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Genomic diversity and antimicrobial resistance in clinical *Klebsiella pneumoniae* isolates from tertiary hospitals in Southern Ghana

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Objectives: Comprehensive data on the genomic epidemiology of hospital-associated *Klebsiella pneumoniae* in Ghana are scarce. This study investigated the genomic diversity, antimicrobial resistance patterns, and clonal relationships of 103 clinical *K. pneumoniae* isolates from five tertiary hospitals in Southern Ghana—predominantly from paediatric patients aged under 5 years (67/103; 65%), with the majority collected from urine (32/103; 31%) and blood (25/103; 24%) cultures.

Methods: We generated hybrid Nanopore–Illumina assemblies and employed Pathogenwatch for genotyping via Kaptive [capsular (K) locus and lipopolysaccharide (O) antigens] and Kleborate (antimicrobial resistance and hypervirulence) and determined clonal relationships using core-genome MLST (cgMLST).

Results: Of 44 distinct STs detected, ST133 was the most common, comprising 23% of isolates ($n=23/103$). KL116 (28/103; 27%) and O1 (66/103; 64%) were the most prevalent K-locus and O-antigen types. Single-linkage clustering highlighted the global spread of MDR clones such as ST15, ST307, ST17, ST11, ST101 and ST48, with minimal allele differences (1–5) from publicly available genomes worldwide. Conversely, 17 isolates constituted novel clonal groups and lacked close relatives among publicly available genomes, displaying unique genetic diversity within our study population. A significant proportion of isolates (88/103; 85%) carried resistance genes for ≥3 antibiotic classes, with the $bla_{\text{CTX-M-15}}$ gene present in 78% ($n=80/103$). Carbapenem resistance, predominantly due to $bla_{\text{OXA-181}}$ and $bla_{\text{NDM-1}}$ genes, was found in 10% ($n=10/103$) of the isolates.

Conclusions: Our findings reveal a complex genomic landscape of *K. pneumoniae* in Southern Ghana, underscoring the critical need for ongoing genomic surveillance to manage the substantial burden of antimicrobial resistance.

Introduction

Klebsiella pneumoniae is notoriously linked with MDR infections, particularly in healthcare settings.[1–8] Within sub-Saharan Africa, *K. pneumoniae* has emerged as the second most frequent causative agent and leading Gram-negative agent in neonatal sepsis cases.[9] In 2019, drug-resistant *K. pneumoniae* contributed to over 600 000 deaths in the region.[4] Thus, *K. pneumoniae* represents a pressing health challenge in the current era of increasing antimicrobial resistance (AMR).

In Ghana, the clinical impact of *K. pneumoniae* is well documented, with increasing resistance to critical antibiotics like third-generation cephalosporins and carbapenems being a notable concern locally,[10–21] as it is globally.[22] However, relatively little is known about the pathogen variants underlying this clinical problem.[10,12–14,16–21]

Despite the paucity of comprehensive local molecular epidemiological data, the information available paints a grim picture of the extensive challenges posed by this pathogen's persistent and complex AMR mechanisms.[15,16,21,23,24] For example, one study observed a 41% prevalence of gut colonization with ESBL-producing *K. pneumoniae* in 435 children under 5 years of age in the Agogo municipality, pinpointing a community-wide reservoir of the $bla_{\text{CTX-M-15}}$ gene.[15] Furthermore, research into poultry meat contamination in Kumasi, Ghana reported 18% of the poultry meat samples tested to carry ESBL-producing *K. pneumoniae*, with $bla_{\text{CTX-M-15}}$ as the predominant gene[25], raising alarm bells about the zoonotic transmission of resistant strains.

A One Health study from Northern Ghana highlighted a 64% prevalence of AMR genes in clinical settings, including the discovery of two carbapenemase-producing isolates (an ST17 clone with $bla_{\text{OXA-181}}$ and an ST874 carrying a $bla_{\text{OXA-48}}$), emphasizing the need for targeted intervention.[21] Moreover, the genomic complexity observed in the Komfo Anokye Teaching Hospital in Kumasi, Ghana, featuring diverse resistance genes on mobile plasmids, highlights the escalating threat of multidrug resistance in hospital-acquired infections.[15,23] Notably, plasmids carrying replicons such as IncF, IncX3 and IncL have been implicated in disseminating critical resistance genes, such as $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-48}}$, without a fitness cost, in *K. pneumoniae* and *Klebsiella quasipneumoniae* isolates from Effia Nkwanta Hospital, Ghana, underscoring their potential for widespread transmission.[24]

WGS has revolutionized pathogen surveillance by providing intricate details of pathogen characteristics, evolution and transmission pathways. However, the resolution offered by conventional short-read technologies like Illumina is often insufficient for thoroughly resolving plasmids and mobile genetic elements, pivotal for a comprehensive understanding of AMR dynamics. In contrast, long-read sequencing platforms such as the Oxford Nanopore MinION offer the capability to unravel complex genetic architectures,[26–28] with hybrid Illumina–Nanopore assemblies leveraging the strengths of both technologies.[29–31]

Here, we utilized a collection of *K. pneumoniae* isolates from major referral hospitals in Southern Ghana to examine the population structure and AMR transmission dynamics in a high-risk setting. By integrating Nanopore and Illumina data, we constructed hybrid reference assemblies to elucidate the genomic diversity and AMR profiles of clinical *K. pneumoniae* isolates from tertiary healthcare facilities in Southern Ghana.

Methods

Sample population and isolate recovery

From January 2021 through to October 2021, we prospectively collected *K. pneumoniae* isolates identified via routine diagnostics from four regions in Ghana: Greater Accra, Ashanti, Central and Western (Figure 1a). The participating facilities included: Korle-Bu Teaching Hospital (KBTH) in Accra, the largest tertiary hospital in Ghana, with a 2000-bed capacity; Greater Accra Regional (Ridge) Hospital, also in Accra, serving as a secondary level facility for the Greater Accra Region; Komfo Anokye Teaching Hospital (KATH) in Kumasi, the second largest with 1200 beds; Cape Coast Teaching Hospital (CCTH) with 400 beds; and Effia Nkwanta Regional Hospital in Sekondi-Takoradi, a significant secondary facility. Blood culture requests were made for all patients with suspected sepsis, although some patients opted to utilize neighbouring private laboratories. Other samples were derived from routine diagnostic processes. Clinical data accompanying these isolates were retrieved from the laboratory records of these hospitals. Additionally, historical isolates from KATH, dating between October 2017 and May 2018, were included in the analysis.

Isolation and identification of *K. pneumoniae* were conducted using conventional microbiological procedures (see File S1, available as Supplementary data at *JAC* Online).

Genomic DNA extraction and sequencing

Isolates were stored at −80°C in skimmed milk tryptone glucose glycerol (STGG) broth until processed for DNA extraction at the University of Cape Coast (UCC) Department of Biomedical Sciences' laboratory, Ghana, as described previously.[32] Aliquots of each DNA sample were sequenced using two approaches: (i) Oxford Nanopore MinION with R9.4.1 flow cells as described previously,[32,33] at UCC; and (ii) Illumina NextSeq 500 platform (Illumina, San Diego, CA, USA), at the Quadram Institute, UK.

Basecalling and genome assembly

For the basecalling of nanopore fast5 files, we applied the ONT Guppy basecaller v4.0.14[34] utilizing the Super accurate model as previously described.[35] Our assembly process integrated both Nanopore and Illumina data, following the hybrid assembly protocol described by Wick *et al.*[36] (further details in File S1).

Plasmid reconstruction and clustering

Using the MOB-suite program,[37] we scrutinized contigs for plasmid indicators like replication and mobilization genes, classifying plasmids into clusters or as novel if they deviated significantly from known references (genomic distances over 0.05 from the closest reference). This tool also classified plasmids by their potential for mobility (the plasmid assemblies are available at figshare: https://doi.org/10.6084/m9.figshare.24631020).

