused by multi-drug resistant (MDR) Enterobacteriaceae, including *Klebsiella pneumoniae*, seriously threatens public health [13, 14].

The emergence and spread of carbapenem resistance in clinical settings leads to high healthcare costs, long hospital stays, and an increase in mortalities associated with such pathogen [15–17]. Furthermore, carbapenem resistance can be harboured on both the chromosome and the plasmid, but most often, plasmid-encoded β -lactamases usually enhance their transmissible activities, leading to the spread of these genes among Enterobacteriaceae, contributing to the rapid dissemination of carbapenem resistance traits to other Enterobacteriaceae genera and species [18, 19].

Evidence from the literature shows that carbapenem resistance in Enterobacteriaceae is common, with the highest prevalence reported in *Klebsiella pneumoniae* [7]. In developed countries, surveillance systems are in place to track the spread and transmission dynamics across the human, animal, and environmental sectors. However, surveillance systems in developing countries are limited and not fully optimised, particularly for tracking the spread of infections within health systems. Limited evidence on CR *Klebsiella pneumoniae* surveillance in developing countries is usually focused solely on human health, without data from animal and environmental isolates.

In particular, there is limited epidemiological data on the genes responsible for carbapenem resistance in *Klebsiella pneumoniae*, with most studies focusing on antibiotic susceptibility testing with imipenem and meropenem. Equally, the mechanisms underlying the spread of carbapenem-resistant bacterial infections remain incompletely elucidated in the Nigerian population. Studies exploring the transmission dynamics of carbapenem-resistant bacteria among humans, animals, and the environment in Nigeria are limited [20–22]. In Nigeria, infection prevention and control (IPC) strategies are not holistic, as they are most often focused on healthcare settings, with no active IPC and biosecurity programmes in the animal, agriculture, and environmental sectors. The problem of quality surveillance data is further worsened in developing countries due to poor access to laboratory facilities for accurate identification and molecular characterisation of MDR pathogens, thereby enhancing clinical diagnosis and the detection of transmissible resistance determinants [23–27].

Therefore, surveillance studies of carbapenem-resistant bacteria, particularly in *Klebsiella pneumoniae*, in humans, animals, and their environment are needed in these settings, as no such study has been conducted to our knowledge. A quick search in two databases (PubMed and Africa Online Journals (AJOLs)) using the keywords (carbapenem, *Klebsiella pneumoniae*, One-Health, Ondo, and Ekiti) in the study area did not return any studies that used a One-Health approach to explore the prevalence of carbapenem-resistant *Klebsiella pneumoniae*. Studies of this nature are necessary to understand the transmission dynamics of resistant bacteria and to inform public health strategies and interventions. There are data gaps in the molecular epidemiology of MDR pathogens and ARGs across sectors, including human, animal, and environmental samples, in developing countries and, in particular, in study areas [12, 28, 29]. This study bridged this gap by providing insights into the prevalence of multidrug-resistant *K. pneumoniae* through a one-health approach. In addition, identified carbapenemase-producing *K. pneumoniae*, detected carbapenemase genes, and conducted whole-genome sequencing on two isolates with similar antimicrobial profiles from the same household in the study settings.

METHODS

Study Area

Study Design and study time frame

The study employed a cross-sectional design and was conducted over 18 months (April 2022–September 2023) in Ekiti and Ondo States, Nigeria.

Sample collection points

The study was carried out at the Federal Medical Centre, Owo, Federal Teaching Hospitals, Ido-Ekiti, The Ekiti State University Teaching Hospital, Ado-Ekiti, Hospital Management Board Hospitals in Ikere-Ekiti, Ikole-Ekiti and Oye-Ekiti, while Mother and Children Hospital, General Hospital Igarra Oke, Ondo State, animal farms in Akure and Ado-Ekiti respectively, Slaughterhouses in Owo, Akure, Ido-Ekiti and Ado-Ekiti and the home and animals of agreed hospitalised participants.

Sample Size

The study analysed a total of 1,329 samples, including 399 clinical human samples, 404 non-clinical human samples, 393 animal samples, and 133 environmental samples. Specifically, environmental samples totalled 91 from Ekiti and 42 from Ondo; animal samples, 253 from Ekiti and 140 from Ondo; clinical samples, 263 from Ekiti and 136 from Ondo; and samples from animal workers, 251 in Ekiti and 153 in Ondo (Table 1).

Sample collections

Samples were collected from three cohorts (Hospitals, farms, and slaughterhouses). Human, animal, and environmental samples were collected from farms, households, and hospital-admitted patients. The residents of each household/farm, their domestic animals, and the water source (well or borehole) used for drinking and washing purposes were sampled. The distribution of the samples collected from each sample cohort in each state is presented in Table 1. In the hospital cohort, clinical samples (blood, urine, stool and rectal swabs) were collected from inpatients after obtaining consent, and the procedures were explained to patients or their guardians who were under the age of consent or

vulnerable. For those who agreed, further samples were collected from participants' homes and, for those who own animals, from their animals. The samples collected were water samples, rectal swabs from their animals, tables, and bench swabs from their residents. In the farm cohort, samples were collected from animal farms and from their handlers, with consent. Also, water samples and swabbing of tables, benches, and pegs from the farms were collected. For the slaughter cohort, rectal swabs were collected from animals to be killed and from butchers after consent was obtained from them. Water from their water sources was collected, and benches and tables used for meat processing were also swabbed. Each participant was randomly assigned a code to maintain confidentiality. All the samples were processed for *K. pneumoniae* isolates.

Table 1 Sample collected from the three sampling cohorts			
Sample Collection Settings and Sample Types		Ekiti	Ondo
Clinical Sample	Blood	89	67
	Urine	146	63
	Stool	2	0
Non-Clinical Sample (Animal Worker) (Rectal Swabs)		238	186
Animals (Rectal Swabs)	Poultry	113	66
	Cow	94	44
	Dogs	51	30
Environment	Water	45	15
	Table, animal peg, Bench Swab	46	27

