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Genomic characterization of multidrug-resistant ESBL-producing *Klebsiella pneumoniae* isolated from a Ghanaian teaching hospital

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ABSTRACT

Objectives: This study delineated the clonal lineages, antibiotic resistome and plasmid replicon types in multidrug-resistant *K. pneumoniae* isolates from a teaching hospital in Ghana.
Methods: Identification and antibiotic susceptibility testing were done using the MALDI-TOF MS and Vitek-2 automated system. Genomic DNA extraction was carried out using the NucliSens easyMAG® (BioMérieux) kits and the DNA was subjected to whole genome sequencing (WGS) using the Illumina MiSeq platform.
Results: Of the 200 isolates obtained, 37 were identified as *K. pneumoniae* of which 9 were resistant to all second and third-generation cephalosporins. These 9 isolates selected for further genomic analysis were characterized by the presence of 8 diverse sequence types (STs), capsular polysaccharide serotypes (*K* types and *wzi* allelic types) and multiple genes encoding resistance to β-lactams ($bla_{CTX\text{-}M\text{-}15}$, $bla_{SHV\text{-}11}$, $bla_{TEM\text{-}1B}$, $bla_{OXA\text{-}1}$), aminoglycosides (*aac(3)-IIa, strB, strA, aadA16*), fluoroquinolones/quinolones (*qnrB66, oqxA, oqxB*) and other antibiotic classes. Resistance genes were associated with plasmids, predominantly IncFIB(K) and ColRNAI. Multiple and diverse mutations in quinolone resistance-determining regions of *gyrA* (S83Y, D87A) and *parC* (S80I, N304S) in isolates resistant to ciprofloxacin (MIC $\geq$ 4 mg/mL) were found. Global phylogenomic analysis affirmed the diverse clonal clustering and origin of these isolates.
Conclusions: The varied clonal clusters and resistome identified in the multidrug-resistant *K. pneumoniae* isolates is a major threat to the management of infections in Ghana. The molecular characterization of antibiotic resistance is thus imperative to inform strategies for containment.

Introduction

The mechanisms of β-lactam antibiotic resistance employed by Gram-negative bacteria including *K. pneumoniae* involve the expression of β-lactamases with/without other non-enzymatic resistance mechanisms such as efflux and/or, outer protein membrane or porin reduction rendering the agents ineffective (Alekshun and Levy, 2007; Lau et al., 2014; Wilson, 2014). The resistance may be intrinsically expressed or acquired. Saravanan and colleagues 2018 reported high rates of resistance to β-lactam antibiotics, particularly second and third generation cephalosporins among Enterobacteriaceae, ranging from 12% to 82.8% in hospital settings in a systematic review on prevalence and drug resistance pattern of extended spectrum β–lactamases (ESBLs) producing Enterobacteriaceae in Africa (Saravanan et al., 2018). ESBL encoding plasmids are commonly associated with genes mediating resistance to other antibiotic classes including fluoroquinolones and aminoglycosides, facilitating the dissemination of multidrug-resistant bacteria in hospital settings. The swift acquisition of plasmid-borne extended-spectrum β-lactamases (ESBLs), especially those belonging to the TEM, SHV and CTX-M β-lactamase families produced by Enterobacteriaceae including

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K. pneumoniae with high preference for oxyimino-cephalosporin hydrolysis is increasing globally (Bonnet, 2004; Gibold et al., 2014; Zhao and Hu, 2013). Plasmids of the IncF group represent one of the most common plasmid types contributing to the spread of antibiotic resistance genes in Enterobacteriaceae with CTX-M-15-positive IncFIIK plasmids commonly characterized in *K. pneumoniae* (Dolejska et al., 2012; Johnson and Nolan, 2009). The spread of these resistant bacteria has compromised the use of β-lactams, considered as safest and most easily available antibiotics for treatment of infections in many parts of the world, including Ghana.

Studies conducted in Ghana have reported *K. pneumoniae* as a major pathogen responsible for UTI (Gyasi-Sarpong et al., 2014). A laboratory-based nationwide surveillance of antimicrobial resistance in Ghana by Opintan and co-workers reported that *K. pneumoniae* represented 1.06% of all bacterial infections and 1.4% of Gram-negative bacilli (Opintan et al., 2015). Agyepong et al. (2018) indicated an increased *K. pneumoniae* resistance of 19% (37/200) of Gram-negative bacteria in their study on MDR bacterial infections in a teaching hospital in Ghana. In spite of the threat posed by multidrug resistant Gram-negative bacteria in health care settings in Ghana, there is paucity of molecular epidemiology studies. This study, which forms part of a broader study on the molecular profile of Gram-negative ESKAPE pathogens in a Ghanaian teaching hospital, delineates the clonal lineages, antibiotic resistome and plasmid replicons of a sub-set of *K. pneumoniae* with resistance to the second and third-generation cephalosporins using whole genome sequencing (WGS).

