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Phenotypic and Genomic Characterisation of Multidrug-Resistant Wound-Associated Gram-negative Bacteria from a Tertiary Hospital in Ghana

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Abstract

Background

Hospital-acquired wound infections due to multi-drug resistant (MDR) Gram-negative pathogens, remain one of the leading causes of clinical complications and mortality. Understanding their resistance patterns and genetic insights is an essential step at reducing this burden in hospital settings. This study determines the resistance patterns of MDR organisms and molecularly characterized extended spectrum beta-lactamase (ESBL)-producers and carbapenem-resistant (CR) E. coli and K. pneumoniae isolated from wound samples from a tertiary hospital in Ghana.

Methods

We analyzed 200 Gram-negative clinical isolates. Species were identified by microbiological biochemical test reactions and confirmed with MALDI-TOF. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method. Isolates with ≥ 5 mm increase in zone of inhibition for cefotaxime/ceftriaxone alone and/or in combination with clavulanate, were considered ESBL-producers whilst those resistant to meropenem were considered carbapenem resistant (CR). Nine ESBL-producers (E. coli, n = 5; K. pneumoniae, n = 4) and 2 CR (E. coli n = 1; K. pneumoniae n = 1) were whole genome sequenced on GridION platform (Oxford Nanopore Technologies).

Results

Clinical resistance to cefuroxime (147/200, 74%), ampicillin (137/200, 69%), gentamicin (126/200, 63%), ceftriaxone (120/200, 60%) and ciprofloxacin (120/200, 60%) was common. However, the majority (178/200, 89%) of the isolates were susceptible to meropenem. In total for all species, the rates of MDR, ESBL production and carbapenem-resistance were 74% (148/200), 53% (106/200) and 10.5% (21/200) respectively. The blaCTX-M-15 was the most predominant ESBL gene (11/11, 100%) and was found to co-exist with other beta-lactamases including blaOXA-181 and blaNDM-1.

Conclusions

The concerning frequency of MDR and ESBL-production among clinical strains calls for strict adherence to antimicrobial stewardship programs. To the best of our knowledge, we report for the first time the emergence of CTX-M-15, NDM-1-producing K. pneumoniae in Ghana. Continued surveillance, coupled with intensive infection prevention and control (IPCs) strategies are essential to control the further spread of these strains in Ghana.

Background

Hospital-acquired wound infections (HAWI) due to multidrug-resistant (MDR) pathogens, particularly Gram-negative organisms, compromise patient care, delay healing, and can lead to loss of limbs and death (1). In sub-Saharan Africa, surgical sites and burns wounds infections accounted for 43.7% and 34% mortalities respectively (2, 3). The predominance of MDR Gram-negative pathogens (defined as resistant to at least three classes of antibiotics) (4) in HAWI, especially MDR *E. coli* and *K. pneumoniae* has been described in Ghana and worldwide (3, 5–12). Antimicrobial resistance (AMR) among Gram-negative pathogens has consistently remained a critical issue, and an urgent public health emergency (13). In Ghana, AMR accounts for 5,900 attributable and 25,300 associated deaths and 4.71 million deaths, globally (14, 15). On a global scale, carbapenem-resistant (CR) and extended spectrum beta-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* have been implicated in severe nosocomial infections, with high mortality rates and substantial global burdens (16). This constitutes a major challenge, especially when treatment of wound infections is mostly empirical in Ghana and other sub-Saharan countries with significant poor treatment outcomes (17). ESBLs and carbapenemases are the most common genetic determinants of resistance to carbapenems and third generation cephalosporins (18). ESBLs and carbapenemase-encoding genes are chiefly acquired through horizontal gene transfer facilitated by mobile genetic elements (MGEs) such as plasmids, transposable elements and integrative and conjugative elements (18–22). Worrisomely, the carriage of these genes by high-risks clones such as *E. coli* ST131, ST410, ST167 and *K. pneumoniae* ST17 has the tendencies to exacerbate the AMR burden (23). *E. coli* high-risk clone, ST131 has received significant global attention due to its association with high levels of MDR, persistence, and effective transmission between patients (24). Nevertheless, *E. coli* ST410, is an emerging high-risk one-health clone, gradually gaining

attention due to its association with the spread of carbapenemases and share similar attributes with *E. coli* ST131 (25). Similarly, *K. pneumoniae* ST17, is another global high-risk clone, found to be frequently associated with the carriage of multiple resistance genes including plasmid-borne $bla_{CTX-M-15}$ and $bla_{OXA-181}$ (26). In Ghana, *E. coli* ST410 and *K. pneumoniae* ST17 carrying ESBLs and multiple carbapenemases have been associated with series of hospital acquired infections, outbreaks and contamination of hospital environment (27–32). Molecular characterization of the genetic contexts of ESBLs and carbapenemase encoding genes like $bla_{CTX-M-15}$, $bla_{OXA-181,}$ bla_{OXA-48} and bla_{NDM-1} in *E. coli* and *K. pneumoniae* strains, has demonstrated the collaborative roles of insertions sequences ISEcp1, ΔISAba125 and IS3000 as well as IS26 composite transposons in the mobilization, dissemination and expression of these genes (33, 34). However, there is a paucity of comprehensive genomic data on ESBL and carbapenemase-producing *E. coli* and *K. pneumoniae* associated with HAWI from the Korle-Bu Teaching Hospital (KBTH), Accra, the third biggest referral center in Africa, with only three published studies in the last decade (5, 8, 35). Here, we determined the resistance profiles of 200 Gram-negative species recovered from in-patients wound sites. We furthermore investigated the genetic determinants of resistance and explored the genetic contexts of ESBL and carbapenemase-encoding genes in *E. coli* and *K. pneumoniae*.

Methods

Study design and setting

This retrospective cross-sectional study, approved by the Korle-Bu Teaching Hospital-Scientific and Technical Committee/Institutional Review Board *(KBTH-STC/1RB/000102/2025)* was conducted between August 2024 and March 2025.

KBTH has 2,000 beds, 21 clinical and diagnostic departments and three centers of excellence. It has an average outpatient attendance of 1,500 with about 250 inpatient admissions. During the study period, 317 non-duplicate Gram-negative organisms were recovered from in-patients' wound sites derived from: surgical, burn, diabetic and ulcerative wounds, and processed for culture and susceptibility testing at KBTH Central Laboratory. The isolates were stored in nutrient broth with 10% glycerol.