Bacteriology of the Isolates

The study involved processing human blood and urine samples. The blood samples were collected and inoculated aseptically into the appropriate blood culture bottles, then incubated at 37°C for up to 5 days. Those that gave a growth signal were cultured on solid media (blood agar, MacConkey agar, and chocolate agar) (Oxoid Ltd, Basingstoke, Hampshire, England). Urine samples were inoculated onto cystine-lactose-electrolyte-deficient (CLED) agar (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated at 37°C. The rectal and environmental swabbed samples were enriched in buffered peptone water for about 3 hours, then plated on MacConkey lactose agar (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated at 37°C for 24 hours. Water samples were processed using membrane filtration to isolate and identify *K. pneumoniae*. Individual sterile filter discs with 0.45 µm pores were used in a filtration apparatus to filter 100 mL of the water sample. The filter membranes were then placed on MacConkey lactose agar plates and incubated at 37°C for 24 hours. In all the sample cohorts, colonies resembling *Klebsiella* species were further processed. Gram reactions were carried out on the bacterial isolates according to the Distinct Laboratory Practices in Tropical Countries [32]. The glass slide was smeared with the test isolate, air-dried, heat-fixed, flooded with crystal violet (the primary stain) for 60 seconds, and rinsed with distilled water. It was mordanted with Lugol's iodine for 30 seconds, rinsed again, decolourised with acetone, counterstained with safranin for 60 seconds, rinsed once more, and air-dried. A drop of immersion oil was added to the stained slide, and the slide was examined under an oil-immersion (X100) objective lens on a microscope. Bacterial isolates were identified using morphology, Gram staining and biochemical tests using API 20E (BioMérieux SA, Lyon, France). The API 20E system (BioMérieux SA, Lyon, France), a plastic strip with 20 microtubes for various biochemical and substrate utilisation tests, was used to characterise and confirm *K. pneumoniae*. Bacterial isolates were stored in 15% glycerol peptone water at – 30°C for further study.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the provided antibiotics according to CLSI guideline [33]. These antibiotics were obtained from Oxoid Ltd, Basingstoke, Hampshire, England. The antibiotic used include tetracycline (TET, 30 µg), ceftazidime (CFZ, 30 µg), cefuroxime (CFX, 30 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CFT, 30 µg), meropenem (MER, 10 µg), trimethoprim-sulfamethoxazole (TRM-SXT, 1.25/23.75 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), amoxicillin-clavulanate (AMC-CLA, 30 µg), colistin (COL, 25 µg), gentamicin (10 µg), amikacin (AMK, 30 µg), cephalothin (CEP, 30 µg), ceftazidime-clavulanate (CFZ-CLA, 30/10 µg), and cefotaxime-clavulanate (CTX-CLA, 30/10 µg). The control strain used for comparison was *E. coli* ATCC 25922. The breakpoint was interpreted by the CLSI (2021) guideline (M100, 31st edition)

Carbapenemase Phenotypic Detection

Carbapenemase production was assessed using a Carba NP/CarbAcineto NP-based assay as previously described by Shinde et al. [34]. Briefly, two solutions (A and B) were prepared. Solution A consisted of phenol red (0.05%) and ZnSO₄·7H₂O (0.1 mmol/L) in distilled water, adjusted to pH 7.8 ± 0.1 and stored at 4°C, while solution B was prepared by adding imipenem–cilastatin injectable to Solution A to achieve an imipenem concentration of 6 mg/mL. For each isolate, 2–3 loops of overnight growth were suspended in NaCl and aliquoted into two tubes: tube A (Solution A, no imipenem) and tube B (Solution B, with imipenem) and incubated for 2 hours at 35°C, carbapenemase production was defined as a colour change from red to yellow/orange in tube B, while tube A remained red, in line with acceptable Carba NP interpretive criteria provided by Shinde et al. [34].

DNA extraction

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands) for genomic DNA. The modified protocol for Gram-negative bacteria DNA extraction, as provided by Yang [35]. was used. The quality of the extracted DNA was assessed using a NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, USA) as described by Yang [35]. The eluted DNA was pipetted into storage tubes and stored in a freezer at -30 degrees Celsius until PCR amplification.

Amplification of Carbapenem Antimicrobial Resistance Gene MDR *K. pneumoniae* Isolates with Positive Carbapenem Phenotypic Result

Real-time PCR (RT-PCR) was used to detect resistance genes in *K. pneumoniae* using specific primers and probes. Specific primers and probes targeting these genes (supplementary table 1) were incorporated into RT-PCRs to potentially identify resistance genes (OXA-48, NDM-1, VIM, and KPC). Primers and probes for RT-PCR were obtained from Biosearch LGC (Novato, California, USA). The primer and probe sequences (supplementary table 1). The reaction mixture utilised PerfeCTa MultiPlex qPCR ToughMix(Quanta Biosciences, Gaithersburg, MD, USA). Amplification was performed using an AriaMx RT-PCR cycler (Agilent Inc., Santa Clara, California, USA). The cycle thresholds were determined from fluorescence signals obtained from amplification plots in the AriaMx system software version 3.1 after the completion of the amplification experiment, following the protocol for real-time PCR, as utilised by the authors in another study [36].

Whole-Genome Sequencing

The DNA extractions and library preparations for whole-genome sequencing (WGS) were performed as previously described by Cookson et al. [37]. Briefly, the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for DNA extraction, and libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA). WGS was undertaken by Novogene Limited (Helios, Singapore) using the Illumina HiSeq paired-end v4 platform (2 by 150 bp). The Nullarbor pipeline was used, including adapter-trimmed read processing and examination of WGS reads for de novo genome assembly using SKESA (v.2.2.1), annotation using Prokka (v.1.13.3), and phylogenetic analysis using Snippy (v.4.2.1).

Bioinformatic analysis of WGS data for the antimicrobial Resistance determinants of *E. coli* isolates

The identification and categorisation of genes that confer resistance were carried out using ResFinder 4.1, a bioinformatics tool developed by the Centre for Genomics Epidemiology (CGE) (<http://www.genomicepidemiology.org/>) (accessed on 2024/03/2). Each isolate's genes were compared to an annotated resistance gene using a threshold of 95–100% identity [38]. The plasmid replicon types of each *E. coli* isolate were identified using PlasmidFinder 2.1. As earlier documented by Joensen et al [39], the strain's O and H serotypes were determined by analysing the generated FASTA files using the Centre for Genomic Epidemiology (CGE) platform (<http://www.genomicepidemiology.org/> accessed on 2024/03/2). Additionally, the CARD pipeline (<https://card.mcmaster.ca/analyze/rgi/> accessed on 2024/06/04) was used to determine the AROs from the sequence data.

Table 2
Basis for Performing Whole Genome Sequencing

SAMPLE ID	Sources	Isolates	Resistance pattern	Remarks
NGEK23	Human (Blood)	<i>Klebsiella pneumoniae</i>	AMC, CIP, CTX, CTM, CRO and TET	WGS DONE
NGEK25	Environment (Water) (home of GNEK 23)	<i>Klebsiella pneumoniae</i>	AMC, CIP, CTX, CTM, CRO and TET	WGS DONE

Data Analysis

Data were entered and validated in Excel 2020 version. Frequencies and descriptive statistics were carried out on all data. Differences in proportions of virulence and resistance genes among humans, animals, and environmental samples were evaluated using two-sample t-tests, ANOVA, and chi-square tests, which were appropriate. Continuous variables were analysed using ANOVA, while categorical variables were analysed using chi-square and Fisher's exact test. P < 0.05 was accepted as statistically significant, while p > 0.05 was accepted as statistically insignificant in all cases. Statistical software for Social Science (SPSS) (IBM, version 27) was used for the analysis. Results were presented in Tables and Figures.

Ethical Considerations

Ethical Clearance from the National Health Research Ethics Committee of Nigeria (NHREC) (NHREC/01/01/2007-21/03/2023), the Federal Teaching Hospital, Ido-Ekiti (ERC/2023/09/20/1034B) and the Ondo State Ministry of Agriculture (MNR/V.384/64) were obtained for this study.

Results

Prevalence of *E. coli* and *K. pneumoniae* in the study

The overall prevalence of *K. pneumoniae* in the study was 7.9% (105/1329). Animal samples had the highest prevalence at 9.4% (38/404), followed by human samples at 7.3% (59/804), and environmental samples at 6.0% (8/121). Among clinical human samples, the prevalence was 8.8% (35/399), compared with 5.7% (24/408) in non-clinical samples. Among animal samples, cows had the highest rate at 13.2% (18/136), followed by poultry at 8.9% (16/180) and dogs at 3.8% (3/80). Environmental isolates were most commonly found in swab samples from surfaces in slaughterhouses, poultry, and patients' homes, 9.8% (6/61), whereas water samples showed a lower prevalence, 3.3% (2/60) (Table 3).