We created heatmaps with Python's Matplotlib to illustrate resistance genes within plasmid clusters, with infrequent clusters consolidated under 'Others' for clarity.

Genotyping and cgMLST clustering with Pathogenwatch

We uploaded our hybrid assemblies to the Pathogenwatch platform v21.3.0[38] for comprehensive genotyping. This included *Klebsiella* species assignments, 7-gene multilocus ST calling,[39] detection of capsular polysaccharide (K) and lipopolysaccharide (O) locus types via Kaptive v2.0.7,[40] implemented via Kleborate, and identification of acquired virulence factors and AMR determinants using Kleborate v2.3.0.[41,42] Furthermore, Pathogenwatch implements the Life Identification Number (LIN) code scheme,[43] facilitating the clustering of core genome

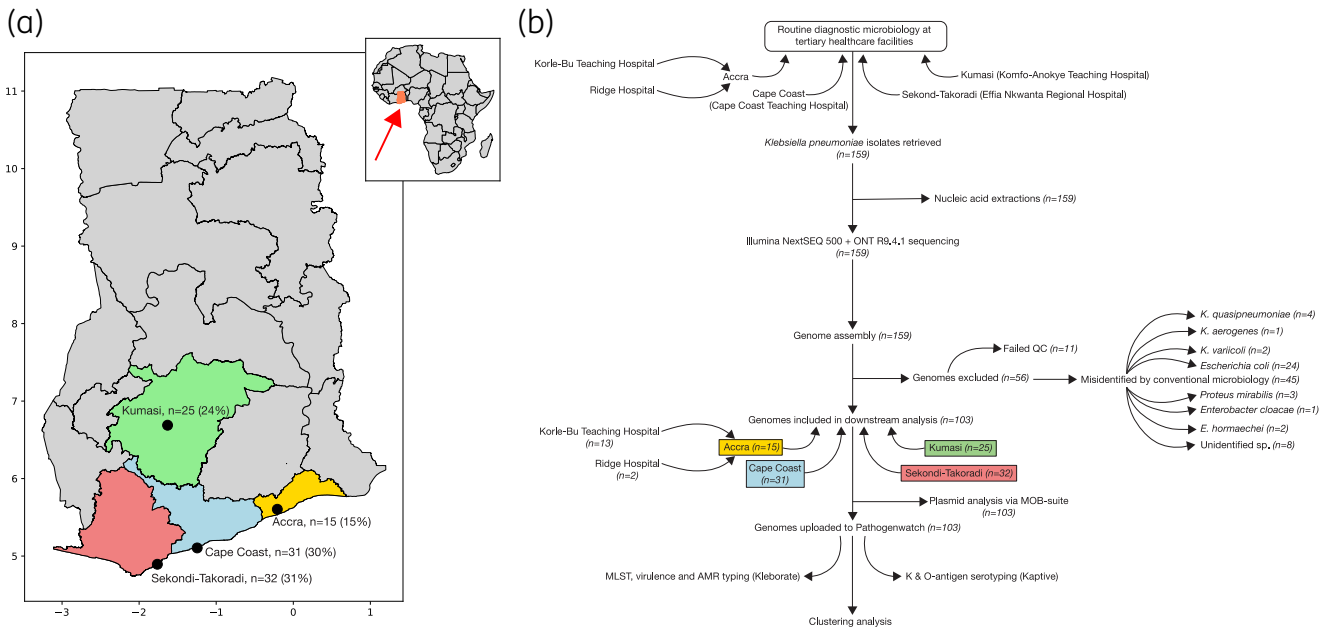

Figure 1. Geographical overview of study sites in Ghana and the study sample processing flow. (a) A map showcasing the study sampling sites in Ghana, with the different regions where these cities are located highlighted in gold (Greater Accra), light green (Ashanti), light blue (Central) and light coral (Western). The number of samples derived from each sampling site is shown as a proportion of the total. The inset depicts Africa with Ghana highlighted in coral (red arrow). (b) The study sample processing flow diagram. The flowchart illustrates the methods utilized for the isolation and genomic characterization of the study isolates, spanning sample collection, culture and isolation, genomic DNA extraction, WGS and analysis aimed at identifying key genetic features, such as AMR markers and virulence factors.

MLST profiles and providing a robust method for identifying and referencing *K. pneumoniae* complex lineages (see File S1).

Using this approach, we determined the closest neighbours to our study isolates within the context of the global *K. pneumoniae* population represented by public data available in Pathogenwatch ($n=32\,642$ genomes as of 20 December 2023).

Data analysis and visualization

We investigated the association between the source of clinical specimens (blood and urine) and the prevalence of predominant STs (ST133, ST39, ST15, ST307, ST1189, ST1207), using the chi-squared test of independence. The expected frequencies were calculated to understand the distribution of STs across specimen types, based on the assumption that the distribution of one variable is independent of the distribution of another.

Data analysis and visualization involved the use of GeoPandas, matplotlib and seaborn libraries in Python to map sample locations and display assembly metrics. Phylogenetic relationships were explored and visualized in R v4.1.0 using packages ggtree v3.0.4, ggplot2 v3.4.4 and phangorn v2.11.1.

Ethics

The institutional review boards of the respective laboratories granted ethical approval. Informed patient consent was waived as samples were obtained from routine diagnostics. Patient data associated with these isolates were anonymized, ensuring no possibility of patient identification based on age, sex or hospital-related information.

Results

Demographic characteristics of the study population

We initially collected 159 non-duplicate isolates identified biochemically as *K. pneumoniae* from the five participating health facilities. WGS showed 45 of these isolates were non-*Klebsiella pneumoniae* (see below) or failed to meet WGS quality control standards (e.g. total genome length of >7.5 Mb or <4.5 Mb); these isolates were therefore excluded from further analysis (Figure 1b). The final genome collection comprised isolates from 103 patients, including 49 females, 51 males, and three individuals of unspecified gender, from Accra ($n=15$), Cape Coast ($n=31$), Kumasi ($n=25$) and Sekondi-Takoradi ($n=32$). Predominantly paediatric, 65% of the isolates were derived from patients under 5 years of age, 26% ($n=27$) from adults aged 46–65 years, and 9% ($n=9$) from over 65-year-olds. Blood and urine were the primary sources of isolates, constituting 24% ($n=25$) and 31% ($n=32$), respectively (Table 1).

Species identification and misidentification

Conventional diagnostics misidentified numerous isolates as *K. pneumoniae*. Post-sequencing verification using Pathogenwatch's Speciator[38] revealed 103 true *K. pneumoniae*, with additional species including *K. quasipneumoniae*, *Klebsiella aerogenes*, *Klebsiella variicola*, *Escherichia coli*, *Proteus mirabilis*, *Enterobacter hormaechei* and *Enterobacter cloacae* among others. Eleven sequences failed quality control (Figure 1b). The subsequent analysis