Data collection

Access to anonymized data on patients' age, sex, ward and clinical diagnosis was granted on 24th September, 2025. Anonymized data on the isolates could not be traced to patients, hence patient consent was not required. The study adhered to the values of the 2013 World Medical Association Declaration of Helsinki.

Identification of isolates

The stored cultures were brought to room temperature to thaw and subsequently sub-cultured on MacConkey (Oxoid Thermo Fisher Scientific, UK) and incubated aerobically for 24 hours at 37°C. Single colonies of Gram-negative bacteria were subjected to in-house prepared conventional biochemical tests (oxidase test, triple sugar iron, citrate, urea, motility, indol) (Oxoid Thermo Fisher Scientific, UK) for species identification (36). Identification was confirmed by Matrix-assisted Laser Desorption/ionization time-of-flight (MALDI-TOF) (Bruker Daltonics, Bremen, German) at the department of Medical Microbiology, University of Ghana Medical School.

DNA extraction, Whole Genome Sequencing and Quality Control

Isolates genomic DNA was extracted from pure overnight cultures on nutrient agar incubated aerobically at 37°C for 24 hours (Oxoid Thermo Fisher Scientific, UK), using the MagAttract High Molecular Weight (HMW) DNA Kit (Qiagen, Hildon, Germany) and purified with NucleoSpin tissue kit according to the manufacturer's instruction (Macherey-Nagel, Düren, Germany) (39). DNA concentration was quantified using a Qubit 4 fluorometer with the Qubit 1X dsDNA HS Assay (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions. Long-read sequencing libraries were made using the Native Barcoding Kit EXP-NBD104 and ligation sequencing kit SQK-LSK109 as per manufacturers protocol (40). Sequencing was performed on Oxford Nanopore GridION platform using R10.4.1 flow cells (Oxford Nanopore Technologies, UK) (41). Raw Nanopore reads were assessed for quality using NanoQC v0.9.4 (42). The reads were preprocessed using fastp v0.23.4 (43) with adapter trimming enabled. Reads were retained if they had a mean Phred quality score of ≥Q10 and a minimum length of 1,000 bp, using --average_qual 10 and --length_required 1000. Post-trimming quality was re-evaluated with NanoQC to ensure data integrity. High-quality trimmed reads were assembled *de novo* using Flye v2.9 (44). Assembly quality metrics, including total contig count, N50, and genome size, were assessed using QUAST v5.2.0 (45).

Assemblies with fewer than 20 contigs and an estimated mean coverage of > 20× after excluding contigs shorter than 1,000 bp were retained for downstream analyses.

Bioinformatics and Data Analysis

Species identification was confirmed using SpeciesFinder v4.0.4 (46) and Kleborate v3.2.4 (47). AMR-encoding genes were detected using Resfinder v3.2 (48) and Kleborate v3.2.4 (47). The rapid annotation using subsystem technology (RAST) tool and basic local alignment search tool (BLAST) were used to annotate and compare the assembled sequences (49). Mobile genetic elements (MGEs) were identified using mge_finder 1.1.2, (50) Uniprot BLAST, (51) and ISfinder (52). SnapGene viewer v4.1.9 and Easyfig v2.2.5 enabled visualization and comparison of genetic contexts of AMR-encoding genes (53, 54). PlasmidFinder v2.1 (55), was used to identify plasmid replicons. Multi-locus sequence typing (MLST) was performed using MLST v2.0 (https://cge.food.dtu.dk/services/MLST/) (Larsen et al., 2012). Default parameters were applied for all analyses unless otherwise specified. The Bactinspector v0.1.3 tool (https://gitlab.com/antunderwood/bactinspector) was used to select the most representative reference genome for phylogenetic analysis using the “closest match” option. The reference sequence with the best hit was selected and subsequently downloaded from the NCBI database. The CSI Phylogeny v1.4 web tool (https://cge.food.dtu.dk/services/CSIPhylogeny/) (56) was used for the construction of a maximum-likelihood phylogenetic tree based on the core genome SNPs using default settings. The constructed phylogenetic tree was visualized and annotated using iTOL v7.0 (https://itol.embl.de) (57). Complete chromosomal and plasmid sequences were deposited in GenBank under Bioproject accession PRJNA1495604. Isolates metadata were entered into Microsoft access and analyzed with Statistical Package for Social Sciences (SPSS) program v28.0 for windows.

Results

Species Diversity of Isolates

Out of the 317 Gram-negative isolates sub-cultured, 200 produced bacterial growth. The remaining 117 isolates failed to recover after storage and were excluded from further analysis.

The most common organisms isolated were *E. coli* (76/200, 38%), *P. aeruginosa* (37/200, 18.5%), *K. pneumoniae* (29, 14.5%) and *A. baumannii* (19/200, 9.5%). The majority of the organisms were isolated from female patients (59.7%), and most isolates were recovered from surgical sites (83/200, 41.5%) wound samples **(Supplementary Table 1).**

Antibiotic Susceptibility Patterns of Isolates

Overall, the multispecies AMR profile of Gram-negative isolates demonstrated high resistance to cefuroxime (147/200, 73%), ampicillin (137/200, 68.5%), cotrimoxazole (135/200, 68.5%), ceftriaxone (120/200, 60%) and ciprofloxacin (116/200, 58%). When tested against the aminoglycosides, most isolates were resistant to gentamicin (126/200, 63%), as compared to amikacin (50/200 25%). However, the majority of isolates were susceptible to meropenem (178/200, 89%) (Table 1).

Table 1

Phenotypic resistance profile of Gram-negative isolates. Clinical isolates (n = 200) from wound sites were cultured as described and AMR characterized by disc-diffusion. Results are shown for resistance frequency according to defined CLSI guidelines. AMP-Ampicillin, AUG-Augmentin; COT-Cotrimoxazole; CXM-Cefuroxime; CRO-Ceftriaxone; CIP- Ciprofloxacin; CAZ-Ceftazidime; LEV-Levofloxacin; GEN-Gentamicin; AMK-Amikacin; MEM-Meropenem.