Antimicrobial Susceptibility Pattern of the Isolated *K. pneumoniae* from Human, Animal and the Environment

The resistance profile of the *K. pneumoniae* from this study is shown in Table 4. The resistance profile of the 59 *K. pneumoniae* from humans shows highest resistance to amoxicillin-clavulanate 49 (83.1%), the resistance to other antibiotics were as follows: 32 (54.2%), 20 (33.9%), 35 (59.3%), 47 (79.7%), 36 (61.0%), 38 (64.4%), 46 (77.9%), 35 (59.3%), 30 (50.8%) and 7 (11.9%) to trimethoprim-sulfamethoxazole, gentamicin, chloramphenicol, cefuroxime, ceftriaxone, cefotaxime, ceftazidime, tetracycline, ciprofloxacin and meropenem, with one isolate of *K. pneumoniae* from humans having intermediate susceptibility to colistin. All 59 *K. pneumoniae* isolates were susceptible to amikacin (Table 4). The resistance profile of the 38 *K. pneumoniae* from animal samples showed highest resistance to cefuroxime 29 (76.3%), while resistance to other antibiotic were as follows; 13 (34.2%), 5 (13.2%), 5 (13.2%), 6 (15.8%), 12 (31.6%), 8 (21.1%), 23 (60.5%), 14 (36.8%), 4 (10.5%) and 1 (2.6%) resistant to amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, gentamicin, chloramphenicol, ceftriaxone, cefotaxime, ceftazidime, tetracycline, ciprofloxacin and meropenem. Of the 8 *K. pneumoniae* isolates from the environment, 2 (12.5%), 3 (37.5%), 1 (12.5%), 2 (25.0%) and 1 (12.5%) were resistant to amoxicillin-clavulanate, cefuroxime, ceftriaxone, ceftazidime and tetracycline, respectively.

MDR and Carbapenemase and Gene Detection

The overall occurrence of MDR *K. pneumoniae* in the study was 65 (61.9%), as shown in Fig. 2a. The occurrence of MDR *K. pneumoniae* in the human samples was 94.9% among the 59 *K. pneumoniae* isolates. The occurrence of MDR *K. pneumoniae* in the animal and environmental samples was 23.7% and 12.5%, respectively. The occurrence of MDR *K. pneumoniae* was significantly higher in the *K. pneumoniae* from human samples compared to those from animals and the environment, as shown in Fig. 2b. The phenotypic detection of carbapenemase among the 65 MDR *K. pneumoniae* was 9.2%, as shown in Fig. 2c. Of the 6 MDR *K. pneumoniae* that were carbapenemase-producing, 5 were from human samples and 1 from animal samples; none were from environmental samples. Of the 5 MDR *K. pneumoniae* that were carbapenemase-producing, 3 (60.0%) harboured the OXA-48 gene, 1 (20.0%) had the VIM genes, and 2 (40.0%) had the KPC gene, as shown in Table 5. Only one of the three genes (OXA-48, VIM, and KPC) was detected in a single MDR *K. pneumoniae* carbapenemase producer from the animal sample.

Gene Mapping and Phylogenetic Analysis of *K. pneumoniae* isolates

Whole-genome sequencing was carried out on two MDR *K. pneumoniae* isolates from human blood culture and from water samples collected from the patients' homes. The two samples exhibited phenotypic similarities in resistance patterns, as shown in Table 2. Figure 3a-f shows the genome maps for the two *K. pneumoniae* strains (NGEK23 and NGK25). The gene mapping of the two sequenced *K. pneumoniae* strains shows different gene intensities across various sections of the genome, as shown in the figure. The gene mapping of the two genes revealed their gene makeup. Looking at the first one, you can see that the intensity was higher in certain regions than in the second genome. Figure 3b shows the coding sequences (CDS) and genome indel length distribution mapping of the two genes. The indel length distribution CDS in the NGK25 was indicated to peak at 3, 12, 15, 18, and 21 base pairs, as against the indel length distribution CDS in NGK23 at 8, 9, and 12 base pairs.

The structure variation (SV) length in base pairs found in the sequenced *K. pneumoniae* NGK23 and 25 showed that NGK23 had a higher SV > 1200 base pairs than that of NGK25 using the reference genome. However, SNPs with base pairs between 100 and 300 were more common in the *K. pneumoniae* NGK25 than in the *K. pneumoniae* NGK23 isolates. The SNV type showed that the deletion, ITX, and insertion were higher in the isolate *K. pneumoniae* NGK25 than in the isolate NGK23. However, SNV inversion was higher in the isolate *K. pneumoniae* NGK23 than in the isolate NGK25, as shown in Fig. 4c.

The phylogenetic analysis showed the evolutionary relationship between the two isolates and other *K. pneumoniae* isolates available in the gene bank, as shown in Fig. 4. The nearest four isolates, the whole genome isolate that came up, were *K. pneumoniae* strain SH209, *K. pneumoniae* strain KP8701, *K. pneumoniae* sub-species *K. pneumoniae* HS11286, and *K. pneumoniae* strain KP294. The phylogenetic analysis

results indicate that the two isolates (*K. pneumoniae* NGEK23 and NGEK25) are not of the same origin. Isolates NGEK23 were more related to *K. pneumoniae* strain NH209 and *K. pneumoniae* strain KP8701.

Resistance Genes, MLST, and Mobile Genetic Element

Table 6 summarises the bioinformatic analysis of the two sequence isolates (*K. pneumoniae* NGEK23 and NGEK25). From the Insilco analysis, the resistance genes found in isolate NGEK23 were aph (6)id, aph(3)ib, OqxB, blaSHV-30, blaSHV-189, blaSHV-14, sul2, fosA5, tetA, blaTEM-1B, Oqx A, qnrS1, dfrA14, while blaSHV-11, blaSHV-110, sul2, fosA6, catA2, OmpK36, pmrB, catll2 genes were found in the isolate. The multilocus sequence (MLST) analysis of the two isolates shows that isolate NGEK23 is of serotype (ST)3157, while isolate NGEK23 is of a new strain, ST 5ee4. The isolates NGEK23 were KL30 K-locus and 01/02 vs2 O-locus, whereas isolates NGEK25 were KL12 and OL103 O-locus. Three (IncFIB(K)_1, IncY_1 and IncFII(K)_1) mobile genetic elements were found in *K. pneumoniae* NGEK23, while two (IncFIB(K)_1 and col(phAD28_1) mobile genetic elements were found in the isolate. Based on the results, the two isolates were from different origins, as they had distinct resistomes, virulomes, and mobilomes. The resistant genes found in *K. pneumoniae* NGEK23 were more diverse than those in NGEK25. The antimicrobial resistance ontology (ARO) data from the WGS analysis indicate that *K. pneumoniae* NGEK23 has 32 ARO outcomes, whereas *K. pneumoniae* NGEK25 has 30, with 25 similar outcomes (Fig. 5).