Table 1. Characteristics of the study population

Characteristic	Female (*N* = 49)	Male (*N* = 51)	Unknown (*N* = 3)	Total (*N* = 103)
Source specimen, *n* (%)				
blood	9 (18.4)	16 (31.4)	0 (0)	25 (24.3)
HVS	6 (12.2)	0 (0)	0 (0)	6 (5.8)
pus	1 (2.0)	0 (0)	0 (0)	1 (1.0)
sputum	13 (26.5)	14 (27.5)	0 (0)	27 (26.2)
swab	3 (6.1)	1 (2.0)	0 (0)	4 (3.9)
urine	15 (30.6)	15 (29.4)	2 (66.7)	32 (31.1)
wound	2 (4.1)	3 (5.9)	0 (0)	5 (4.9)
tracheal aspirate	0 (0)	2 (3.9)	0 (0)	2 (1.9)
unknown	0 (0)	0 (0)	1 (33.3)	1 (1.0)
Source location, *n* (%)				
Accra	4 (8.2)	8 (15.7)	3 (33.3)	15 (14.6)
Cape Coast	14 (28.6)	17 (33.3)	0 (0)	31 (30.1)
Kumasi	12 (24.5)	13 (25.5)	0 (0)	25 (24.3)
Sekondi-Takoradi	19 (38.8)	13 (25.5)	0 (0)	32 (31.1)
Age group, *n* (%)				
0–6 days	1 (2.0)	3 (5.9)	0 (0)	4 (3.9)
7–27 days	2 (4.1)	2 (3.9)	0 (0)	4 (3.9)
28–364 days	1 (2.0)	3 (5.9)	0 (0)	4 (3.9)
1–4 years	1 (2.0)	4 (7.8)	0 (0)	5 (4.9)
5–9 years	1 (2.0)	0 (0)	0 (0)	1 (1.0)
10–14 years	1 (2.0)	1 (2.0)	0 (0)	2 (1.9)
15–19 years	0 (0)	4 (7.8)	0 (0)	4 (3.9)
20–24 years	2 (4.1)	1 (2.0)	0 (0)	3 (2.9)
25–59 years	36 (73.5)	25 (49.0)	2 (66.7)	63 (61.2)
60–99 years	4 (8.2)	8 (15.7)	1 (33.3)	13 (12.6)
Collection year, *n* (%)				
2017	12 (48.0)	13(52.0)	0 (0)	25 (24.3)
2021	37 (47.4)	38 (48.7)	3 (3.8)	78 (75.7)

focused on the confirmed *K. pneumoniae sensu stricto* isolates (*n* = 103).

ST and K loci diversity

The 103 *K. pneumoniae* isolates were diverse, comprising 44 unique STs (Figure 2) distributed across 25 different clonal groups (CGs). Remarkably, 18% (*n* = 18) of our study isolates were assigned novel CGs, suggesting that these groups may represent emergent or region-specific lineages.

ST133 (CG 10031) emerged as the predominant ST, accounting for 24 (23%) of the isolates and was present in three of the four geographic regions sampled. This indicates a widespread distribution of ST133 in our study population, with potential implications for its role in disease transmission and persistence in these areas. Following closely was ST39 (CG 39), representing 9 (9%) of the isolates and ST15 (CG 15) with 7 (7%), while ST307 (CG 307), ST1189 (CG 10004) and ST1207 (CG 1207) each contributed 4 isolates (4%). We did not find any significant association between the source of the specimen and the prevalence of the predominant STs ($\chi^2 = 8.8348$, $P = 0.1158$, df = 5) (File S2).

Geographically, Sekondi-Takoradi exhibited the highest diversity, featuring 21 unique STs out of 32 isolates, followed by Cape Coast with 16 STs out of 31 isolates, Accra with 13 STs, and Kumasi with just 6 STs. Notably, no ST was detected across all four study regions, although ST1207, ST133, ST15, ST39, ST469 and ST147 were found in two or more locations. Each pair of sites shared between one and five STs (mean, 2.6).

We identified a total of 27 K locus antigens, with KL116 being the most prevalent (*n* = 29; 28%, including 23/29 ST133 and 6/29 other STs), followed by KL2 (*n* = 9, 9%, including *n* = 6/9 ST39, *n* = 2/2 ST25 and *n* = 1/9 ST86-1LV), KL102 (*n* = 8; 8%, including 4/8 ST307 and 4/8 others), KL127 (*n* = 5; all ST1207) and KL25 (*n* = 3/3 ST17; 5% each), as well as KL112 and KL7 (4% each). These seven K loci collectively represented 64% of the study strains. Of seven O types identified, O1 (*n* = 66/103; 64%), O2afg (*n* = 9/103; 9%), and O2a (*n* = 8/103; 8%) were the most frequently encountered (File S3).

Genetic diversity of clinical K. pneumoniae from Southern Ghana

Using Pathogenwatch's single-linkage clustering search, we identified several (*n* = 18) isolates that were genetically distinct and lacked close relatives among publicly available genomes within the 50-allele threshold for cgMLST clustering (File S4). These

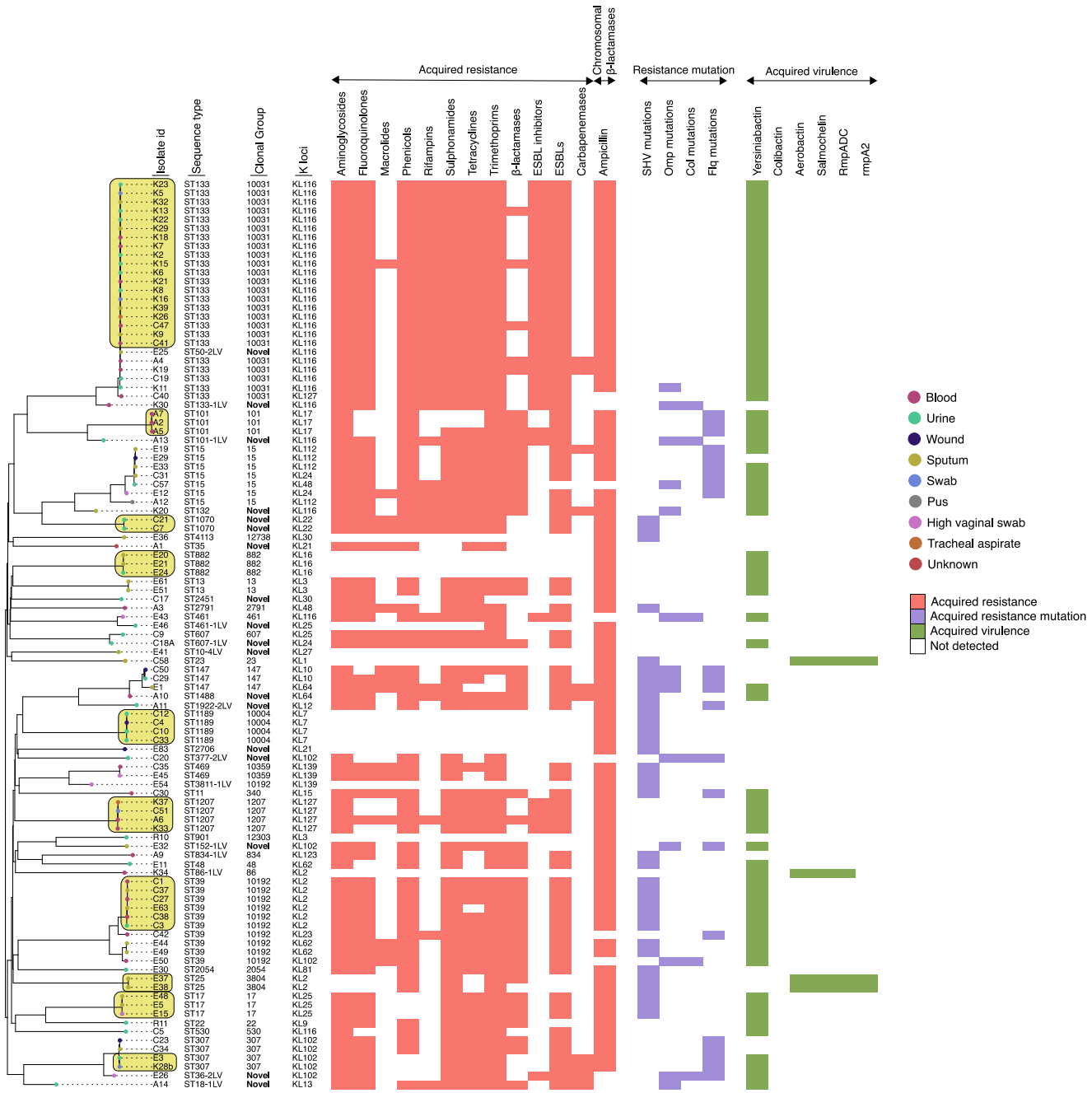

Figure 2. Phylogenetic analysis of the study isolates with AMR and virulence annotations. The figure depicts the evolutionary relationships among the study isolates, as determined by phylogenetic inference. The tree was reconstructed using the APE package via Pathogenwatch[44] based on a concatenated alignment of 1972 genes (2 172 367 bp) that constitute the core-gene library for *K. pneumoniae* in Pathogenwatch. Each tip of the tree corresponds to a unique isolate, coloured by the source of infection (indicated in the legend), with annotations indicating the presence of acquired AMR genes, resistance mutations and virulence factors. Putative transmission clusters are highlighted in light khaki. The tree was rooted using the midpoint method using the phangorn package, which places the root at the midpoint of the longest distance between any two terminal nodes, balancing the tree and aiding in the interpretation of evolutionary paths. The figure was generated using the ggtree package in R and annotated using Adobe Illustrator.

included isolates belonging to ST1488, ST35, ST901, ST2451, ST1070 and 11 other STs.