Isolates	**Antimicrobial Resistance Patterns of Isolates**										
		AMP	**AUG**	**COT**	**CXM**	**CRO**	**CIP**	**LEV**	**GEN**	**AMK**	**CAZ**	**MEM**
	n = 200	137 (69%)	116 (58%)	135 (68%)	147 (74%)	120 (60%)	116 (58%)	101 (51%)	126 (63%)	50 (25%)	38 (68%)	22 (11%)
E. coli	76	71	58	69	70	59	58	54	57	19	-	5
K. pneumoniae/ oxytoca	29	29	23	24	29	23	18	16	21	8	-	1
C. diversus/freundii/koseri	15	10	8	9	15	10	8	5	11	4	-	0
E. bugandensis /hormaechei	11	9	8	10	9	7	7	4	8	3	-	0
S. paratyphi	1	0	0	0	1	0	0	0	1	0	-	0
M. morganii	2	1	1	2	2	1	1	0	1	0	-	0
P. mirabilis	9	1	2	2	2	2	2	2	4	2	-	0
A. *baumannii*	19	15	15	18	18	18	15	12	15	7	-	11
P. aeruginosa	37	-	-	-	-	-	6	7	7	6	11	4
S. maltophilia	1	1	1	1	1	1	1	1	1	1	1	1

Comparatively, across species, higher drug resistance was observed among *K. pneumoniae* (27/27, 100%), *A. baumannii* (18/19, 94.7%), and *E. coli* (70/76, 92%). Overall, MDR, ESBL production and carbapenem resistance found in isolates were 74.5% (149/200), 53% (106/200) and 11% (22/200) respectively. ESBL production was commonly observed in *E. coli* (66%), and CR was predominantly seen in *A. baumannii* (50%) **(**Fig. 1**).**

Genomic Characteristics of Isolates

Twelve isolates (10 ESBL-producing and 2 carbapenem-resistant isolates) were initially selected for whole-genome sequencing. Following sequencing, one isolate originally identified as *Acinetobacter* spp. by MALDI-TOF was re-classified as *Escherichia coli* based on genomic analysis. Since the discrepant identification could not be resolved with confidence, this isolate was excluded from subsequent genomic analyses, resulting in a final dataset of 11 genomes. All isolates harbored the plasmid-based $bla_{CTX-M-15}$ gene in addition to other beta-lactamases **(Supplementary Table 2)**. Whereas OXA and TEM beta-lactamases were predominantly produced by *E. coli*, *K. pneumoniae* isolates mainly carried *SHV beta-lactamases* (Table 2). Fluroquinolone resistance correlated with the presence of *qnrS1*, *qnrB1* and *qepA4* as well as mutations within the quinolone resistance determining regions; *gyrA* (D874N), *parC* (S801), *parE* (8458A/8458T) and *acrR* (F172S, F197I, G164A, K201M, L195V, P161R, and R173G) in *E. coli* and *K. pneumoniae* isolates as described (58, 59). Other AMR-encoding genes conferring resistance to trimethoprim, aminoglycosides, sulfonamides, tetracycline, macrolides, and rifamycin as well as efflux pumps such as major facilitator superfamily (MFS); *emrR, emrB, mdtH, mdtG*; resistance nodulation-cell division (RND); *acrB, mdtB, marA, mdtM*, and disinfectants/antiseptics (*leuO, mdtO*, mdtN) were present in the isolates' genomes (Table 2).

The carbapenem-resistant *E. coli* strain, CR-*Ec*-124 carried plasmid replicons; ColKP3, IncFIA, IncFIB (AP001918), IncFII (pAMA1167-NDM-5), IncHI1A, IncHI1B (R27), IncX3 and p0111. Additionally, the isolate harbored two plasmids designated as pEc124kb_2 and pEc124kb_3. Plasmid pEc124kb_2 of size 204,935bp and GC content 49%, encoded bla_{NDM-1}, $bla_{CTX-M-15}$, $bla_{TEM-1A,}$ and bla_{OXA-9}. Plasmid pEc124kb_3 encoded $bla_{OXA-181}$ and the ciprofloxacin resistance gene, *qnrS1* embedded within IS26 composite transposon

(Table 2). The isolate also harbored CMY-2, the most common imported *Amp*C beta-lactamases among *E. coli* isolates as described (25).

By comparison, the carbapenem-resistant *K. pneumoniae* strain CR-*Kp*-1894, carried plasmid replicons Col(pHAD28), Col440I, Col440II, IncFIB(K), IncFIB(pQil), IncFII(K) and IncR.The isolate also harbored two distinct plasmids pkp1894kb_7 and pkp1894kb_8, which harbored diverse resistance genes including $bla_{NDM-1,}$ $bla_{CTX-M-15}$ and bla_{TEM-1B} (pkp1894kb_8); $bla_{CTX-M-15}$, bla_{OXA-9}, bla_{TEM-1A} and aminoglycoside resistant genes *ANT (3")-Ia* and *AAC (6')-Ib* (pkp1894kb_7). The $bla_{SHV-172}$ and fosfomycin resistance genes (*fosA, fosA6*), were chromosomally encoded **(**Table 2**).**

Table 2

Genomic characteristics of ESBL producing and Carbapenem-resistant isolates. Of 200 Gram-negative clinical isolates derived from wound-site infections, a subset of phenotypically AMR *E. coli* and *K. pneumoniae* samples were selected for whole-genome sequencing. Plasmid-borne and chromosomally-encoded genetic features with known association with AMR are summarized.