Table 3
Occurrence of *K. pneumoniae* in the Study

Sample Sources	<i>Klebsiella pneumoniae</i> n(%)	No Growth n(%)	Total n(%)
Human	59 (7.3)	744 (92.7)	803 (60.4)
Clinical	35 (8.8)	364 (91.2)	399 (49.7)
Non-Clinical	24 (5.9)	380 (94.1)	404 (50.3)
Animal	38 (9.4)	355 (90.6)	393 (29.6)
Cow	18 (13.2)	118 (86.8)	136 (34.6)
Dog	3 (3.8)	75 (96.2)	78 (19.8)
Poultry	16 (8.9)	163(91.1)	179 (50.4)
Environment	8 (6.0)	125(94.0)	133 (10.0)
Water	2 (2.8)	70 (97.2)	72 (54.1)
Table Swab	6 (9.8)	55 (90.2)	61 (45.9)
Total	105 (7.9)	1223 (92.0)	1329 (100.0)

Distribution of *Klebsiella pneumoniae* isolates and samples with no bacterial growth across human, animal, and environmental sources. Data are presented as numbers (percentages) of isolates or samples within each category, with clinical and non-clinical subgroups detailed for human samples, and specific sources indicated for animal and environmental samples.

Table 4
Antibiotic Susceptibility Pattern of Isolated *K. pneumoniae* from Humans, Animals and their Environment

Antibiotics	<i>Human</i> n = 59			<i>Animal</i> n = 38			<i>Environmental Sample</i> n = 8		
	Sensitive n (%)	Immediate n (%)	Resistant n (%)	Sensitive n (%)	Immediate n (%)	Resistant n (%)	Sensitive n (%)	Immediate e n (%)	Resistant
Amoxicillin-clavulanate	10 (16.9)	0 (0.0)	49 (83.1)	25 (65.8)	0(0.0)	13 (34.2)	7 (87.5)	0 (0.0)	1 (12.5)
Trimethoprim-Sulfamethoxazole	17 (28.9)	10 (16.9)	32 (54.2)	31 (81.6)	2 (5.3)	5 (13.2)	8 (100.0)	0 (0.0)	0 (0.0)
Gentamicin	20 (33.9)	19 (32.2)	20 (33.9)	26 (68.4)	7 (18.4)	5 (13.2)	7 (87.5)	1 (12.5)	0 (0.0)
Chloramphenicol	18 (30.5)	6 (10.2)	35 (59.3)	32 (84.2)	0 (0.0)	6 (15.8)	8 (100.0)	0 (0.0)	0 (0.0)
Cefuroxime	4 (6.8)	8 (13.6)	47 (79.7)	3 (7.9)	6 (15.8)	29 (76.3)	3 (37.5)	2 (25.0)	3 (37.5)
Ceftriaxone	15 (25.4)	8 (13.6)	36 (61.0)	22 (57.9)	4 (10.5)	12 (31.6)	7 (87.5)	0 (0.0)	1 (12.5)
Cefotaxime	9 (15.3)	12 (20.3)	38 (64.4)	5 (13.2)	25 (65.7)	8 (21.1)	2 (25.0)	6 (75.0)	0 (0.0)
Ceftazidime	5 (8.5)	8 (13.6)	46 (77.9)	4 (10.5)	11 (29.0)	23 (60.5)	2 (25.0)	4 (50.0)	2 (25.0)
Tetracycline	5 (8.5)	19 (32.2)	35 (59.3)	14 (36.8)	10 (26.4)	14 (36.8)	4 (50.0)	3 (37.5)	1 (12.5)
Amikacin	59 (100.0)	0 (0.0)	0 (0.0)	38 (0.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)
Ciprofloxacin	27 (45.8)	2 (3.4)	30 (50.8)	34 (89.5)	0 (0.0)	4 (10.5)	8 (100.0)	0 (0.0)	0 (0.0)
Meropenem	43 (72.9)	9 (15.2)	7 (11.9)	37 (97.4)	0 (0.0)	1 (2.6)	8 (100.0)	0 (0.0)	0 (0.0)
Colistin	58 (98.3)	1 (1.7)	0 (0.0)	37 (97.4)	1 (2.6)	0 (0.0)	8 (100.0)	0 (0.0)	0 (0.0)

Antimicrobial susceptibility profiles of *Klebsiella pneumoniae* isolates from human, animal, and environmental samples. The table presents the number and percentage of isolates classified as sensitive, intermediate, or resistant to each antibiotic tested, across all three sources. Human (n = 59), animal (n = 38), and environmental (n = 8) data are shown. Results are expressed as n (%).

Table 5
Detection of Carbapenem Genes from Carbapenemase-Producing MDR *K. pneumoniae*

Genes	<i>MDR K. pneumoniae</i>			
	Human n = 5	Animal n = 1	Environment n = 0	Total n = 6
<i>OXA-48</i>	3 (60.0)	0 (0.0)	0 (0.0)	3 (50.0)
<i>VIM</i>	1 (20.0)	0 (0.0)	0 (0.0)	1 (16.7)
<i>KPC</i>	2 (40.0)	0 (0.0)	0 (0.0)	2 (33.3)

Distribution of carbapenemase genes (*OXA-48*, *VIM*, *KPC*) among multidrug-resistant (*MDR*) *Klebsiella pneumoniae* isolates sourced from humans (n = 5), animals (n = 1), and the environment (n = 0). Values are presented as counts with percentages in parentheses, representing the proportion of positive isolates within each source category and overall.

Table 6
Summary of the Bioinformatic results for *K. pneumoniae* isolates

Parameter	NGEK23	NGEK25
Resistance genes	aph (6)id, aph(3)ib, Oqx B, blaSHV-30, blaSHV-189, blaSHV-14, sul2, fosA5, tetA, blaTEM-1B, Oqx A, qnrS1, dfrA14	blaSHV-11, blaSHV-110, sul2, fosA6, catA2, OmpK36, pmrB, catII2
Clonal -group	ST 3157	New (5ee4)
K-locus	KL30	KL12
O locus	01/02vs2	OL103
Plasmid Inc types	IncFIB(K)_1, IncY_1 and IncFII (K)_1	IncFIB(K)_1 and col(phAD28)_1
The table compares the genotypic and plasmid profiles of multidrug-resistant <i>Klebsiella pneumoniae</i> isolates NGK23 (human blood) and NGK25 (environmental water). This table shows the key molecular features of two <i>K. pneumoniae</i> isolates, highlighting differences in resistance genes, sequence types, capsular and O-antigen loci, and plasmid types. The findings illustrate the genetic diversity and varied resistance profiles between clinical and environmental strains, emphasising the importance of integrated surveillance. Note: these results are based on the analysis from the pipeline of http://www.genomicepidemiology.org/)		

DISCUSSION

This study aimed to isolate and characterise MDR *K. pneumoniae* from humans, animals, and their environment in the study area using a one-health approach. Understanding the transmission of MDR *K. pneumoniae* through One Health surveillance across human, animal, and environmental sources is vital for identifying reservoirs, tracking transmission, and guiding targeted interventions against antimicrobial resistance.