The dominant ST in our study, ST133 (uniformly $bla_{\text{CTX-M-15}}$ positive), shared its closest genetic relation with a $bla_{\text{CTX-M-15}}$-positive ESBL-producing neonatal sepsis isolate from Nigeria, as reported in the BARNARDs study.[45] This relative differed by 20 alleles within the cgMLST framework, suggesting spread within the West African region. By contrast, ST39, the second-most prevalent ST, was genetically close to isolates from Senegal and the UK, differing by merely four alleles, highlighting this clone's regional and global

dissemination. Both isolates from Senegal and the UK carried the $bla_{CTX\text{-}M\text{-}15}$ gene.

Isolates of globally disseminated MDR clones found in our study, including ST15, ST307, ST17, ST11, ST101 and ST48, all had close relatives (≤5 allele differences) amongst public genomes from other countries and continents (File S4), consistent with widespread global dissemination.

SNP differences in isolates within and across sampling sites

We downloaded the Pathogenwatch pairwise distance matrix (File S5), which facilitated a focused investigation into the SNPs present within *K. pneumoniae* genomes across the sampling sites. Our analysis revealed instances of pairwise SNP differences of less than 10 SNPs, suggestive of potential nosocomial transmission events,[35,46–48] within and between the sampling locations as follows.

Among the isolates sampled in Accra, two genomes, designated A2 and A7, displayed a pairwise SNP difference of merely 8 SNPs, indicating a very close genetic relationship typical of recent divergence. Among the isolates from Cape Coast, a more diverse set of genomes (C10, C12, C1, C33, C4, C27, C37 and C38) showed SNP differences ranging from 4 to 9 SNPs. Similarly, in Kumasi, several genomes (K8, K9, K7, K6, K5, K39, K32, K29, K23, K21, K18, K22, K16 and K13) exhibited SNP differences between 2 and 9 SNPs, pointing to a clonal expansion likely facilitated by nosocomial vectors. A parallel trend was discerned in the following isolates from Sekondi-Takoradi, where genomes E5, E48, E15, E20, E21, E24, E37 and E38 displayed SNP differences ranging from 3 to 9 SNPs. The genetic proximity observed in these genomes exceeds the expected diversity from community-acquired strains and aligns more closely with the genomic homogeneity expected of potential nosocomial transmission clusters[35,46–48] (Figure 2, highlighted).

Analysis of SNP differences between isolates from the different sites also highlighted several instances of close genetic relatedness (below the threshold of 10 SNPs), indicating potential shared or parallel sources of infection, or the movement of strains between these locations. For example, isolates E63 (from Sekondi-Takoradi) and C1 (sourced from Cape Coast) showed SNP differences as low as 3 SNPs, with similar closeness observed in pairs E63–C27 and E63–C38. This degree of closeness warrants further investigation into the epidemiological connections between these isolates.

Similarly, multiple isolates from Kumasi (K13, K16, K18, K21, K22, K23, K29, K32, K39, K5, K6, K7, K8 and K9) exhibited SNP differences ranging from 2 to 7, when compared with isolate C41 from Cape Coast, suggesting a cluster of closely related strains circulating within or between these sites. Additionally, isolate K37 differed from C51 by only 9 SNPs (Figure 2, highlighted).

Distribution of resistance genes by antibiotic class

We identified 94 distinct AMR genes spanning 11 antibiotic classes (File S3). Most isolates (89/103; 86%) harboured acquired AMR genes, predominantly against aminoglycosides, trimethoprim, sulfamethoxazole (86/103; 83% each), and chloramphenicol (81/103; 79%). The ESBL gene $bla_{CTXM\text{-}15}$ was present in 78% ($n=80/103$) of isolates (Figure 2, File S3). Carbapenemases were identified in 10% ($n=10/103$) of isolates, comprising the $bla_{OXA\text{-}181}$, $bla_{NDM\text{-}1}$ and $bla_{OXA\text{-}69}$ genes (7/10; 70%, 2/10; 20% and 1/10; 10%, respectively). These were derived from blood (3/10; 30%), sputum (3/10; 30%), urine (2/10; 20%), high vaginal swab (HVS) (1/10; 10%) and another swab specimen (1/10; 10%). In all instances, the carbapenemase genes co-occurred with $bla_{CTX\text{-}M\text{-}15}$. Four out of the 10 carbapenemase-positive isolates also exhibited porin mutations (including 3 $bla_{OXA\text{-}181}$-carrying isolates and 1 NDM-positive isolate) and belonged to various STs, including such lineages known for MDR as ST15, ST307 and ST147,[41,44,49,50] as well as lesser-known STs like ST133, ST1488, ST18, ST36 and ST132. No convergence of acquired virulence traits associated with increased risk of invasiveness and ESBL and/or carbapenemase production was observed in our study population.

Acquired β-lactamases such as OXA-1, CMY-2, LAP-2, TEM-1D and SCO-1 were present in 48% ($n=49$), while rifamycin (*arr*-3) and macrolide resistance genes [*mph*(A), *erm*(B) and *lsa*(A)] were found in 40% ($n=41$) and 17% ($n=18$) of the isolates, respectively. Tetracycline and fluoroquinolone resistance were prominent, with 76% ($n=78$) and 74% ($n=76$) of isolates harbouring resistance genes, respectively.

The bulk of the resistance genes were plasmid-borne. Of the observed resistance rates above, the following proportions were chromosomally encoded: aminoglycosides [*aac(3)-IIa*, *aac(6′)-Ib-cr*, *aadA16*, *aadA2*, *ant(3′)-Ia*, *ant(6)-Ia*, *aph(3′)-Ib*, *aph(3′)-III* and *aph(6)-Id*], 30% ($n=26/86$); sulphonamides (*sul1*, *sul2*), 11% ($n=9/83$); tetracyclines [*tet*(A), *tet*(C), *tet*(D), *tet*(G) and *tet*(M)], 13% ($n=10/78$); fluoroquinolones (*qnr*S1), 3% ($n=2/76$); macrolides [*erm*(B)], 6% ($n=1/18$); rifampicin (*arr*-3), 2% ($n=1/41$); chloramphenicol (*catA1*, *catA2* and *catB3* and *floR*), 14% ($n=11/81$), trimethoprim (*dfrA12*, *dfrA14*, *dfrA1*, *dfrA8* and *dfrA7*), 7% ($n=6/86$); ESBL ($bla_{CTX\text{-}M\text{-}15}$), 10% ($n=8/80$), and carbapenemase ($bla_{OXA\text{-}181}$ and $bla_{OXA\text{-}69}$), 30% ($n=3/10$) and 10% ($n=1/10$), respectively. Intrinsic chromosomal β-lactamases (SHVs) occurred in 91% ($n=94/103$) of the study isolates (File S6).

Ten isolates demonstrated mutations in porin genes OmpK35 ($n=8$) and OmpK36 ($n=2$) in the absence of acquired carbapenemases. Fluoroquinolone resistance-associated mutations in the *gyrA* and *parC* genes were detected in 21% ($n=22$) of isolates. No determinants for colistin or tigecycline resistance were detected.