Materials and methods

Ethical approval and voluntary informed consent

Ethical clearance was granted by the Joint Committee of Human Research Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University of Technology, Research and Development Unit of the Hospital Administration (ref: CHRPE/AP/015/15) and the Biomedical Research Ethics Committee of University of Kwa-Zulu Natal (ref: BE 494/14). Voluntary, informed consent was obtained from all participants and from parents or guardians for minors in written form either signed or by a thumb print after explaining the procedure and purpose of the study, using an interpreter as appropriate.

Study setting

The study was conducted between February and August 2015 in Komfo Anokye Teaching Hospital (KATH) in Kumasi, in the Ashanti region of Ghana. The facility is a 1000-bed tertiary care government hospital. The average daily primary care and specialist outpatient attendance was 169 and 954 patients respectively during the period of study. The population of the region is concentrated in a few districts, with the Kumasi metropolis accounting for nearly one-third of the region's population of 4,780,380 (Owusu and Oteng-Ababio, 2015). KATH is the only regional and referral hospital that takes care of about 80% of both emergencies and regular medical cases in the region and serves as referral hospital for parts of Brong Ahafo, Western, Eastern and the Northern regions of Ghana.

Bacterial selection and identification

The *K. pneumoniae* isolates included in this study was a subset from a larger collection of 200 clinical, non-duplicate Gram-negative bacterial samples (Agyepong et al., 2018). Of the 200 isolates, 37 were identified as *K. pneumoniae*, nine were resistant to all second and third-generation cephalosporins and thereby selected for genomic characterization by whole genome sequencing (WGS). Seven of these isolates were obtained from urine and one each from gastric lavage and tracheal aspirate. Eight of the nine isolates were obtained from in-patients and one from an out-patient. Date of collection, diagnosis, sex, age and ward type were obtained from patient records (Information on all 37 isolates appear in the Table S1).

Bacterial identification and antibiotic susceptibilities were determined by the Vitek-2 (BioMérieux, France) automated system. Identity and MICs were further confirmed by using MALDI-TOF MS (Bruker Daltonic Gmbh, Bremen, Germany) broth micro-dilution in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines respectively (Testing). *K. pneumoniae* ATCC700603 was used as the control strain.

DNA extraction and genome sequencing

DNA extraction was carried out using the NucliSens easyMAG® (BioMérieux) kits according to the manufacturers' instructions. The genomic DNA libraries were generated using the Nextera® kit (Illumina) followed by sequencing on an Illumina MiSeq platform at the Genomics Resource Center at the University of Tromso, the Arctic University of Norway. Raw sequence reads were adaptor and quality-trimmed using Trimmomatic (Bolger et al., 2014). Assembly using SPAdes 3.9.1 (Bankevich et al., 2012), quality scores were assessed by QUAST version 4.6.0 software (Bankevich et al., 2012). The assembled reads were annotated using the Bacterial Analysis Pipeline (BAP) of software revision 4.2 and National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) searches (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The antibiotic resistance genes and plasmids were identified by mapping the sequence data to an online database using ResFinder (Zankari et al., 2012) and PlasmidFinder (Carattoli et al., 2014) respectively. Comparative genomic analysis was further performed using *K. pneumoniae* ATCC 13883 (PRJNA244567) as reference strain to elucidate the chromosomal mutation resulting in quinolone resistance. Multi-locus Sequence Typing (MLST) was also determined from the assembled genomes (https://github.com/tseemann/mlst) which also predicted the allelic profiles of the 7 housekeeping genes, *gapE, infB, mdh, pgi, phoE, rpoB,* and *tnoB* of *K. pneumoniae*. The reference Klebsiella WGS data online platform tool, Kaptive-web (http://kaptive.holtlab.net/) was used to infer the serotypes (*K types* and *wzi* allelic types) of the isolates.

Phylogenetic analyses

A genome-wide gene-by-gene comparison approach was used to assess the genetic relatedness between isolates within and across wards. The core genes were determined from the annotated genome assemblies, predicted coding regions were extracted and converted into protein sequences. A phylogeny was drawn for *K. pneumoniae* using Rapid large-scale prokaryote pangenome analysis (Roary; https://sanger-pathogens.github.io/Roary/) to estimate the tree for the core genome. The genome of *K. quasipneumoniae* strain P27-02 (accession number: NXHG00000000.1) served as the outgroup to root the tree to enable easy configuration of the phylogenetic distance between the strains on the branches. Altogether, 3,492 core genes were extracted with an alignment length of 3,484,711 bp shared by the nine *K. pneumoniae* genomes. The allelic distance from the cgMLST was visualized using Figtree v1.4.3 (https://tree.bio.ed.ac.uk/sofware/figtree/) in a maximum likelihood phylogenetic tree using optimized parameters as follows: nucleotide substitution model,

Jukes-Cantor; transition/transversion ratio, 2; estimate substitution rate, yes; number of substitution rate, 4; perform bootstrap analysis, yes; replicates, 1,000. A metadata (including isolate name, ward, ST, K type and *wzi* allelic type) were imported to provide a comprehensive analysis of the generated phylogenetic tree.