The overall prevalence of *K. pneumoniae* (7.9%) and its distribution across animal (9.4%), human (7.3%), and environmental (6.0%) samples indicate its ubiquitous nature. The prevalence found in our study closely matches the 8.6% reported in a 2025 meta-analysis in sub-Saharan Africa using the one-health approach [40]. In the Olaitan et al. [40], the pooled prevalence estimates of 12.1%, 8.6%, and 6.2% were reported for animal, human, and environmental samples of ESBL-producing *K. pneumoniae*, respectively. The country-specific pooled prevalence of *K. pneumoniae* ranged from 8.1% in Tanzania to 23.3% in South Africa, with *K. pneumoniae* from Nigeria showing a pooled prevalence of 11.1%. The finding of a higher prevalence of *K. pneumoniae* in clinical isolates (8.5%) versus 6.4% in non-clinical human samples shows a trend in line with the evidence documented in the previous studies in Nigeria [22, 41–44]. The higher prevalence in clinical human samples (8.5%) compared to non-clinical samples (6.4%) aligns with the established role of *K. pneumoniae* as a leading opportunistic nosocomial pathogen. This disparity suggests that the healthcare environment exerts selective pressure that favours the colonisation and persistence of this bacterium, likely exacerbated by compromised host immunity and frequent exposure to antimicrobial agents.

However, the higher prevalence in animals in cows (13.2%) supports the reservoir hypothesis, as shown recently by Hetland et al. [45] and previously by Hu et al. [46] and Wareth and Neubauer [47] which posits that livestock may serve as significant natural reservoirs for *Klebsiella* species, potentially facilitating zoonotic spillover to humans through the food chain, direct contact, or environmental contamination. The finding of high resistance to amoxicillin–clavulanate and second/third-generation cephalosporins in our study supports recent One Health and clinical studies, which show high resistance to amoxicillin–clavulanate and second/third-generation cephalosporins in human *K. pneumoniae* isolates, reflecting widespread ESBL production and high multidrug resistance (MDR) in humans compared to lower MDR in animals. For example, Marzouk et al. [48] reported 97% resistance to cefotaxime, ceftazidime, and amoxicillin–clavulanate in ESBL-producing *K. pneumoniae*, which is consistent with the 83.1% resistance to amoxicillin–clavulanate and similarly high resistance to other cephalosporins observed in our human isolates. In addition, Bayaba et al [49], in Cameroon reported that 82% of Enterobacterales isolates were MDR, similar to the high MDR burden identified in our study. Olaitan et al [40], also found that ESBL-producing *K. pneumoniae* is widespread, with MDR rates markedly higher in humans (94.9%) compared to animals (23.7%), reinforcing our observation of greater selection pressure from antibiotic use in human clinical settings. The animal *K. pneumoniae* isolates in our study showed substantial resistance to certain cephalosporins, particularly cefuroxime (76.3%), which was lower than the 95% resistance reported by Aslam et al [50] for the 115 *K. pneumoniae* isolates in Pakistan. These further demonstrated the high resistance to cephalosporin associated with *K. pneumoniae* isolates globally. The 11.9% meropenem resistance in *K. pneumoniae* from clinical human isolates in this study further aligns with recent WHO and global reports, which highlight the rise of hypervirulent, carbapenem-resistant *K. pneumoniae* and raise global concerns about resistance to last-line antibiotics [51]. The report on meropenem resistance from CHINET Surveillance networks reveals a sharp increase in carbapenem resistance in *K. pneumoniae*, with meropenem resistance rising from about 3% in 2005 to over 26% by 2018 in China [52], which show a gradual increase in meropenem resistance over the years.

The detection of *blaOXA-48*, *blaKPC*, and *blaVIM* among MDR *K. pneumoniae* isolates in this study provides molecular confirmation of diverse carbapenem resistance mechanisms, encompassing class D, A, and B carbapenemases, respectively. The predominance of *blaOXA-48*, frequently located on conjugative IncL/M-type plasmids, is consistent with reports of efficient horizontal transfer of these plasmids between

Enterobacterales in clinical and experimental models [53–55]. The identification of a carbapenemase-producing isolate in livestock, in the absence of such isolates from the sampled environment, raises the hypothesis of reverse zoonosis (anthropozoonosis), whereby resistant strains or mobile genetic elements move from humans into animal populations [56]. However, without genomic comparisons of human and animal isolates, temporal linkage, and more extensive environmental sampling, the direction of transmission remains uncertain, and this interpretation should be regarded as speculative [56, 57]. OXA-48-producing *K. pneumoniae* are associated with reduced susceptibility to ertapenem and meropenem and have been linked in multiple cohorts to delayed effective therapy, prolonged hospital stay, and increased mortality compared with carbapenem-susceptible infections [55, 58]. VIM metallo- β -lactamases confer resistance to most β -lactams [59, 60]. They are often detected in high-risk patients, including those in intensive care or with significant immunosuppression, where they are associated with severe invasive infections [61]. KPC enzymes mediate resistance to a broad spectrum of β -lactams, including carbapenems, and infections due to KPC-producing *K. pneumoniae* have historically required prolonged courses of toxic agents such as colistin or aminoglycosides [62–64]. Although newer β -lactam/ β -lactamase inhibitor combinations have partially improved outcomes, there remains a need to develop a new antibiotic to treat emerging infections caused by carbapenem-resistant pathogens, including those caused by MDR *K. pneumoniae*.

Whole-genome sequencing of *K. pneumoniae* NGEK23 from human blood and *K. pneumoniae* NGEK25 obtained from a water sample from the home of the patient NGEK23 (environmental water) provides insights into *K. pneumoniae* genomic plasticity, including differences in core-genome phylogeny, structural variation, and resistome composition. Despite similar multidrug-resistant phenotypes (Table 2), phylogenetic analysis indicates that the two isolates are not closely related. This is consistent with previous work demonstrating that similar resistance profiles can emerge in distinct *K. pneumoniae* lineages through horizontal acquisition of shared mobile genetic elements [65–67].

Differences in Indel length distribution and structural variation between *K. pneumoniae* NGEK23 and *K. pneumoniae* NGEK25 indicate substantial genome remodelling. The higher number of insertions and deletions in the environmental isolate (*K. pneumoniae* NGEK25) may reflect recent recombination and mobile element activity, potentially linked to adaptation to fluctuating environmental conditions, as previously documented in the studies by Thorpe et al [67] and Rocha et al [68]. However, this remains speculative and could have also been driven by other lineage-specific evolutionary phenomena. *K. pneumoniae* NGEK23 harbours a broader resistome than *K. pneumoniae* NGEK25, with more annotated resistance determinants. The above finding in our study agrees with large-scale studies showing that clinical *K. pneumoniae* isolates often carry expanded AMR gene repertoires compared with environmental counterparts, likely due to intensive antibiotic selection in healthcare settings [69, 70]. This pattern supports the widely described model in which clinical isolates accumulate resistance genes through the acquisition of plasmids and other mobile genetic elements [65]. However, it should be interpreted with caution, given that resistome size is also influenced by sequence type and plasmid background [69].