Correlation between phenotypic resistance rates and genotypic predictions

Among the isolates subjected to phenotypic disc diffusion antimicrobial susceptibility testing ($n=101/103$; 98%), the observed resistance rates were as follows: gentamicin, 55% ($n=55/101$); amikacin, 4% ($n=4/101$); ampicillin, 100% ($n=101/101$); ceftriaxone, 83% ($n=84/101$); chloramphenicol, 72% ($n=73/101$); trimethoprim/sulfamethoxazole, 87% ($n=88/101$); tetracycline, 77% ($n=78/101$); ciprofloxacin, 78% ($n=79/101$); and meropenem, 10% ($n=10/101$) (Figure S1 and File S7). There was substantial concordance between the genotypic predictions and the observed phenotypic resistance, with agreement rates ranging from 65% to 100% for the various antimicrobials tested (File S7).

Plasmid diversity and gene cargo

A striking 97% ($n=100/103$; 97%) of isolates harboured plasmids—with an average of four each—comprising 393 unique plasmids with 42 replicon markers, half of which were of six common types (File S8).

MOB-suite analysis showed 91% ($n=358/393$) of plasmids could be mobilized, categorizing them into 118 clusters, including 16 new ones. The distribution of these clusters varied considerably; for instance, two clusters were widespread across 36 genomes each, while 59 were unique to single genomes (File S9).

Plasmid sizes varied widely, highlighting the genetic diversity within clusters (File S9 and Figure S2). Certain clusters, notably 'plasmid_AA274' [encompassing Inc types Col440I_1/IncFIB(K)_1_Kpn3/IncFII_1_pKP91 ($n=1/36$), IncFIA(HI1)_1_HI1/IncFIB(K)_1_Kpn3/IncFII_1_pKP91/IncR_1 ($n=1/36$), IncFIB(K)_1_Kpn3/IncFII_1_pKP91 ($n=28/36$), IncFIB(K)_1_Kpn3/IncFII_1_pKP91/IncR_1 ($n=4/36$), IncFII_1_pKP91 ($n=1/36$) and a single unknown Inc type], 'plasmid_AA277' [encompassing the IncFIB(K)_1_Kpn3 ($n=3/13$) and IncFIB(K)_1_Kpn3/IncFII_1_pKP91 ($n=10/13$) incompatibility types], 'plasmid_AA553' [comprising the IncFIA(HI1)_1_HI1 ($n=1/20$), IncFIA(HI1)_1_HI1/IncR_1 ($n=16/20$), IncR_1 ($n=1/20$) and $n=2/20$ plasmids of unknown replicons], and 'plasmid_AA556' [consisting of the following Inc types or combinations: IncFIA(HI1)_1_HI1 ($n=2/13$), IncFIA(HI1)_1_HI1/IncFII_1_pKP91/IncR_1 ($n=1/13$), IncFIA(HI1)_1_HI1/IncR_1 ($n=3/13$), IncFIB(pQil)_1_pQil/IncR_1 ($n=1/13$), IncR_1 ($n=1/13$) and 5/13 plasmids of unknown replicons] harboured numerous AMR genes, potentially acting as AMR hotspots (Figure 3). Clusters like 'plasmid_AA406' [comprising the IncFIB(K)_1_Kpn3/IncHI1B_1_pNDM-MAR incompatibility types ($n=2/2$)], 'plasmid_AE314' [belonging to the rep9a_1_repA (pAD1) incompatibility type] and 'plasmid_AE437' of an unknown replicon type were marked by virulence genes, suggesting a role in pathogenicity.

Acquired virulence traits

We found few acquired virulence traits, with the yersiniabactin siderophore being the most common, detected in 70% ($n=72/103$) of isolates, mainly from the ybt16-ICEKp12 lineage. Other lineages like ybt10-ICEKp4, ybt15-ICEKp11 and ybt14-ICEKp5 were less common, and 6% ($n=6/10$) of isolates had potentially novel ybt lineages. Notably, four isolates carried hypervirulence genes from well-known hypervirulent clones (1 ST86, isolated from a blood specimen in Kumasi, 1 ST23 and 2 ST25 derived from sputum from Effia-Nkwanta Regional Hospital and Cape Coast Teaching Hospital, respectively) (Figure 2).

Discussion

The population structure and genomic diversity of *K. pneumoniae* in Ghana, as in many sub-Saharan countries, remain poorly characterized, yet they are fundamental to the effective management and containment of infections. Our investigation sheds light on these critical aspects by analysing *K. pneumoniae* isolates from tertiary hospitals in Southern Ghana, providing new insights into their epidemiology, genetic diversity and AMR. This enhanced understanding is a crucial step toward developing targeted interventions to combat the spread of this formidable pathogen.

In line with previous investigations, our study adds to the growing body of evidence indicating that conventional microbiology struggles to distinguish between members of the *K. pneumoniae* species complex.[46,51–55] A recent study in southwestern Nigeria reported a 25% misidentification rate of *Klebsiella* as other Enterobacterales, including *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.[56] Our results echo these findings, underscoring the pressing need to strengthen and enhance conventional microbiological diagnostics to accurately identify pathogens and inform treatment strategies.

We have uncovered a troublingly complex picture of AMR in Southern Ghana, with most *K. pneumoniae* isolates harbouring multiple resistance genes. This is consistent with global trends of increasing multidrug resistance in hospital-acquired infections.[1,6,51,57]

The distribution of resistance genes—chromosomal versus plasmid-based—highlights the different evolutionary pressures and mechanisms at play, suggesting intrinsic resistance as well as the potential for horizontal gene transfer.[58,59]

Our data add to the mounting evidence of the $bla_{\text{CTX-M-15}}$ ESBL genotype's prevalence in healthcare and community settings in our setting.[15,16,21,23–25,60–63] The detection of carbapenem resistance genes, such as $bla_{\text{OXA-181}}$ and $bla_{\text{NDM-1}}$, is particularly concerning and echoes findings from other regional studies.[12,21,24] The presence of these genes indicates the challenging reality of treating infections with limited antimicrobial options.

The diversity of the plasmids carrying these resistance genes reflects *K. pneumoniae*'s genomic plasticity and potential to act as a reservoir for AMR. IncFIB and IncX3 plasmids, in particular, have been implicated in the spread of resistance, underscoring the role of mobile genetic elements in resistance gene dissemination.[6,15,23,24]

Our analysis also highlights the complexity of resistance mechanisms, with porin mutations known to confer an increase in MICs of carbapenems and cephalosporins[64,65] co-occurring with carbapenemase and or ESBL genes in certain isolates. Our findings warrant further investigation to ascertain the clinical implications and the precise role of porin mutations in the development of carbapenem resistance within our context. Although virulence traits were less diverse, the prevalence of yersiniabactin suggests it may play a significant role in the pathogenicity of *K. pneumoniae* in this region.[56]

We observed a significant diversity of sequence types and K loci among *K. pneumoniae* isolates, which could inform alternative control measures like vaccines, monoclonal antibodies and phage therapies.[66–69] The predominance of specific K and O antigens may reflect their role in pathogen survival and virulence, highlighting potential targets for preventive strategies.[6,66–69]

Identifying MDR clones such as ST15, ST307 and ST17, alongside unique genetic profiles among our isolates, points to a dynamic and evolving landscape of *K. pneumoniae* in Ghana. The disparity in ST distribution across various study sites reinforces the genetic diversity of these pathogens, with some STs being widespread while others are unique to specific locales. Previous studies corroborate our findings, with certain STs such as ST17 being recurrent in clinical settings and others being more geographically dispersed.[18,19,23,24] This genetic variability across regions emphasizes the need for localized infection control