To investigate the global phylogeny of the *K. pneumoniae* isolates, genome assembly datasets including metadata were downloaded from the Pathosystems Resource Integration Center (PATRIC) database (https://www.patricbrc.org/). Genomes with less than 400 contigs and with MLST and isolation country available were selected and run through parsnp (software designed for intraspecific or core genome alignment for high quality assemblies) v.1.2 (Treangen et al., 2014) with "–c" -flags enabled to include all the selected genomes in the phylogenetic tree, and random reference selection among the included samples. FigTree (https://tree.bio.ed.ac.uk/software/figtree/) and R-ape package (v5.1) were used to visualize and edit the phylogenetic trees.

Accession numbers

The raw read sequences and the assembled whole genome contigs have been deposited in GenBank. The data is available under project number PRJNA411997.

Results

The clinical data indicated that *K. pneumoniae* was frequently implicated in UTI. Antibiotic susceptibility profiles showed that all the isolates were resistant to cefuroxime, cefotaxime and ceftazidime but sensitive to imipenem, ertapenem, meropenem, amikacin and colistin. Seven of the nine isolates were additionally resistant to gentamicin, nitrofurantoin and trimethoprim-sulfamethoxazole and six were sensitive to ciprofloxacin (Table 1). WGS analysis revealed that all the isolates were predominantly characterized by the presence of multiple resistome encoding for resistance within and between antibiotic classes. The isolates carried 3-5 β-lactamase genes, 3-6 aminoglycoside resistance genes, 2-5 fluoroquinolone resistance genes in different permutations and combinations (Table 2). *bla*$_{\text{CTX-M-15}}$ *and bla*$_{\text{TEM-1B}}$), (*aac(3)-IIa-like, aph(3')-Ia* and *aac(6')Ib-cr)* and (*oqxA-like, oqxB-like, qnrB10-like* and *qnrB2*) were the most common β-lactam, aminoglycoside and fluoroquinolone resistance genes observed respectively. The isolates also carried resistance genes for other antibiotic classes including *sul2, fosA, dfrA14 and catB7-like* encoding resistance for sulphonamide, fosfomycin, trimethoprim and phenicol respectively. MLST analysis showed a high variation among the strains identifying 8 different sequence types including; ST2171, ST2816, ST17, ST152, ST397, ST1788, ST798 and ST101 (Table 2 and Figure 1) evident by the different allelic profiles of the 7 housekeeping genes between the isolates (Table 3). This indicates the circulation of multiple *K. pneumoniae* sequence types in a single hospital. The high diversity was confirmed by the epidemiological typing scheme via the Kaptive database which also predicted 8 different capsular polysaccharide serotypes (KL15-*wzi*50, KL30-*wzi*122, KL155-*wzi*173, KL149-*wzi*110, KL158-*wzi*475, KL2-*wzi*2, KL18-*wzi*18 and KL17-*wzi*137) (Table 2 and Figure 1) for the isolates. IncFIB(K) and ColRNAI were the most prevalent plasmid replicon types among *K. pneumoniae* isolates (Table 2).

cgMLST analysis using *K. quasipneumoniae* strain P27-02 as the outgroup collaborated the high genetic variation in the strains. The tree was divided into many subclades showing the differentiation of the isolates (Figure 1). Phylogenetics coupled with metadata analysis provided a deeper insight into the diversity of the clones between the wards in the hospital (Figure 1). Specifically, three different clonal types (ST101-KL17-*wzi*137, ST17-KL155-*wzi*173 and ST397-KL158-*wzi*475) were found in the child health ward whiles two other clones (ST152-KL149-*wzi*110 and ST2171-KL15-*wzi*50) were identified in the obstetrics and gynaecology ward. Phylogenomic analyses including the genomes from this study (n = 9) and from a global strain collection (n = 1158, including 20 South African isolates) were performed to ascertain clustering and the likely origins the isolates from our study showed a dispersed or wider distribution on the global tree (Figure 1). As visualized, the two ST101-isolates from Ghana (P26-75 and P26-81) are closely related to each other and belong to the same distinct phylogenetic cluster as the South African lineage of ST101. Moreover, isolates P26-71 (ST397) and P26-66 (ST152) are closely related to the South African ST14 and South African ST323 isolates, respectively. As shown, most branches constitute isolates from diverse geographic origin. The branch **A** isolates, P27-01 and P26-63 are closely related to each other and to ST17-isolates from the USA. In branch **B**, P26-71 clusters with isolates (mainly ST14) from eight countries, including

Table 1
Antibiotic susceptibility profiles of the MDR *K. pneumoniae* (n = 9).