The detection of ST3157 and a putatively novel sequence type (ST 5ee4) highlights the high genetic diversity of *K. pneumoniae* in the study area and mirrors international genomic surveillance data that report numerous STs, including many rare or newly described lineages [71, 72]. While the identification of a novel ST underscores the dynamic local emergence of previously undocumented lineages, its epidemiological significance and potential to become a high-risk clone will depend on evidence of dissemination, convergence of resistance and virulence, and association with outbreaks, which require ongoing genomic and epidemiological surveillance [70]. The presence of multiple serotypes and unique combinations of K- and O-loci suggest variations in virulence factors and distinct interactions with hosts, indicating that they are not of the same lineage or origin. Afolayan et al [73] asserted that performing K- and O-locus typing of *K. pneumoniae* is essential for public health and clinical management. It aids in identifying clonal lineages, monitoring transmission, comprehending virulence and pathogenesis, and predicting antibiotic resistance profiles. K-loci are responsible for encoding capsular polysaccharide (CPS), which aids the bacteria in evading the immune defences of the host and facilitating infection [73–76]. Various K-loci are associated with varying levels of virulence and may affect the types of infections. Specific antibiotic resistance profiles are influenced by certain K-loci, which in turn affect treatment choices. K-locus typing is crucial for identifying precise capsular antigens that vaccines can selectively target against *K. pneumoniae* [12, 73]. It has also been found to aid in understanding the evolutionary process of *K. pneumoniae*. It provides valuable insights for public health policies regarding infection control, antibiotic management, and vaccine advancement [75]. Differences among mobile genetic elements indicate varying capacities for horizontal gene transfer, enabling the rapid dissemination of resistance genes and virulence factors among bacterial populations. Monitoring the dissemination of these components among various ecosystems and host populations is crucial. The resistome, virulome, and mobilome profiles found in the two isolates indicate the potential for the transmission and evolution of bacteria resistant to antibiotics and highly infectious across species. This finding is consistent with the literature, which reports diversity in the mobilome of *K. pneumoniae* and other Gram-negative bacteria [77–79].

Conclusion

The study demonstrates high levels of antibiotic resistance among *K. pneumoniae* isolates from humans and environmental samples in the study area. At the same time, the detection of carbapenemase genes in human and animal samples suggests that resistance is not confined within hospital boundaries but extends into surrounding ecosystems. The high diversity of mobile genetic elements and the identification of new sequence types indicate a dynamic, evolving resistome that can traverse human, animal, and environmental reservoirs, underscoring the

risk of wider dissemination. These findings highlight the urgent need for integrated antimicrobial stewardship that explicitly targets the hospital–livestock interface, including prudent antibiotic use in clinical care and animal husbandry, coordinated infection-prevention practices, and control of clinical and faecal waste. Sustained genomic and epidemiological surveillance within a One Health framework is essential to detect emerging high-risk lineages early and to inform targeted interventions before they become entrenched in human and animal populations.

Declarations

DATA AVAILABILITY AND WGS DATA-GENERATED INFORMATION.

The WGS data generated in this study have been deposited at NCBI under the project accession PRJNA1295602. An additional request can be made to the corresponding author.

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Author Contributions

ULI-Conceptualisation, investigation, software, formal analysis, write first draft, OBS-Supervision, data validation, Editing, Writing. OTM-Supervision, data validation, Editing, Writing, OOE-Supervision, data validation, Editing, Writing, AFA-investigation, formal analysis, writing, project administration, OB-investigation, formal analysis, writing, project administration, FOS-investigation, formal analysis, writing and editing, AEC-Supervision, data validation, Editing, Writing and OSK-Supervision, data validation, Editing, Writing.

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Ethics and consent to participate

Ethical Clearance from the National Health Research Ethics Committee of Nigeria (NHREC) (NHREC/01/01/2007-21/03/2023), the Federal Teaching Hospital, Ido-Ekiti (ERC/2023/09/20/1034B) and the Ondo State Ministry of Agriculture (MNR/V.384/64) were obtained for this study. Consent was gotten from the participants to participate in the study.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

Clinical Trial Number

Not applicable

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Figures

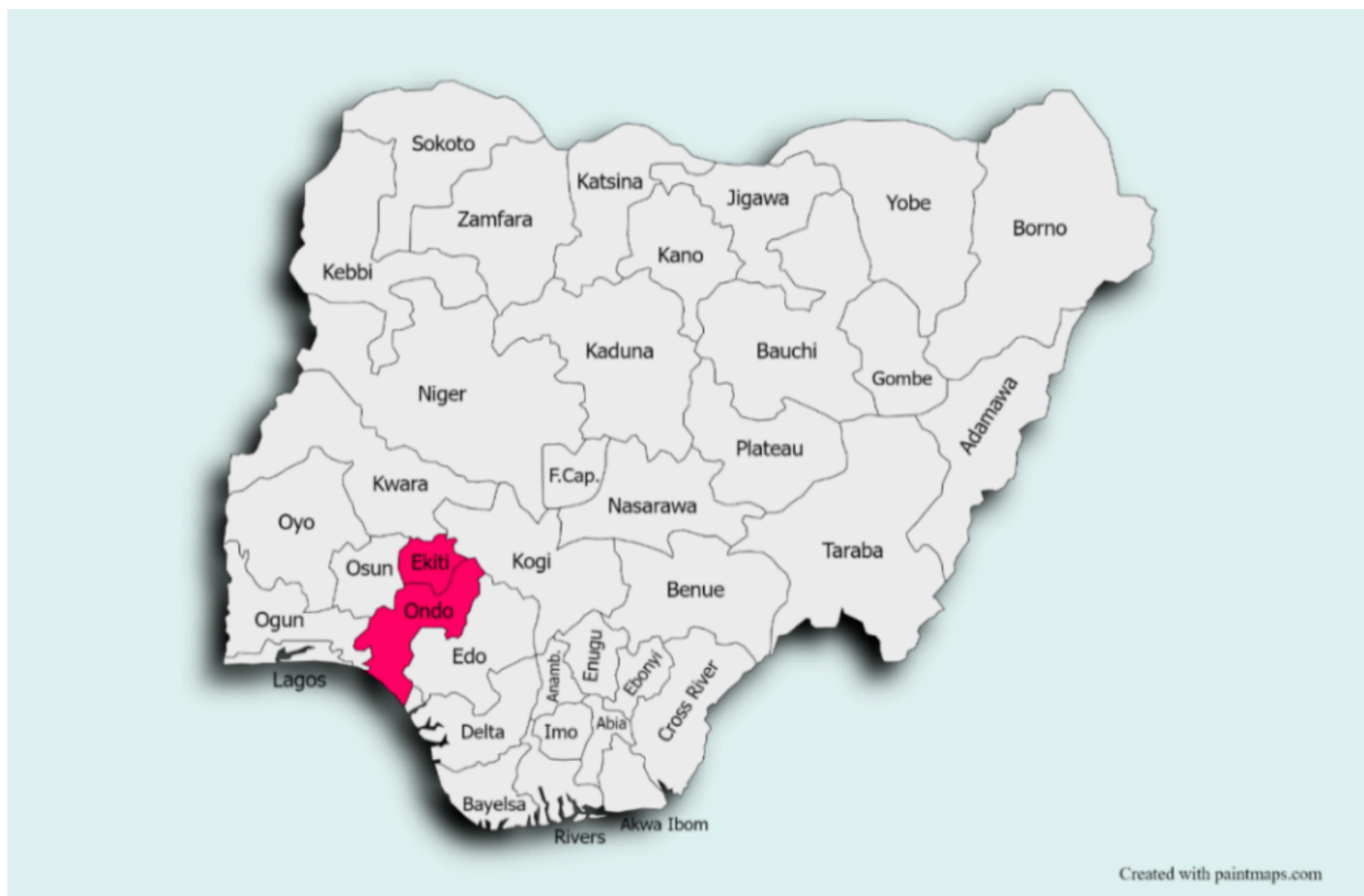


Figure 1

Map Diagram of Nigeria, highlighting Ekiti and Ondo States Google Map, 2019).