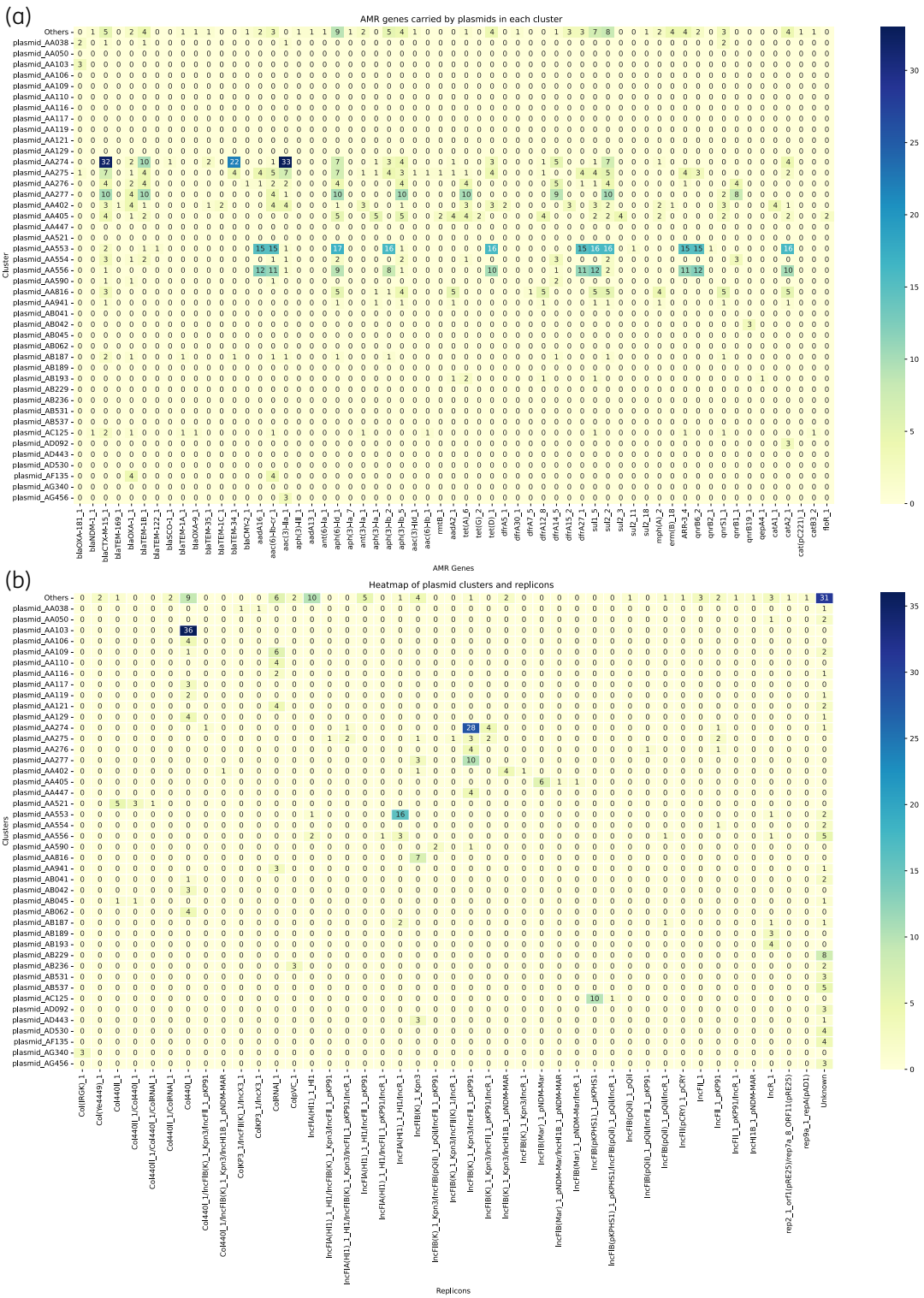

Figure 3. Heatmap of AMR genes carried by plasmids in each cluster and replicon types contained in each cluster. (a) Each cell reflects the count of specific AMR genes found within a given cluster. The colour intensity correlates with the number of genes present, with darker shades representing higher gene counts. The scale ranges from light yellow (fewer genes) to dark blue (more genes). The numerical values in each cell denote the total count of AMR genes detected for that specific cluster. The rows are labelled with the names of plasmid clusters, while the columns correspond to specific AMR genes or groups of genes. The specific genes represented by the columns are provided in File S8. (b) The different replicon types contained in the plasmid clusters. Similar to (a), the colour intensity reflects the count of each replicon type within the clusters.

strategies, which take into account the regional differences in ST prevalence and the potential for localized spread of specific clones.

In a hospital setting, a genome-wide SNP difference of 21–25, corresponding to 10 Pathogenwatch SNPs, is indicative of nosocomial transmission.[35,46–48] The observation of SNP differences less than 10 in certain pairs of genomes suggests missed opportunities in identifying and preventing hospital-acquired infections. This underscores the need for enhanced surveillance and infection control measures within hospitals and reinforces the importance of genomic surveillance in identifying potential hospital-acquired infections,[56] which is paramount for informing public health interventions and antibiotic stewardship strategies.

Limitations

The phenotypic susceptibility assessment was limited to disc diffusion methods, primarily due to logistical constraints. The inclusion of MICs could have offered a more nuanced view of how the predicted porin mutations might influence the MICs of carbapenems and cephalosporins. Furthermore, storage complications led to the loss of some isolates, which might have resulted in an underrepresentation of the genetic diversity within the studied *K. pneumoniae* population. It is also important to note that our study encompassed only those *K. pneumoniae* isolates accessible from the participating laboratories. As some patients may have opted for private laboratory services, our findings potentially do not fully capture the breadth of *K. pneumoniae* diversity present in our study region.

Conclusions

Our research indicates a highly diverse and antimicrobial-resistant *K. pneumoniae* population in Southern Ghana. The dominance of $bla_{\text{CTX-M-15}}$ is particularly alarming, necessitating robust local and global surveillance and action. This study underscores the critical need for ongoing genomic surveillance and reinforces the importance of antimicrobial stewardship and infection prevention strategies adapted to local epidemiology.

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Transparency declarations

The authors declare that they have no conflicts of interest.

Data availability

WGS data for this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA1052100 (https://shorturl.at/esWZ2). The individual accession numbers for the sequencing reads are available in File S3. The interactive tree and Kleborate output are available to explore at https://microreact.org/project/gfkmA5Q2gUHQ2w1QRvjTRY-genomic-diversity-and-antimicrobial-resistance-in-clinical-klebsiella-pneumoniae-isolates-from-tertiary-hospitals-in-southern-ghana.

Supplementary data

Files S1 to S7 and Figures S1 and S2 are available as Supplementary data at *JAC* Online.

References

1 Wyres KL, Nguyen TNT, Lam MMC *et al*. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. *Genome Med* 2020; **12**: 11. https://doi.org/10.1186/s13073-019-0706-y

2 Holt KE, Wertheim H, Zadoks RN *et al*. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc Natl Acad Sci U S A* 2015; **112**: E3574–81. https://doi.org/10.1073/pnas.1501049112

3 Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev* 2017; **41**: 252–75. https://doi.org/10.1093/femsre/fux013

4 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022; **399**: 629–55. https://doi.org/10.1016/S0140-6736(21)02724-0

5 Wyres KL, Holt KE. *Klebsiella pneumoniae* as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Curr Opin Microbiol* 2018; **45**: 131–9. https://doi.org/10.1016/j.mib.2018.04.004

6 Wyres KL, Holt KE. *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. *Trends Microbiol* 2016; **24**: 944–56. https://doi.org/10.1016/j.tim.2016.09.007

7 Vading M, Nauclér P, Kalin M *et al*. Invasive infection caused by *Klebsiella pneumoniae* is a disease affecting patients with high comorbidity and associated with high long-term mortality. *PLoS One* 2018; **13**: e0195258. https://doi.org/10.1371/journal.pone.0195258

8 Anderson DJ, Moehring RW, Sloane R *et al*. Bloodstream infections in community hospitals in the 21st century: a multicenter cohort study. *PLoS One* 2014; **9**: e91713. https://doi.org/10.1371/journal.pone.0091713