ID	Sample type (swab)	ESBLS *(bla)*	Carbapenemases *(bla)*	Other AMR-Encoding Genes	STs
Ec01	**Surgical site**	***CTX-M-15, CTX-M-3***	***Absent***	***aadA2, mph(A), gepA* (4), *sul1, tet (B), cat A***	**167**
Ec02	Surgical site	*CTX-M-15, TEM-1B*	*Absent*	*aph (3")-1b, aph (6')-Id, dfrA14, qnrS1, sul* (2), *tet(A)*	38
Ec03	Ulcerative wound	*CTX-M-15, OXA-1*	*Absent*	*aac (6')-Ib-cr, aadA5, catB3, dfr17, sul1*	44
Ec04	Surgical site	*CTX-M-15, OXA-1, TEM-1B*	*Absent*	*aac (6')-1b-cr, aadA5, catB3, dfrA17*	448
*Ec*05	Surgical site	*CTX-M-15, OXA-1, TEM-35*	*Absent*	*aac (3')-IIa, aac (6'), catB3, dfrA17, erm (B), mph(A), sul1, tet (B)*	405
CR-Ec-124	Surgical site	*CTX-M-15, TEM-1A*	*NDM-1*, *OXA-181*	*aac (6')-1b, aadA1, aadA5, CMY-2, dfrA17, erm(B), mph (A), qnrS1*	410
Kp01	Burns wound	*CTX-M-15, OXA-1, SHV-27, TEC-1C*	*Absent*	*fosA, aac (3')-IIa, aac (6')-Ib-cr, aad (A1), catB3, drfA15, sul1*	967
Kp02	Surgical site	*CTX-M-15, OXA-1, SHV-172, TEM-1B*	*Absent*	*fosA, aac* (3)*-IIa, aac (6')-Ib-cr, aph* (3)*-Ib, aph* (6)*-Id, catB3, dfrA14, qnrB1, sul2, tet (A)*	48
Kp03	Ulcerative wound	*CTX-M-15, SHV-75, TEM-35*	*Absent*	*fosA, aac* (3)*-IIa, aac (6')-Ib-cr, aph* (3)*-Ib, aadA16, aph (3')-Ib, catA2, drfA27, qnrB6, sul1, sul2, tet (D)*	133
Kp04	Surgical site	*CTX-M-15, OXA-1, SHV-11, SHV-67*	*Absent*	*fosA, aac* (3)*-IIa, aac (6')-Ib-cr, aadA16, catA2 drfA27, qnrB6, sul1, sul2, tet (D)*	147
CR-Kp-1984	Insertion site	*CTX-M-15, OXA-9, SHV-72, TEM- (IA & IB)*	*NDM-1*	*fosA6, aac* (3)*-IIa, aac (6')-Ib-cr, aadA16, catA2 qnrS1, sul1*,	17

Genetic contexts of $bla_{NDM-1,}$ $bla_{OXA-181}$ and bla_{OXA-48} in CR-Ec-124

The bla_{NDM-1} gene was identified on a plasmid (pEc124kb_2). The gene was flanked by ΔISAba125 upstream, and bleomycin resistant gene ble_{MBL}-phosphoribosylanthranilate isomerase, downstream. A BLAST alignment analysis of the genetic contexts of bla_{NDM-1}, showed it shared 100% identity and query coverage with p-dm7409_NDM-1, a plasmid identified in *K. pneumoniae* from Bangladesh (CP095646.1). $bla_{CTX-M-15,}$ also located on pEc124kb_2, was flanked by ISEcp1 upstream and downstream by ISEc21-tryptophan-IS26 and a phage integrase inserted between bla_{TEM-1A} and bla_{OXA-9}. Furthermore, IS1380 and two copies of ISEc1 flanked bla_{TEM-1A} downstream, and *ANT (3")-Ia-AAC (6")*- Tn2-*like*-IS15DII were arrayed upstream of bla_{OXA-9}. BLAST analysis of the entire backbone structure of pEc124kb_2 indicated, 99.99% homology with plasmids pCO_Ec4457-1 from New Zealand (CP049968.1) and p2189-NDM from Ghana (CP029631.1; Ayibieke et al., 2018).

The $bla_{OXA-181}$ gene was flanked by ISEcp1 upstream and ISKpn19 downstream. BLAST analysis showed pEc124kb_3, shared 100% identity and query coverage with plasmids pEc1079_3 isolated from strain ST410 *E. coli* in Ghana (CP081309.1, Mahazu et al, 2021,)

and pRIVM_C01425_2 recovered from *K. pneumoniae* from the Netherlands (CP068910.1). Comparing the backbone structure of the three international plasmids (pEc124kb_3, pEc1079_3 and pRIVM_C01425_2) with Easyfig, showed they were highly similar (Fig. 2A**)**.

Interestingly, resfinder did not identify any carbapenemases in isolate Ec01, however, further analysis with BLAST and SnapGene viewer identified the presence of bla_{OXA-48} on a plasmid pEckb1403_2. Analysis of the genetic environment of bla_{OXA-48} showed similar genetic arrangement as seen for $bla_{OXA-181}$, except for the presence of ISEc63 found in the upstream region of bla_{OXA-48}. Plasmid pEckb1403_2 also shared 100% identity and query coverage with plasmids p17a17002_B_OXA (PP320286.1) and p32A19014_A_OXA (PP320308.1; Lerminiaux et al., 2024), all recovered from *E. coli* strains from Canada **(**Fig. 2B**)**.

The genetic environments of bla_{NDM-1} in CR-Kp-1894

bla_{NDM-1} carried by pkp-1894_1, was flanked by two copies of ISkpn19 upstream and bleomycin resistant gene ble_{MBL}, downstream. Additionally, quinolone resistance gene *qnrs1*, was positioned upstream of ISkpn19. Further analysis revealed the presence of bla_{CTM-15} flanked by ISEcp1 upstream. Plasmid pkp-1894_1 shared 100% identity and 85% query coverage with plasmid p4-42Gasa2023 isolated from *K. pneumoniae* from Gaza (CP162111.1; Mellman & Schaumburg, unpublished) **(**Fig. 2C**)**.

Genetic diversity of isolates

E. coli isolates were assigned to seven different sequence types (STs); ST167 (1/6, 16.7%), ST648 (1/6, 16.7%), ST38 (1/6, 16.7%), ST44 (1/6, 16.7%), ST410 (1/6, 16.7%), ST448 (1/6, 16.7%), and ST405 (1/6, 16.7%). Core-genome SNP phylogenetic analysis among the study isolates and publicly available genomes with corresponding STs revealed that the ST44 study isolate clustered with two human-related isolates from Ethiopia and Australia with pairwise SNP differences of 26, showing close relatedness. They exhibited similar profiles with respect to the presence of beta-lactam resistance determinants including ESBL genes, fluoroquinolone resistance determinants, and IncF, IncR, and IncH plasmid replicons **(**Fig. 3**).**