Isolate code	Demographics				Susceptibility profile - MICs (mg/L)															
	Date	SPM	Diagnosis	WT	AMC	TZP	CXM	FOX	CTX	CAZ	CFP	ETP	IMP	MEM	AMK	GEN	CIP	TET	NIT	COL	SXT
P27-01 [20]	06/06/2015	Urine	UTI	O&G	≥32	≥64	≥64	4	≥64	16	8	≤0.5	≤0.25	≤0.25	≤2	≥16	≤0.2	1	64	≤0.5	≥320
P26-62 [70]	13/06/2015	Urine	UTI	Surg.	≥32	16	≥64	16	≥64	16	16	≤0.5	≤0.25	≤0.25	≤2	≥16	≤0.25	2	64	≤0.5	≥320
P26-63 [76]	02/06/2015	Urine	UTI	CH	≥32	32	≥64	≤4	32	16	2	≤0.5	≤0.25	≤0.25	≤2	≥16	≤0.25	1	128	≤0.5	≥320
P26-66 [117]	11/03/2015	Urine	UTI	O&G	16	32	≥64	≤4	≥64	16	2	≤0.5	≤0.25	≤0.25	≤2	≥16	≥4	4	≥128	≤0.5	≥320
P26-71 [155]	14/09/2015	Gastric lavage	Gastritis	CH	16	≥64	≥64	≥64	16	16	2	≤0.5	≤0.25	≤0.25	≤2	≥16	≤0.25	1	≤16	≤0.5	≥320
P26-75 [183]	01/07/2015	Urine	UTI	CH	16	≥64	≥64	16	≥64	16	2	≤0.5	≤0.25	≤0.25	≤2	≥16	≥4	4	>256	≤0.5	≥320
P26-78 [201]	19/03/2015	Aspirate	Sinusitis	ICU	≥32	32	≥64	≤4	≥64	16	2	≤.5	≤.25	≤.25	≤2	≥16	≤.25	≤1	≤16	≤0.5	≥320
P26-79 [202]	11/03/2015	Urine	UTI	OPD	≥32	128	≥64	≤4	≥64	16	2	≤0.5	≤0.25	≤0.25	≤2	≥16	≤0.25	≤0.5	32	≤0.5	≥320
P26-81 [206]	14/03/2015	Urine	UTI	Med	16	32	≥64	16	≥6	16	2	≤0.5	≤0.25	≤0.2	≤2	≥16	≥4	≤0.5	≥512	≤0.5	≥320
Resistant MICs breakpoint (EUCAST, 2017)					>8	>16	>8	NA	>2	>4	>4	>1	>8	>8	>16	>4	>0.5	>2	>64	>2	>4

AMC- Amoxicillin-Clavulanate, TZP- Piperacillin Tazobactam, CXM-Cefuroxime, FOX-Cefoxitin CTX- Cefotaxime, CAZ- Ceftazidime, CFP- Cefepime, ETP- Ertapenem, IMP-Imipenem, MRP-Meropenem, AMK- Amikacin, GEN-Gentamicin, CIP-Ciprofloxacin, TET- Tetracycline, NIT- Nitrofurantoin, COL-Colistin, SXT- Trimethoprim-sulfamethoxazole, Fluoro- Fluoroquinolones, Tet- Tetracycline, CH- Child Health, O&G-Obstetrics and Gynaecology, Med-Medicine, Surg- Surgery, WT-Ward type, SPM-Specimen.

Table 2
Genomic characterizations of multidrug-resistant *K. pneumoniae* isolates from WGS Analysis.

Isolate code	WGS *in-silico* typing			Plasmid replicons	Antibiotic classes/resistance genes				Chromosomal mutation		
	MLST	K type	*Wzi type*		β-lactamases	Aminoglycosides	Fluoroquinolones/ Quinolones	Other resistance	*gyrA*	*gyrB*	*parC*
P27-01 [20]	ST2171	KL15	wzi50	IncFIA(HI1),IncFIB(K),IncFII (K),IncR	*blaTEM-1B, blaCTX-M-15, blaSHV-11*	*aac(3)-IIa, strB, strA*	*oqxA, oqxB*	*fosA, sul2, tet(D), dfrA14*	-[1]	-	-
P26-62 [70]	ST2816	KL30	wzi22	IncFIA(HI1),IncFIB(K),IncFII (K),IncR,ColRNAI	*blaTEM-1B, blaCTX-M-15,*	*strA, aac(3)-IIa, strB,*	*oqxA, oqxB*	*fosA, catA2, sul2, tet(D), dfrA14*	-	-	-
P26-63 [76]	ST17	KL155	wzi173	IncFIA(HI1),IncFIB(K),IncFII (K),Col(MGD2),IncR, ColRNAI	*blaCTX-M-15, blaTEM-1B*	*strA, aac(3)-IIa, strB,*	*oqxA, oqxB,*	*fosA, catA2, sul2, tet(D), dfrA14,*	-	-	-
P26-66 [117]	ST152	KL149	wzi110	IncFIB(K),IncFII(K),ColRNAI	*blaCTX-M-15, blaOXA-1,*	*aac(6')Ib-cr, aac (3)-IIa, aac(6')Ib-cr,*	*oqxB, oqxA, qnrB66*	*fosA, catB4, sul2, tet(A), dfrA14*	S83F D87A	NM[2]	S80I
P26-71 [155]	ST397	KL158	wzi475	IncFIB(pKPHS1),IncFIB(K), IncFII(K),ColRNAI	*blaTEM-1B, blaCTX-M-15*	*aac(6')Ib-cr, aac (3)-IIa, strB, strA,*	*oqxB, oqxA*	*fosA, catB4, sul2, dfrA14*	-	-	-
P26-75 [183]	ST101	KL17	wzi137	IncFIA(HI1), IncFII,IncFIB(K), ColRNAI	*blaCTX-M-15, blaOXA-1*	*aac(6')Ib-cr, aac (3)-IIa, strA, strB*	*qnrB66, oqxA, oqxB,*	*fosA, catB4, sul1, sul2, dfrA14, dfrA5*	S83Y D87A	NM	S80I, N304S
P26-78 [201]	ST1788	KL2	wzi2	IncFIB(pKPHS1),IncFIB(K), IncFII(K),ColRNAI	*blaCTX-M-15, like, blaTEM-1B*	*aac(3)-IIa,strA, strB*	*oqxA, oqxB*	*FosA sul2 dfrA14*	-	-	-
P26-79 [202]	ST789	KL18	wzi18	IncFIA(HI1),IncFIB(K),IncFII (K), Col(MGD2),IncR, ColRNAI	*blaCTX-M-15, blaTEM-1B*	*aac(3)-IIa, aadA16-strA,strB*	*oqxA, oqxB*	*fosA sul1,sul2 tet (D) dfrA14 catA2*	-	-	-
P26-81 [206]	ST101	KL17	wzi137	IncFIA(HI1),IncFII,IncFIB(K), ColRNAI	*blaCTX-M-15, blaOXA-1, blaTEM-1B*	*aac(3)-IIa-strA, strB*	oqxA-like,oqxB-like,qnrB66 aac(6') Ib-cr	*fosA sul1,sul2 dfrA14 dfrA5*	S83Y D87A	NM	S80I, N304S