9 Okomo U, Akpalu ENK, Le Doare K *et al*. Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. *Lancet Infect Dis* 2019; **19**: 1219–34. https://doi.org/10.1016/S1473-3099(19)30414-1

10 Osei MM, Dayie NTKD, Azaglo GSK *et al*. Alarming levels of multidrug resistance in aerobic gram-negative bacilli isolated from the nasopharynx of healthy under-five children in Accra, Ghana. *Int J Environ Res Public Health* 2022; **19**: 10927. https://doi.org/10.3390/ijerph191710927

11 Opintan JA, Newman MJ, Arhin RE *et al*. Laboratory-based nationwide surveillance of antimicrobial resistance in Ghana. *Infect Drug Resist* 2015; **8**: 379–89. https://doi.org/10.2147/IDR.S88725

12 Owusu FA, Obeng-Nkrumah N, Gyinae E *et al*. Occurrence of carbapenemases, extended-spectrum beta-lactamases and AmpCs among beta-lactamase-producing Gram-negative bacteria from clinical sources

in Accra, Ghana. *Antibiotics (Basel)* 2023; **12**: 1016. https://doi.org/10.3390/antibiotics12061016

13 Labi AK, Enweronu-Laryea CC, Nartey ET *et al*. Bloodstream infections at two neonatal intensive care units in Ghana: multidrug resistant Enterobacterales undermine the usefulness of standard antibiotic regimes. *Pediatr Infect Dis J* 2021; **40**: 1115–21. https://doi.org/10.1097/INF.0000000000003284

14 Dayie NT, Bannah V, Dwomoh FP *et al*. Distribution and antimicrobial resistance profiles of bacterial aetiologies of childhood otitis media in Accra, Ghana. *Microbiol Insights* 2022; **15**: 11786361221104446. https://doi.org/10.1177/11786361221104446

15 Agyekum A, Fajardo-Lubián A, Ansong D *et al*. $bla_{\text{CTX-M-15}}$ carried by IncF-type plasmids is the dominant ESBL gene in *Escherichia coli* and *Klebsiella pneumoniae* at a hospital in Ghana. *Diagn Microbiol Infect Dis* 2016; **84**: 328–33. https://doi.org/10.1016/j.diagmicrobio.2015.12.010

16 Akenten CW, Khan NA, Mbwana J *et al*. Carriage of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* among children in rural Ghana: a cross-sectional study. *Antimicrob Resist Infect Control* 2023; **12**: 60. https://doi.org/10.1186/s13756-023-01263-7

17 Dwomoh FP, Kotey FCN, Dayie NTKD *et al*. Phenotypic and genotypic detection of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Accra, Ghana. *PLoS One* 2022; **17**: e0279715. https://doi.org/10.1371/journal.pone.0279715

18 Pankok F, Taudien S, Dekker D *et al*. Epidemiology of plasmids in *Escherichia coli* and *Klebsiella pneumoniae* with acquired extended spectrum beta-lactamase genes isolated from chronic wounds in Ghana. *Antibiotics (Basel)* 2022; **11**: 689. https://doi.org/10.3390/antibiotics11050689

19 Labi AK, Nielsen KL, Marvig RL *et al*. Oxacillinase-181 carbapenemase-producing *Klebsiella pneumoniae* in neonatal intensive care unit, Ghana, 2017–2019. *Emerg Infect Dis* 2020; **26**: 2235–8. https://doi.org/10.3201/eid2609.200562

20 Egyir B, Nkrumah-Obeng N, Nyarko EO *et al*. 898. Prevalence of extended spectrum beta-lactamase producing *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from hospital acquired surgical site infections in Ghana. *Open Forum Infect Dis* 2020; **7**: S483. https://doi.org/10.1093/ofid/ofaa439.1086

21 Calland JK, Haukka K, Kpordze SW *et al*. Population structure and antimicrobial resistance among *Klebsiella* isolates sampled from human, animal, and environmental sources in Ghana: a cross-sectional genomic one health study. *Lancet Microbe* 2023; **4**: e943–52. https://doi.org/10.1016/S2666-5247(23)00208-2

22 WHO. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including Tuberculosis. 2017. https://apps.who.int/iris/handle/10665/311820

23 Agyepong N, Govinden U, Owusu-Ofori A *et al*. Genomic characterization of multidrug-resistant ESBL-producing *Klebsiella pneumoniae* isolated from a Ghanaian teaching hospital. *Int J Infect Dis* 2019; **85**: 117–23. https://doi.org/10.1016/j.ijid.2019.05.025

24 Mahazu S, Prah I, Ota Y *et al*. *Klebsiella* species and *Enterobacter cloacae* isolates harboring $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-48}}$: resistome, fitness cost, and plasmid stability. *Microbiol Spectr* 2022; **10**: e0332022. https://doi.org/10.1128/spectrum.03320-22

25 Eibach D, Dekker D, Gyau Boahen K *et al*. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana. *Vet Microbiol* 2018; **217**: 7–12. https://doi.org/10.1016/j.vetmic.2018.02.023

26 Ashton PM, Nair S, Dallman T *et al*. MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. *Nat Biotechnol* 2015; **33**: 296–300. https://doi.org/10.1038/nbt.3103

27 Loman NJ, Pallen MJ. Twenty years of bacterial genome sequencing. *Nat Rev Microbiol* 2015; **13**: 787–94. https://doi.org/10.1038/nrmicro3565

28 Arredondo-Alonso S, Willems RJ, van Schaik W *et al*. On the (im)possibility of reconstructing plasmids from whole-genome short-read sequencing data. *Microb Genom* 2017; **3**: e000128. https://doi.org/10.1099/mgen.0.000128

29 Land M, Hauser L, Jun SR *et al*. Insights from 20 years of bacterial genome sequencing. *Funct Integr Genomics* 2015; **15**: 141–61. https://doi.org/10.1007/s10142-015-0433-4

30 Wick RR, Judd LM, Gorrie CL *et al*. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb Genom* 2017; **3**: e000132. https://doi.org/10.1099/mgen.0.000132

31 De Maio N, Shaw LP, Hubbard A *et al*. Comparison of long-read sequencing technologies in the hybrid assembly of complex bacterial genomes. *Microb Genom* 2019; **5**: e000294. https://doi.org/10.1099/mgen.0.000294

32 Foster-Nyarko E, Nabil-Fareed A, Anuradha R *et al*. Genomic diversity of *Escherichia coli* isolates from non-human primates in the Gambia. *Microb Genom* 2023; **9**: mgen000428. https://doi.org/10.1099/mgen.0.000428

33 Foster-Nyarko E, Alikhan NF, Ikumapayi UN *et al*. Genomic diversity of *Escherichia coli* from healthy children in rural Gambia. *PeerJ* 2021; **9**: e10572. https://doi.org/10.7717/peerj.10572

34 Oxford Nanopore Technologies. Guppy 4.0.14. https://github.com/nanoporetech/pyguppyclient

35 Foster-Nyarko E, Cottingham H, Wick RR *et al*. Nanopore-only assemblies for genomic surveillance of the global priority drug-resistant pathogen, *Klebsiella pneumoniae*. *Microb Genom* 2023; **9**: mgen000936. https://doi.org/10.1099/mgen.0.000936

36 Wick RR, Judd LM, Holt KE. Assembling the perfect bacterial genome using Oxford nanopore and Illumina sequencing. *PLoS Comput Biol* 2023; **19**: e1010905. https://doi.org/10.1371/journal.pcbi.1010905

37 Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microb Genom* 2018; **4**: e000206. https://doi.org/10.1099/mgen.0.000206

38 Argimón S, David S, Underwood A *et al*. Rapid genomic characterization and global surveillance of *Klebsiella* using Pathogenwatch. *Clin Infect Dis* 2021; **73**: S325–35. https://doi.org/10.1093/cid/ciab784

39 Diancourt L, Passet V, Verhoef J *et al*. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; **43**: 4178–82. https://doi.org/10.1128/JCM.43.8.4178-4182.2005