K. pneumoniae isolates belonged to five different sequence types; ST17 (1/5, 20%), ST 967 (1/5, 20%), ST48 (1/5, 20%), ST147 (1/5, 20%), and ST133 (1/5, 20%). Phylogenetic analysis based on core-genome SNPs showed that the ST967 study isolate clustered with a human-associated isolate from Nigeria, differing by 48 pairwise SNPs, indicating close genetic relatedness. They shared similar antimicrobial resistance profiles, carrying aminoglycoside, beta-lactam, folate pathway antagonist, and quinolone resistance determinants, together with Col and IncF plasmid replicons **(**Fig. 4**).**

Discussion

The management of HAWIs caused by MDR pathogens, has often resulted in serious health complications and mortality (60). This is largely due to limited knowledge on the bacteriological profile of HAWI and their corresponding appropriate antibiotic therapy. Failure to identify and monitor prevalence of drug-resistance at the local level entrenches and exacerbates MDR, and ultimately undermines national AMR-control efforts. In the present study, the predominant species isolated from wound-site infections included: *E. coli* (38%), *P. aeruginosa* (18.5%), *K. pneumoniae* (14.5%) and *A. baumannii* (9.5%). These results were similar to reports from Ghana and Saudi Arabia (5, 61). The overall prevalence of MDR was 75%. This was higher than the 63.3% reported in Egypt (62) and 70.2% reported from another hospital in Ghana (63). Yet, higher prevalences of 78% and 80.5% were reported from Saudi Arabia and Northwest Ethiopia, respectively, (61, 64). *P. aeruginosa* was the predominant organism isolated from burns wounds. This observation concurred with Addison *et al* report, also from Korle-Bu Teaching Hospital (35), and findings reported from Nigeria, Palestine and Bangladesh (65–67). It has been suggested that *P. aeruginosa* ubiquitous nature in hospitals constitutes a reservoir for burns wound' colonization and infection (68).

In this study, ESBL-production among isolates was 53% and meropenem was the most effective antibiotic against ESBL-producers. Consequently, the carbapenems still remain the most reliable therapeutic option for the treatment of MDR infections. However, excessive reliance on carbapenems could inversely increase selective pressure and drive resistance. The $bla_{CTX-M-15}$ is the most prevalent ESBL-gene described in Ghana and a major correspondence to cephalosporins resistance (69–74). Notably, three *E. coli* ESBL-producing isolates belonged to sequence types; ST 405, ST 167 and ST 38. These are globally dominant high-risk clones, known for extensively drug-resistance, hypervirulence and can be responsible for severe urinary tract infections, urosepsis and bloodstream infections (75, 76).

In our study, the occurrence of carbapenem resistance was low (10.5%), nevertheless, we noted with great concern that, these carbapenem-resistant isolates, harbored multiple plasmids encoding a wide range of antibiotic resistance genes including $bla_{CTX-M-15}$, $bla_{OXA-181}$, and bla_{NDM-1}. This observation was consistent with findings previously reported in Ghana (27, 34, 64, 71) and echoes the widespread nature of these mobile genetic elements in Ghanaian hospitals. More importantly, we identified ST410 *E. coli* strain, co-harboring bla_{NDM-1} and $bla_{OXA-181}$ on two different plasmids, pEc124kb_2 and pEc124kb_3 respectively. *E. coli* ST410 strains, are highly virulent, have robust transmission and the potential to escalate carbapenem-resistance in *E. coli* and other bacterial species (24, 25, 77). In Ghana, ST410 *E. coli* coharboring bla_{NDM-1} and $bla_{CTX-M-15}$, on plasmid p2189_NDM was first identified at the Tamale teaching hospital (78) and subsequently Mahazu et al, characterized ST410 *E. coli* carrying IncX3 plasmid pEc1079_3 encoding $bla_{CTX-M-15}$, bla_{NDM-1} and $bla_{OXA-181}$ from the Effia Nkwanta Regional Hospital (34). Comparing our plasmids with these revealed, sequence identity between 99.99–100%. We can conclude that ST410 *E. coli* strains harboring transferable plasmid-encoding multiple AMR-genes including $bla_{CTX-M-15}$, bla_{NDM-1} and $bla_{OXA-181}$ are present in Ghana, and further sampling to establish their potential dissemination urgently required.

To the best of our knowledge, we report for the first time, the presence of ST17 CTX-M-15, NDM-1-producing *K. pneumoniae* in Ghana. ST17 *K. pneumoniae* is a globally widespread opportunistic pathogen known to cause MDR infections and outbreaks (79). They are also known to harbor $bla_{CTX-M-15}$ and $bla_{OXA-181}$, and thus far, the carriage of bla_{NDM-1} by this clone is uncommon (80). So far, studies from Ghana, have reported on ST17 *K. pneumoniae* harboring $bla_{OXA-181}$ and $bla_{CTX-M-15}$ being responsible for various infections and outbreaks (28, 32), and this study may be the first to describe ST17 CTX-M-15, NDM-1-producing *K. pneumoniae* in Ghana. Reports from China have described ST17 NDM-1-producing *K. pneumoniae* strains causing infection outbreaks in neonatal units (81, 82); its presence in Ghana could be as a result of international travel. The bla_{NDM-1} and $bla_{CTX-M-15}$ cohabited on plasmid pKp-1894_1. Interestingly, in analyzing the genetic environment of bla_{NDM-1}, we noticed the absence of ISAba125 known to frequently flank bla_{NDM-1} upstream, instead, two copies of ISKpn19 flanked the gene upstream. This genetic arrangement has been described in some NDM-type-producing *K. pneumoniae* strains (83) and affirms the significant role of ISKpn19 and its type in the mobilization of AMR genes on plasmids and chromosomes (84). Strikingly, pKp-1894_1 shared 100% identity and 85% query coverage with plasmid p4-42Gasa2023 isolated from *K. pneumoniae* from Gaza, even though the Gaza strain was ST147 bla_{NDM-5}-producing *K. pneumoniae* (85). In view of these findings, further dissemination of these high-risk clones carrying plasmid-encoded potent ESBLs and carbapenemases is an emergent danger that could trigger outbreaks and escalate AMR and its burden in Ghana and the sub-region.

In summary, this study contributes to the identification of modern resistance patterns of Gram-negative clinical isolates, which can be used to inform Ghana's AMR Action Plan phase 2 and treatment guidelines. Furthermore, the study also has contributed detailed genomic analysis on a subset of ESBL-producing and carbapenem-resistant *E. coli* and *K. pneumoniae* isolates in Ghana.