Unless otherwise stated in the footnote, *K. pneumoniae* ATCC 13883 (PRJNA244567) was used as reference strain in the comparative genomic analysis. MLST-multi locus sequence typing, *K typing-Klebsiella* surface polysaccharide capsule characterization and wzi type- *wzi* allelic typing scheme.
[1] Susceptible to ciprofloxacin.
[2] NM-No mutation.

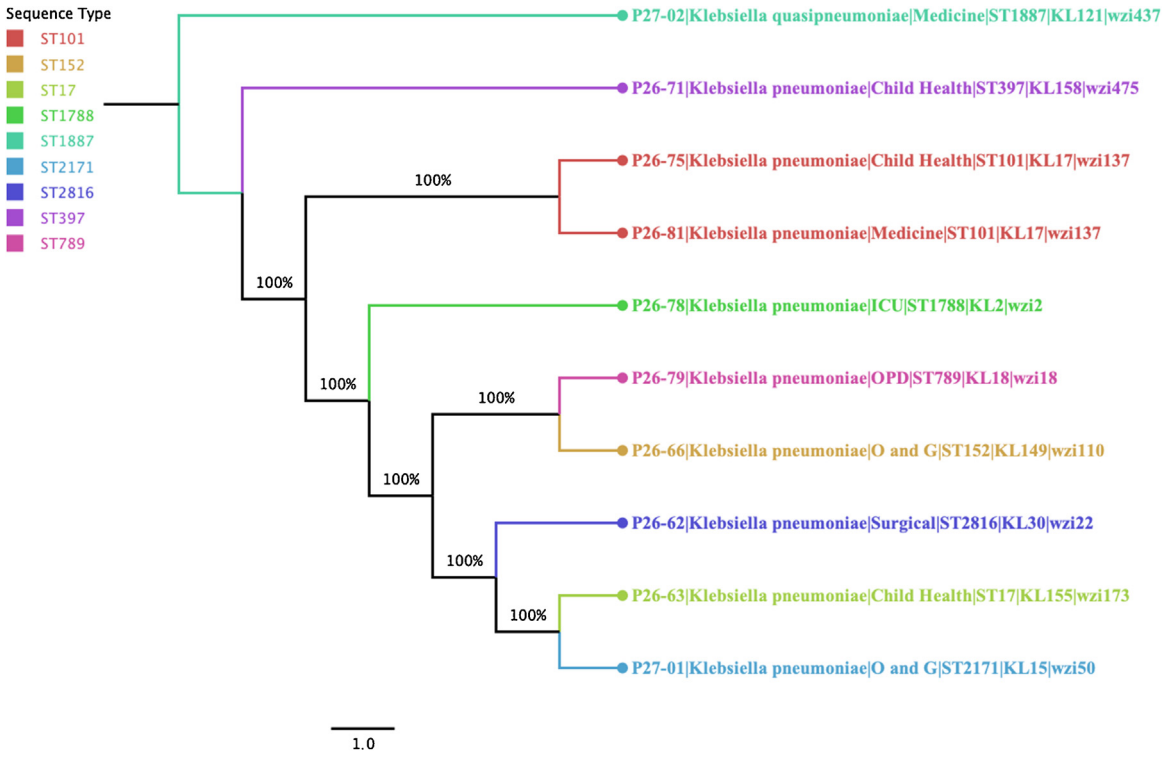

Figure 1. A phylogeny based on core genome multilocus sequence typing genes of the 9 *K. pneumoniae* genomes. The *K. quasipneumoniae* strain P27-02 (accession number: NXHG00000000.1) was rooted and used as the outgroup in the tree. The following information is provided for each isolate: name/reference, ward, MLST (**ST types**), K type and *wzi* allelic type. The tree was divided into many subclades showing the differentiation of the isolates in the phylogenetic tree. The colour codes depict the diversity of the isolates on the phylogenetic tree. The bootstrap values (%) for the nodes has been indicated on the tree. The scale bar represents one nucleotide substitution per 1000 sequence positions.

Table 3
A table showing the diversity of MLST (**ST types**) and allelic profiles of the 7 housekeeping genes in the multidrug-resistant *K. pneumoniae* isolates (n = 9).

Isolate	MLST	*gapE*	*infB*	*mdh*	*pgi*	*phoE*	*rpoB*	*tnoB*
P27-01 [20]	ST2171	2	6	1	1	4	4	4
P26-62 [70]	ST2816	7	40	169	26	1	1	398
P26-63 [76]	ST17	2	1	1	1	4	4	4
P26-66 [117]	ST152	2	3	2	1	1	4	56
P26-71 [155]	ST397	2	1	1	1	21	44	9
P26-75 [183]	ST101	2	6	1	5	4	1	6
P26-78 [201]	ST1788	2	6	160	1	226	111	299
P26-79 [202]	ST789	25	10	1	1	20	1	22
P26-81 [206]	ST101	2	6	1	5	4	1	6

South Africa. In branch **C**, P26-79 relates to isolates from UK and Norway (diverse STs), and in branch **D**, P26-78 is most closely related to isolates of ST493 from the Netherlands and the USA. In branch **E**, P26-66 is located together with isolates of diverse origin and STs, while isolates from four countries (diverse STs) collocate with P26-62 in branch **F**. The ST101-cluster situates on branch **G,** which in addition to the Ghanaian and South African isolates includes isolates from UK and Pakistan.

Discussion

We report on the complexity of multidrug resistance in ESBL-producing *K. pneumoniae* isolates from a referral hospital in Ghana. The isolates were phylogenetically diverse in terms of their alignment to geographical distinct clusters. They were characterized with diverse and multiple permutations and combinations of antibiotic resistance genes. A high prevalence of CTX-M-15 β-lactamases was observed, which mediated high-level phenotypic resistance to the second- and third-generation cephalosporins as indicated in the MICs profile (Tables 1 and 2). The resistance genes were mainly correlated with IncFIB(K) plasmids, with ColRNAI also being common among the isolates.