40 Lam MMC, Wick RR, Judd LM *et al*. Kaptive 2.0: updated capsule and lipopolysaccharide locus typing for the *Klebsiella pneumoniae* species complex. *Microb Genom* 2022; **8**: 000800. https://doi.org/10.1099/mgen.0.000800

41 Lam MMC, Wick RR, Watts SC *et al*. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. *Nat Commun* 2021; **12**: 4188. https://doi.org/10.1038/s41467-021-24448-3

42 Wyres KL, Wick RR, Gorrie C *et al*. Identification of *Klebsiella* capsule synthesis loci from whole genome data. *Microb Genom* 2016; **2**: e000102. https://doi.org/10.1099/mgen.0.000102

43 Hennart M, Guglielmini J, Bridel S *et al*. A dual barcoding approach to bacterial strain nomenclature: genomic taxonomy of *Klebsiella pneumoniae* strains. *Mol Biol Evol* 2022; **39**: msac135. https://doi.org/10.1093/molbev/msac135

44 Naha S, Sands K, Mukherjee S *et al*. OXA-181-like carbapenemases in *Klebsiella pneumoniae* ST14, ST15, ST23, ST48, and ST231 from septicemic neonates: coexistence with NDM-5, resistome, transmissibility, and genome diversity. *mSphere* 2021; **6**: e01156-20. https://doi.org/10.1128/mSphere.01156-20

45 Sands K, Carvalho MJ, Portal E *et al*. Characterization of antimicrobial-resistant Gram-negative bacteria that cause neonatal sepsis in seven low- and middle-income countries. *Nat Microbiol* 2021; **6**: 512–23. https://doi.org/10.1038/s41564-021-00870-7

46 Gorrie CL, Mirceta M, Wick RR *et al*. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. *Clin Infect Dis* 2017; **65**: 208–15. https://doi.org/10.1093/cid/cix270

47 Sherry NL, Lane CR, Kwong JC *et al*. Genomics for molecular epidemiology and detecting transmission of carbapenemase-producing Enterobacterales in Victoria, Australia, 2012 to 2016. *J Clin Microbiol* 2019; **57**: e00573-19. https://doi.org/10.1128/JCM.00573-19

48 David S, Reuter S, Harris SR *et al*. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat Microbiol* 2019; **4**: 1919–29. https://doi.org/10.1038/s41564-019-0492-8

49 Peirano G, Chen L, Kreiswirth BN *et al*. Emerging antimicrobial-resistant high-risk *Klebsiella pneumoniae* clones ST307 and ST147. *Antimicrob Agents Chemother* 2020; **64**: e01148-20. https://doi.org/10.1128/AAC.01148-20

50 Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. *Nat Rev Microbiol* 2020; **18**: 344–59. https://doi.org/10.1038/s41579-019-0315-1

51 Gorrie CL, Mirčeta M, Wick RR *et al*. Genomic dissection of *Klebsiella pneumoniae* infections in hospital patients reveals insights into an opportunistic pathogen. *Nat Commun* 2022; **13**: 3017. https://doi.org/10.1038/s41467-022-30717-6

52 Gorrie CL, Mirceta M, Wick RR *et al*. Antimicrobial-resistant *Klebsiella pneumoniae* carriage and infection in specialized geriatric care wards linked to acquisition in the referring hospital. *Clin Infect Dis* 2018; **67**: 161–70. https://doi.org/10.1093/cid/ciy027

53 Long SW, Linson SE, Ojeda Saavedra M *et al*. Whole-genome sequencing of human clinical isolate of the novel species *Klebsiella quasivariicola* sp. nov. *mSphere* 2017; **2**: e01057-17. https://doi.org/10.1128/genomeA.01057-17

54 Fontana L, Bonura E, Lyski Z *et al*. The brief case: *Klebsiella variicola*—identifying the misidentified. *J Clin Microbiol* 2019; **57**: e00826-18. https://doi.org/10.1128/JCM.00826-18

55 Fontana L, Bonura E, Lyski Z *et al*. Closing the brief case: *Klebsiella variicola*-identifying the misidentified. *J Clin Microbiol* 2019; **57**: e00825-18. https://doi.org/10.1128/JCM.00825-18

56 Afolayan AO, Oaikhena AO, Aboderin AO *et al*. Clones and clusters of antimicrobial-resistant *Klebsiella* from southwestern Nigeria. *Clin Infect Dis* 2021; **73**: S308–15. https://doi.org/10.1093/cid/ciab769

57 Chang HH, Cohen T, Grad YH *et al*. Origin and proliferation of multiple-drug resistance in bacterial pathogens. *Microbiol Mol Biol Rev* 2015; **79**: 101–16. https://doi.org/10.1128/MMBR.00039-14

58 Ito R, Mustapha MM, Tomich AD *et al*. Widespread fosfomycin resistance in Gram-negative bacteria attributable to the chromosomal *fosA* gene. *mBio* 2017; **8**: e00749-17. https://doi.org/10.1128/mBio.00749-17

59 Li J, Zhang H, Ning J *et al*. The nature and epidemiology of OqxAB, a multidrug efflux pump. *Antimicrob Resist Infect Control* 2019; **8**: 44. https://doi.org/10.1186/s13756-019-0489-3

60 Ahmed ZB, Ayad A, Mesli E *et al*. CTX-M-15 extended-spectrum β-lactamases in Enterobacteriaceae in the intensive care unit of Tlemcen hospital, Algeria. *East Mediterr Health J* 2012; **18**: 382–6. https://doi.org/10.26719/2012.18.4.382

61 Breurec S, Guessennd N, Timinouni M *et al*. *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clin Microbiol Infect* 2013; **19**: 349–55. https://doi.org/10.1111/j.1469-0691.2012.03805.x

62 Rodrigues C, Machado E, Ramos H *et al*. Expansion of ESBL-producing *Klebsiella pneumoniae* in hospitalized patients: a successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, IncFIIK). *Int J Med Microbiol* 2014; **304**: 1100–8. https://doi.org/10.1016/j.ijmm.2014.08.003

63 Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed Res Int* 2016; **2016**: 2475067. https://doi.org/10.1155/2016/2475067

64 Fajardo-Lubián A, Ben Zakour NL, Agyekum A *et al*. Host adaptation and convergent evolution increases antibiotic resistance without loss of virulence in a major human pathogen. *PLoS Pathog* 2019; **15**: e1007218. https://doi.org/10.1371/journal.ppat.1007218

65 Wong JLC, Romano M, Kerry LE *et al*. OmpK36-mediated carbapenem resistance attenuates ST258 *Klebsiella pneumoniae in vivo*. *Nat Commun* 2019; **10**: 3957. https://doi.org/10.1038/s41467-019-11756-y

66 de Sousa JAM, Buffet A, Haudiquet M *et al*. Modular prophage interactions driven by capsule serotype select for capsule loss under phage predation. *ISME J* 2020; **14**: 2980–96. https://doi.org/10.1038/s41396-020-0726-z

67 Feldman MF, Bridwell M, Scott AE *et al*. A promising bioconjugate vaccine against hypervirulent *Klebsiella pneumoniae*. *Proc Natl Acad Sci U S A* 2019; **116**: 18655–63. https://doi.org/10.1073/pnas.1907833116

68 Ravinder M, Liao KS, Cheng YY *et al*. A synthetic carbohydrate–protein conjugate vaccine candidate against *Klebsiella pneumoniae* serotype K2. *J Org Chem* 2020; **85**: 15964–97. https://doi.org/10.1021/acs.joc.0c01404

69 Campbell WN, Hendrix E, Cryz S *et al*. Immunogenicity of a 24-valent *Klebsiella* capsular polysaccharide vaccine and an eight-valent *Pseudomonas* O-polysaccharide conjugate vaccine administered to victims of acute trauma. *Clin Infect Dis* 1996; **23**: 179–81. https://doi.org/10.1093/clinids/23.1.179