Conclusions

Our findings provide baseline phenotypic and genomic surveillance data for wound-associated MDR Gram-negative pathogens in Ghana. The high MDR and ESBL-producing Gram-negative organisms associated with HAWI, underscores the critical need for routine culture and sensitivity testing to guide effective treatment. Moreover, MGEs such as transferable plasmids and key insertion sequences were closely associated with ESBLs and carbapenemase encoding genes, highlighting their significant roles in the spread of AMR genes in Ghana and across the globe. To the best of our knowledge, we report for the first time, the presence of ST17 CTX-M-15, NDM-1-producing *K. pneumoniae* in Ghana. Continuous surveillance with molecular epidemiological tools, coupled with rigorous infection control practices is urgently needed to track and monitor their potential spread within Ghana and the African subregion.

Declarations

Ethics approval and consent to participate

Approval for this study was granted by the Korle-Bu Teaching Hospital-Scientific and Technical Committee/Institutional Review Board *(KBTH-STC/1RB/000102/2025)*. Access to anonymized data on patients' age, sex, ward and clinical diagnosis was granted on 24th September, 2025. The study adhered to the values of the 2013 World Medical Association Declaration of Helsinki. Consent to participate not applicable.

Availability of data and materials

Supplementary datasets are available here (link to be added). Whole-genome sequencing data generated and analysed during the current study have been deposited in the NCBI BioProject database under accession number **PRJNA1484964**. The dataset is currently under embargo and is not yet publicly available. The data will be released in NCBI and made publicly accessible upon publication of this manuscript.

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Figures

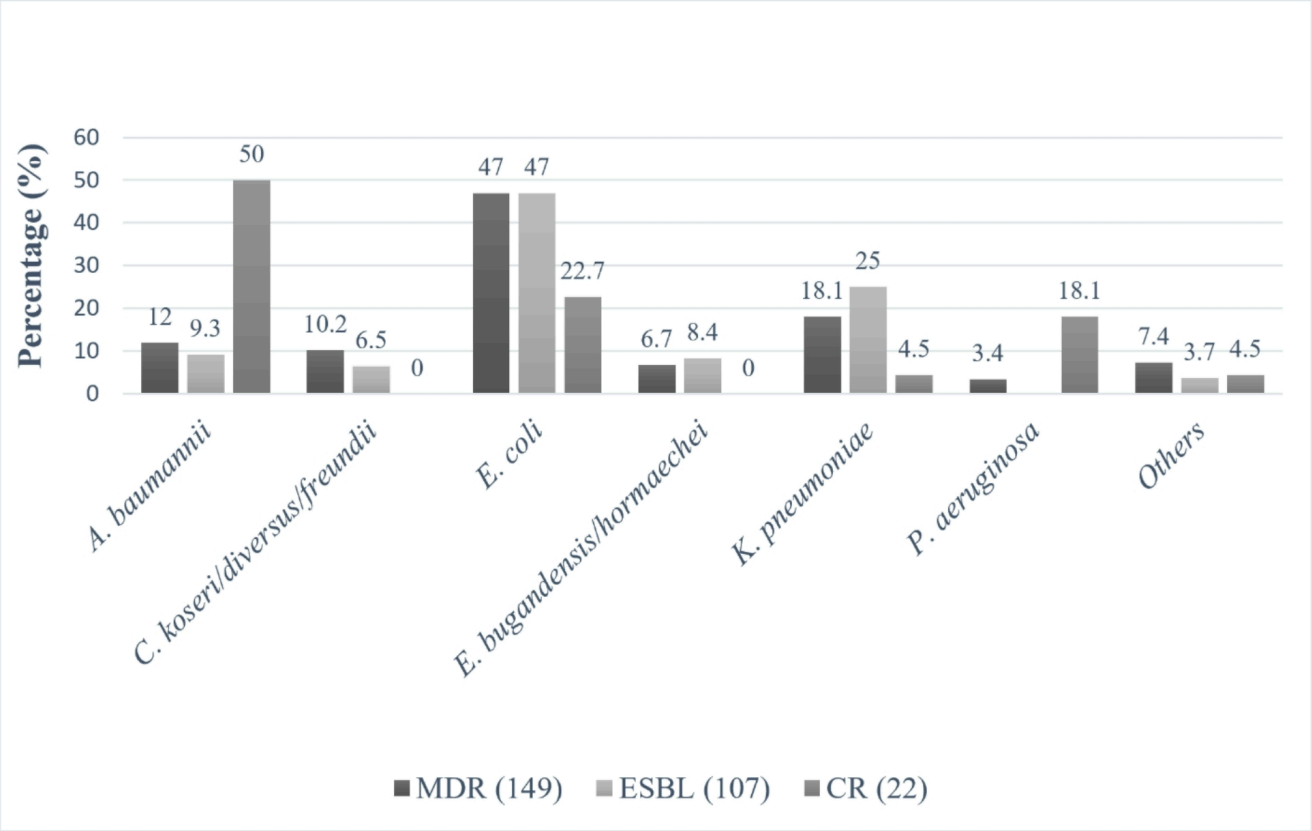


Figure 1

Proportion of Multidrug resistance (MDR), Extended Spectrum Beta-lactamase (ESBL) and Carbapenem-resistance (CR) by species.
Others: *K. oxytoca* (n=2); *M. morganii* (n=2); *P. mirabilis* (n=9); *S. paratyphi (*n=1); *S. maltophilia* (n=1).