The isolates were resistant to cefuroxime, cefotaxime ceftazidime, amoxicillin-clavulanate, piperacillin-tazobactam, gentamicin, nitrofurantoin and trimethoprim-sulfamethoxazole. This poses a serious challenge to antibiotic therapy as these agents are commonly used as empirical treatment in Ghana (Hackman et al., 2014). The phenotypic profile was corroborated by the whole genome sequencing results as evident from Tables 1 and 2. This is comparable to studies from many parts of the world, which reported CTX-M class of β-lactamases as a major resistance mechanism among Gram-negative bacteria to oxyimino-cephalosporins, particularly cefotaxime (Bonnet, 2004; Tofteland et al., 2007), with CTX-M-15 being the most common allele in *Enterobacteriaceae* in Africa (Ahmed et al., 2012; Breurec et al., 2013), including Gram-negative ESKAPE bacteria and (Breurec et al., 2013; Rodrigues et al., 2014; Santajit and Indrawattana, 2016), particularly in *K. pneumoniae* (Baraniak et al., 2013).

Multiple *K. pneumoniae* STs were identified in lineage with other isolates from a global strain collection, although from different geographical sources and genetic exchange suggest high diversity and clonal expansion of this species, as reported in other studies (Breurec et al., 2013; Brisse et al., 2009). The high variation between the isolates was in concordance with the Klebsiella capsular serotypes which also predicted 8 different *K types* and *wzi* allelic types for the strains. This highlights the ability of WGS to accurately predict different epidemiological typing techniques. The CTX-M-15-producing *K. pneumoniae* ST type 101 was first reported in Greece in an ICU infections outbreak caused by ertapenem-resistant *K. pneumoniae* (Poulou et al., 2013) and then in other countries including Spain, Italy, France and Tunisia in hospital outbreaks associated with carbapenem resistance (Marcade et al., 2013). In contrast, our isolates were sensitive to carbapenems as these agents have only recently been introduced into the Ghanaian clinical practice, are comparatively more expensive than the mainstay antibiotics and used as last-resort agents in treating serious infections. Thus, there is relatively low selection pressure for the development of carbapenem resistance. Global phylogeny investigation indicated that two of the isolates (P26-75 and P26-81) were of the same sequence type (ST101) as the main cluster of carbapenemase-producing *K. pneumoniae* found in Durban, South Africa. Interestingly, the cgMLST analysis coupled with metadata confirmed that the two isolates; P26-75 (child-health) and P26-81 (Medicine) of ST101 clone were genetically related with 100% identity, an allelic distance of zero (Figure 1 and Table 3). This reiterates the importance of visualizing phylogenetic structures in relation to their metadata as it offers valuable insight into the identification, characterization spread and evolution of pathogens. However, the lineages of ST101 were the same in our isolates but have evolved in different local environments and resistance patterns including carbapenem resistance. Also the P26-66 and P26-71 isolates were phylogenetically related to the South African ST323 isolate and ST14 isolate respectively (Sekyere and Amoako, 2017). This could indicate regional transmission, perhaps due to international travel between the two countries facilitating the dissemination of specific *K. pneumoniae* STs (Figure 2).