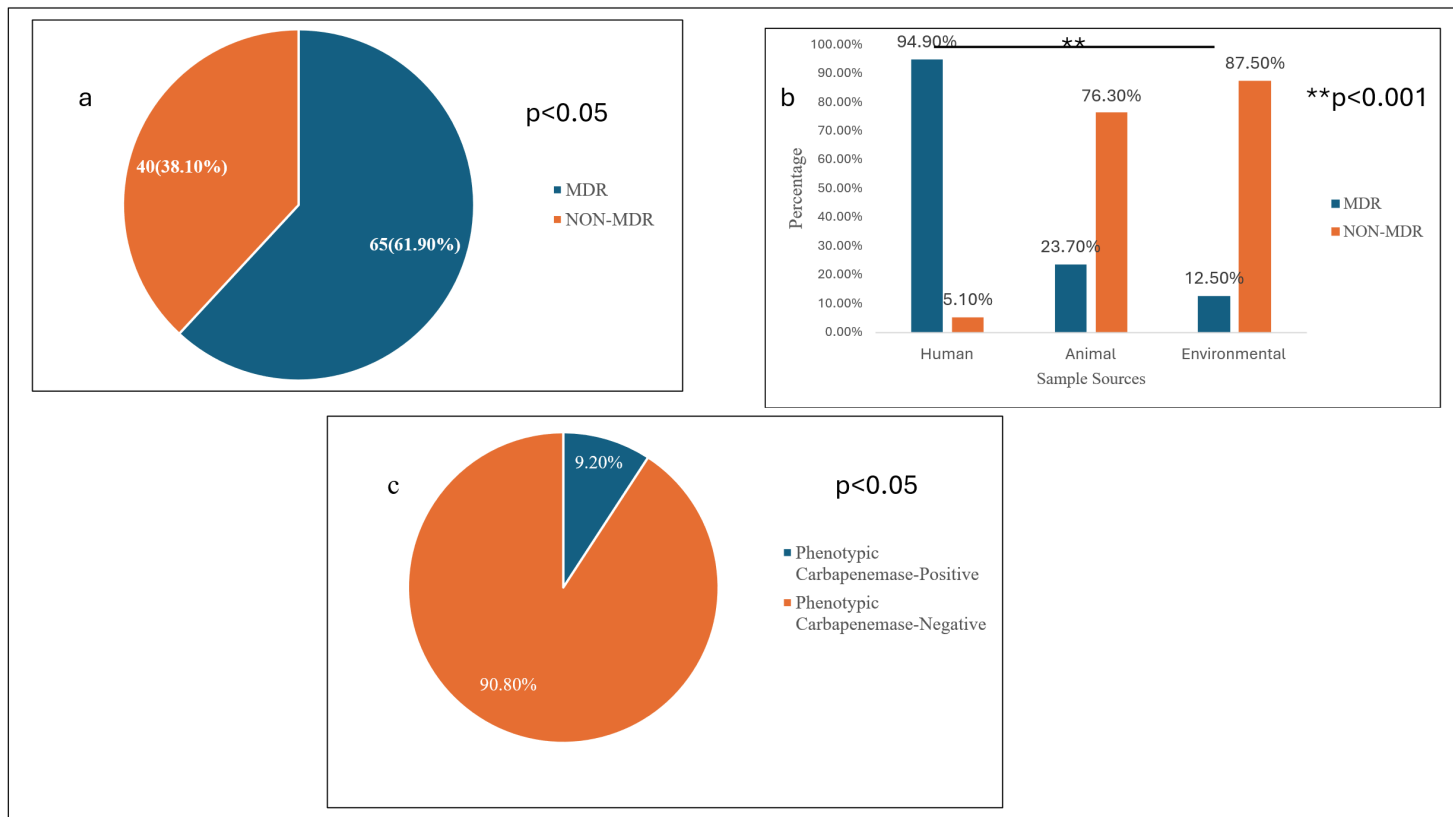


Figure 2

Distribution of multidrug resistance and carbapenemase phenotypes among isolates. (a) Pie chart showing the overall proportion of multidrug-resistant (MDR) versus non-MDR isolates. (b) Bar chart depicting percentages of MDR and non-MDR *K. pneumoniae* isolates stratified by human, animal, and environmental sample sources. (c) Pie chart illustrating the proportion of phenotypically carbapenemase-positive and carbapenemase-negative isolates among the MDR-*K. pneumoniae*.