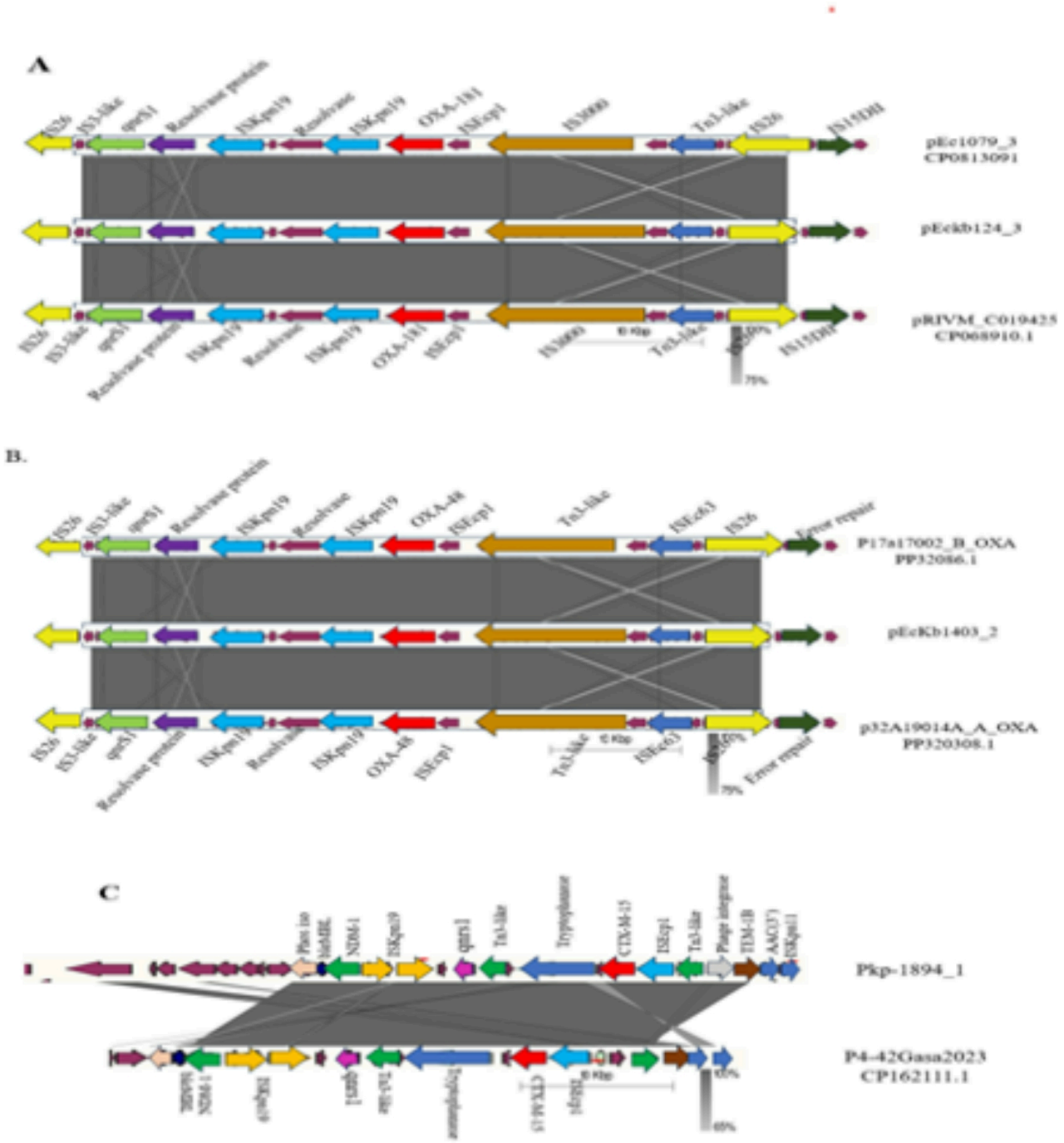


Figure 2

Linear comparison of backbone structures of plasmids and genetic environments of *bla*$_{OXA-181}$ (A), *bla*$_{OXA-48}$ (B) in *E. coli* and *bla*$_{NDM-1}$ in *K. pneumoniae* (C). Colored arrows represent genes and indicate their orientation.