The predominance of CTX-M-15 and different TEM-types found in this study is associated with multidrug-resistance in *Enterobacteriaceae.* The CTX-M-15 and TEM-, SHV- and OXA-types of β-lactamases are plasmid encoded with the tendency to disseminate among various species to confer resistance to β-lactams and other non-β-lactam antibiotics including quinolones, chloramphenicol, tetracyclines and aminoglycosides (Shaikh et al., 2015) as reflected in this study.

The IncFIB(K) and ColRNAI plasmids were found in all the isolates and were associated with CTX-M-15 and other resistance genes. This finding is consistent with studies that reported that CTX-M-15 is mainly harboured on IncFII(K) plasmids in ESBL-producing *K. pneumoniae* isolates (Coelho et al., 2010; Tokajian et al., 2015). Reports from others studies have described IncFIB(K) plasmids as dynamic in nature, with the capacity to disseminate antibiotic resistance genes among *Enterobacteriaceae* (Carattoli, 2013; Coelho et al., 2010; Dolejska et al., 2013).

Our study found *qnrB66* variant of the *qnrB* gene in the isolates (P26-66, P26-75 and P26-81) which mediated quinolone resistance, consistent with a study that reported this gene as predominantly encoding for fluoroquinolone/quinolone resistance among *K. pneumoniae* in Africa (Breurec et al., 2013). *oqxA* and *oqxB* genes were found together in all the isolates, suggesting that the *oqxA* and *oqxB* genes cannot be a major mechanism, particularly as they were detected in isolates of both susceptible and much higher MICs. Perhaps *oqxAB* in synergy with other mechanisms increased fluoroquinolone resistance in the isolates as other studies have indicated *oqxA* and *oqxB* genes encoding oqxAB protein to mediate high fluoroquinolone resistance commonly in *K. pneumoniae* (El-Badawy et al., 2017; Sekyere and Amoako, 2017). We also identified multiple aminoglycoside (*aac(6')Ib-cr, StrA, StrB*) and quinolone (*qnrB66*) resistance genes which have been reported in other studies to mediate resistance to gentamicin and ciprofloxacin (Breurec et al., 2013).

Analysis of quinolone resistance-determining regions of *gyrA* and *parC* genes revealed the presence of multiple and diverse mutations in *gyrA* (S83Y, S83F, D87A)and *parC* (S80I, N304S) in isolates that were clonally distinct. Mutations in *gyrA* and *parC* genes have been reported as major mechanisms of fluoroquinolone/quinolone resistance associated with DNA gyrase and topoisomerase IV alterations in Enterobacteriaceae (Alvi et al., 2018; Piekarska et al., 2015; Sekyere and Amoako, 2017), as the plasmid-mediated