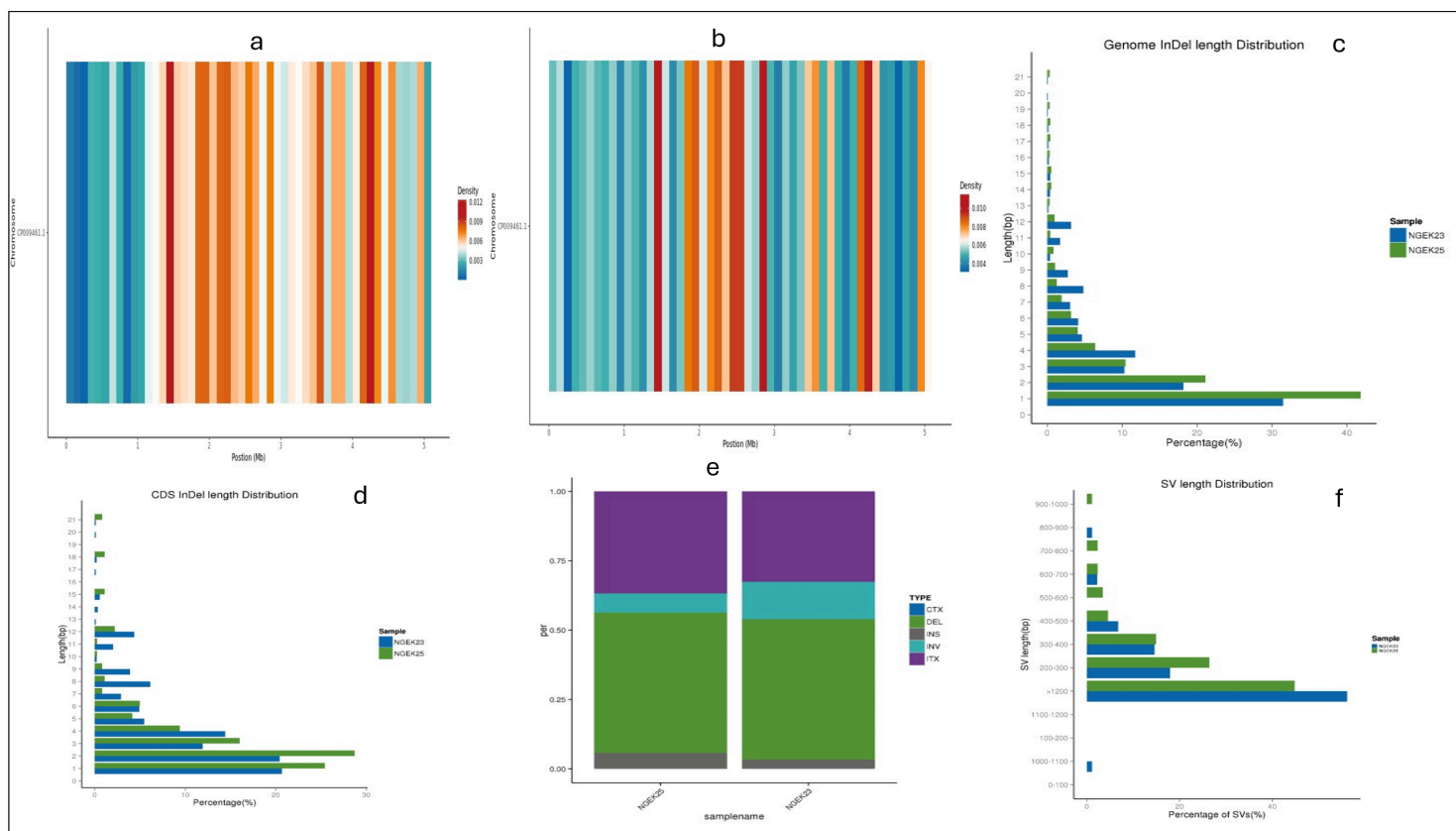


Figure 3

Comparative genome analysis of *K. pneumoniae* NGEK23 and NGEK25. Heatmaps show the distribution of insertion sequences and integron cassettes across individual genomes a=NGEK23 and b=NGEK25. Genomic island and chromosomal cassette length distributions are presented for (c) intact and (d) truncated elements. (e) Stacked bar plots compare the proportional abundance of mobile element categories between carbapenem-resistant and susceptible isolates. (f) The bar plot shows the percentage contribution of each insertion sequence family to the total mobile element content.

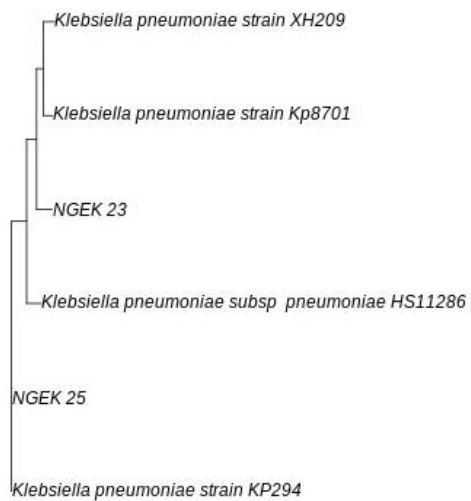


Figure 4

Phylogenetic Analysis of the *Two K. pneumoniae* with reference genomes: Phylogenetic tree illustrating the genetic relationships between the two *K. pneumoniae* isolates (NGEK23 and NGEK25) and selected reference genomes.

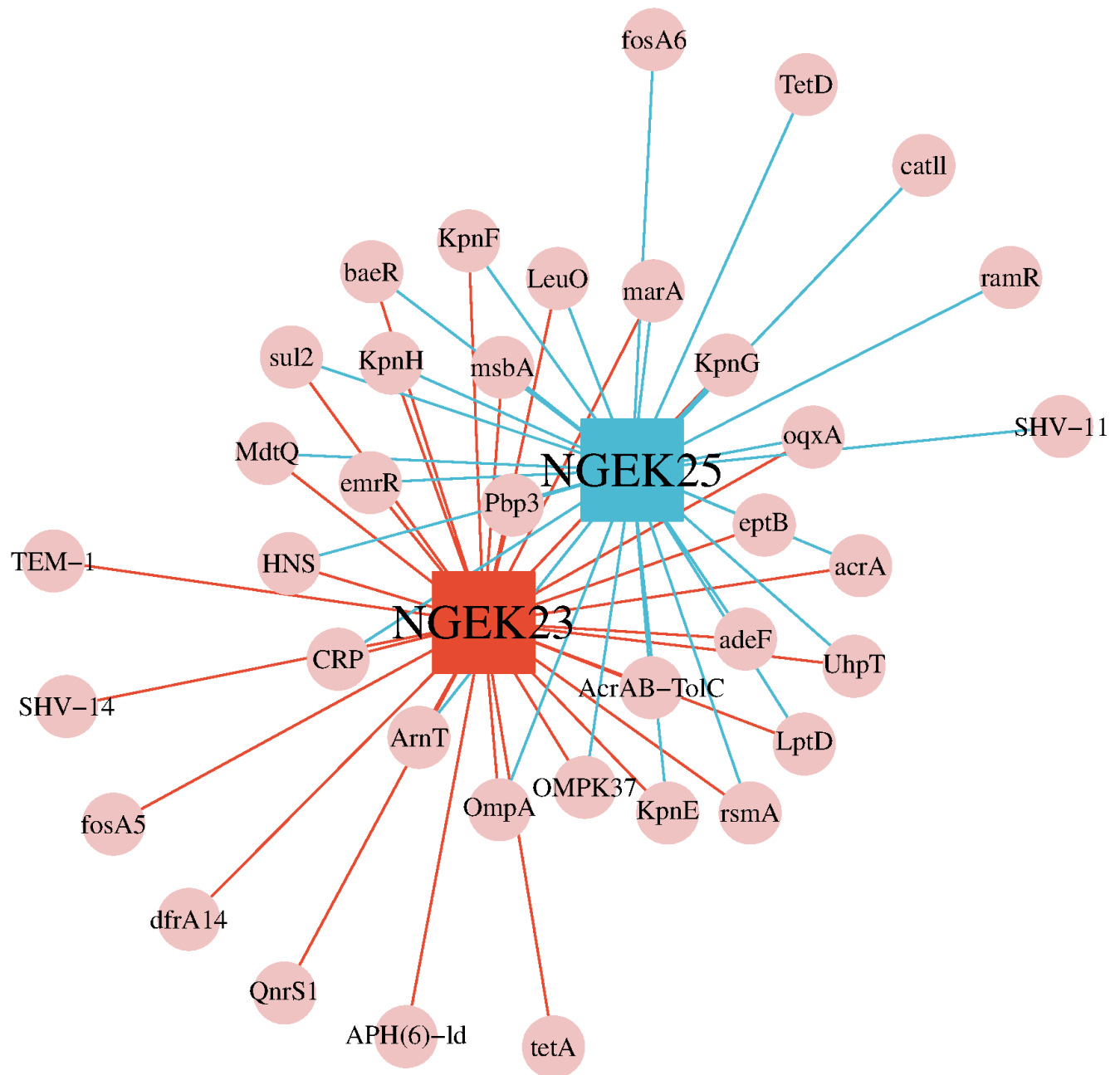


Figure 5

Venn diagram of antimicrobial resistance gene profiles using the CARD pipeline for *Klebsiella pneumoniae* isolates NGEK23 (human blood) and NGEK25 (environmental water). *pneumoniae* isolates NGEK23 (human blood) and NGEK25 (environmental water). Venn diagram illustrating the distribution and overlap of antimicrobial resistance (AMR) gene profiles identified in *Klebsiella pneumoniae* isolates NGEK23 (recovered from human blood) and NGEK25 (recovered from environmental water) using the CARD pipeline. Unique and shared AMR genes between the two isolates are depicted, highlighting both isolate-specific resistance determinants and common elements, thereby providing insight into the genetic basis of resistance in clinical versus environmental sources. The Venn diagram was created from <http://www.bioinformatics.com.cn/>

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