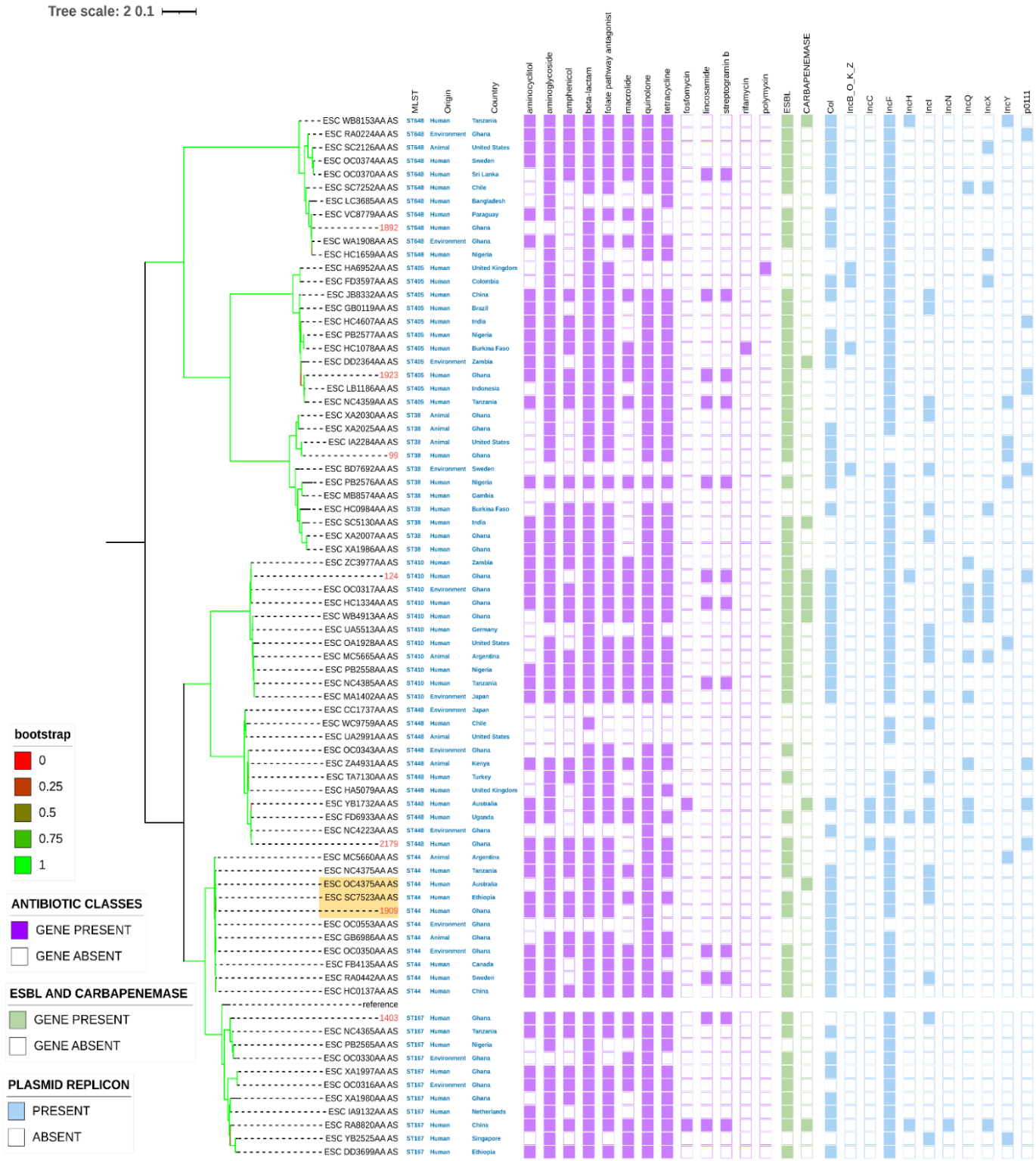


Figure 3

Maximum-likelihood phylogenetic tree of study (*Escherichia coli*) isolates (highlighted in red) and publicly available genomes belonging to the same sequence types (ST44, ST167, ST405, ST410, ST448, ST648, and ST38). Tree annotations include MLST, host origin, country of isolation, antibiotic resistance determinants grouped by antimicrobial class, ESBL and carbapenemase genes, and plasmid replicon profiles. Purple squares indicate the presence of antibiotic resistance genes, green squares represent ESBL and carbapenemase genes, and blue squares indicate plasmid replicons. Branch colours correspond to bootstrap support values. Clusters containing study and publicly available genomes with <50 core-genome SNP differences.

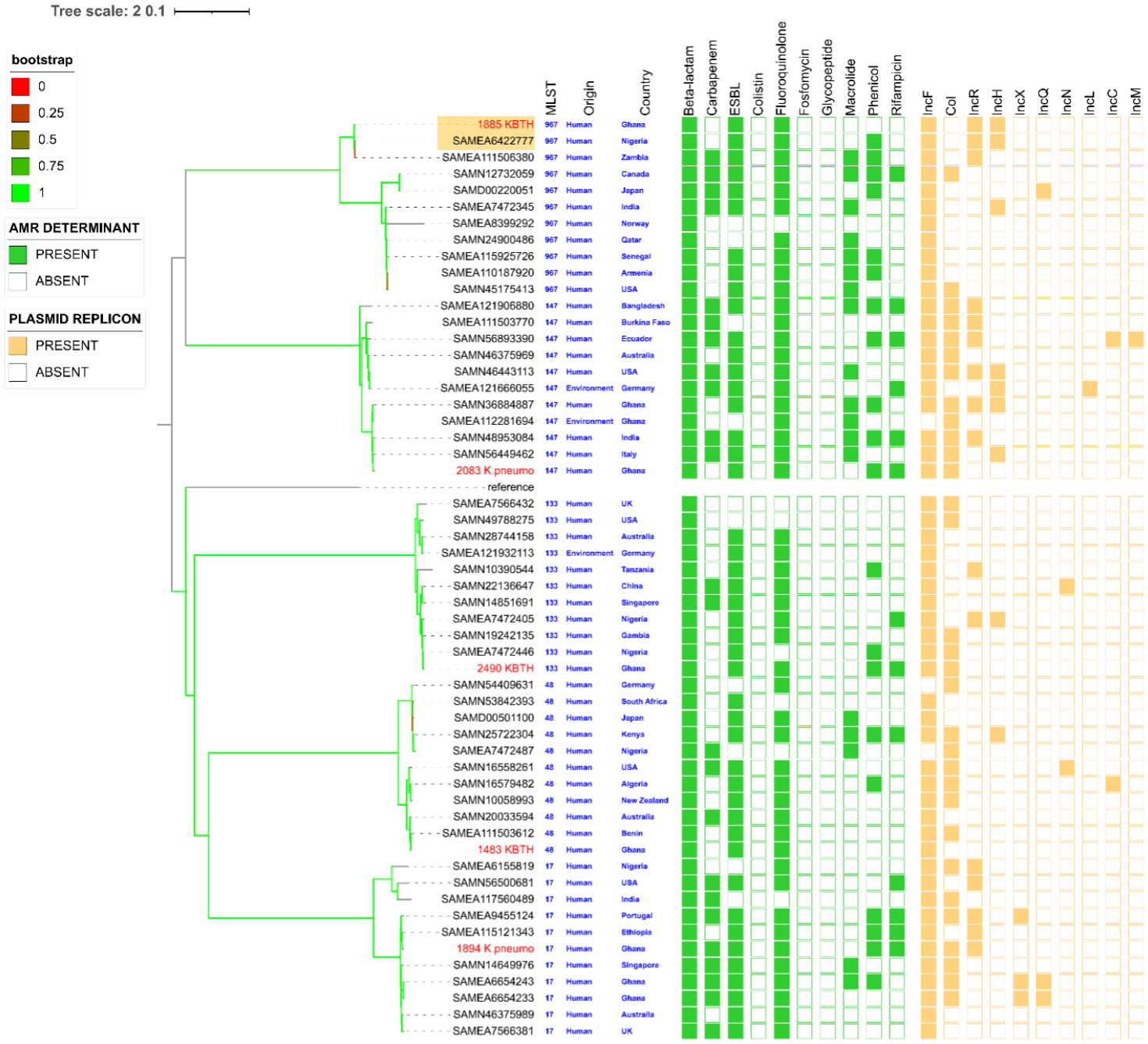


Figure 4

Maximum-likelihood phylogenetic tree showing the relationship between study (*Klebsiella pneumoniae*) isolates (highlighted in red) and publicly available genomes from the corresponding sequence types (ST17, ST48, ST133, ST147, and ST967). The tree is annotated with MLST, host origin, country of isolation, AMR determinants, and plasmid replicon profiles. Green squares indicate the presence of AMR determinants, while orange squares denote the presence of plasmid replicons. Bootstrap support values are represented by branch colours. Clusters containing study and publicly available genomes with <50 core-genome SNP differences are highlighted in yellow.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryData.docx