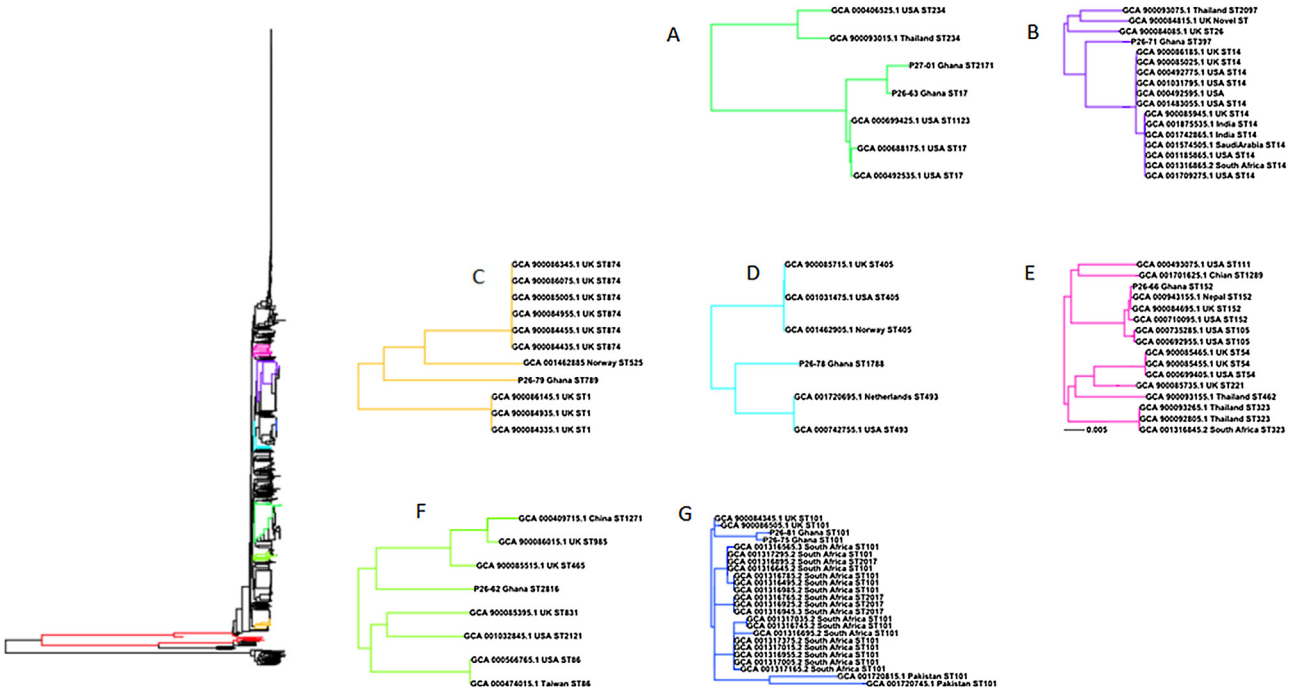

Figure 2. *Klebsiella pneumoniae* global phylogeny as revealed by rapid core genome multi-alignment (https://github.com/marbl/parsnp Assembly dataset from this study was analyzed together with datasets from the PATRIC database (n = 1158) and from South Africa (ref. 35). Subtrees showing the distribution of the Ghanaian isolates in eight in distinct phylogenetic branches (A-G) as indicated by the color codes in the global tree. The subtrees rooted (includes branch lengths) and scale of 0.005 (for all the trees) using (https://cran.r-project.org/web/packages/ape/ape.pdf). For the isolates included in each of these branches, assembly ID, isolation country and MLST are shown.

quinolone resistance genes (PMQR) and extrusion by intrinsic efflux pumps commonly mediate low-level fluoroquinolone/quinolone resistance (Sekyere and Amoako, 2017). However, mutations in both *gyrA* and *parC* are often common and associated with high-level quinolone resistance in Enterobacteriaceae compared with alterations in *gyrB* (Piekarska et al., 2015) as evident in this study. ST101 (P26-75, P26-81) and ST152 (P26-66) isolates were of the same mutation codons 83 and 87 of the *gyrA* and at 80 in *parC* genes with no mutation in *gyrB* gene among isolates with a ciprofloxacin MIC of ≥4 mg/L. Mutations at 83 and 87 in *gyrA* and 80 in *parC* genes have been reported as the most common mutation points which display major alterations among clinical isolates, associated with fluoroquinolone resistance (Sekyere and Amoako, 2017), with codon 83 commonly identified in fluoroquinolone resistant *K. pneumoniae* (Sekyere and Amoako, 2017). The combined effect of S83Y/F, D87A and S80I detected in *gyrA* and *parC* genes in the isolates, P26-66, P26-75 and P26-81 (MIC ≥ 4 mg/L) in our study could be associated with increased level of ciprofloxacin resistance as previously reported (Minarini and Darini, 2012). This is because fluoroquinolones have been the main stay in the management of UTIs in Ghanaian teaching hospitals including Komfo Anokye Teaching Hospital (KATH) as levofloxacin and ciprofloxacin remain the commonest fluoroquinolones prescribed for UTIs leading to recent reports of the emergence of resistance in these settings (Agyepong et al., 2018; Afriyie et al., 2015; Feglo et al., 2013). The complexity and diversity of resistance gene combinations detected among *K. pneumoniae* strains in this study and their potential for dissemination poses a serious threat to the management of infections by this species in Ghana.

Conclusion

This study identified genes encoding resistance for β-lactams, fluoroquinolones, aminoglycosides and other antibiotics in diverse permutations and combinations among multidrug-resistant *K. pneumoniae* bacteria in Komfo Anokye Teaching Hospital. There is thus an urgent need for epidemiological and molecular studies to understand the dynamics of antibiotic resistance transmission to inform strategies for containment.

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Authors' contribution

The study was co-conceptualized and jointly designed by NA, UG, AO, DGA, MA, JJ, TP, AS and SE. NA collected the data undertook the preliminary laboratory work and present result as tables. AO supervised the sampling and preliminary laboratory work. UG supervised the isolates phenotypic screening and analysis. DGA and MA contributed to the bioinformatics data analysis. TP contributed to isolation of high-quality DNA and illumina bioinformatics analyses. JJ, TP and AS contributed to data analyses and assuring the quality of the result. SE contributed to assuring the quality of the final manuscript. All the authors contributed in preparation and submission of manuscript.

Competing interests

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.05.025.

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