

Original Research Article

Prevalence, antibiogram and whole genome sequence of multidrug-resistant *Klebsiella pneumoniae* isolated from community-onset urinary tract infections in Calabar, Southern Nigeria

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Abstract

Purpose: To carry out genome characterization of clinical multidrug-resistant *Klebsiella pneumoniae* strains isolated from urine samples and determine the clones implicated in community onset urinary tract infection (UTI), with a focus on antimicrobial resistance genes, multi-locus sequence typing (MLST), plasmid content, virulence gene and capsule typing.

Methods: Three hundred and eighty-four urine samples were collected from outpatients in General Hospital, Calabar and the General Hospitals in Calabar Municipal and Akampka Local Government Area of Cross River State, Nigeria. Isolates were confirmed using 16S rRNA sequencing and were evaluated for antibiotic susceptibility using CLSI protocols. Whole-genome sequencing (WGS) was performed using Illumina technique.

Results: The prevalence of ESBL and carbapenemase-producing *K. pneumoniae* strains was predominant in Calabar (21.87 %) and Akampka (22.00 %). Fourteen *K. pneumoniae* strains isolated showed acquired resistance genes including; *tet(A)*; *aph(3')-Ia*, *aadA1*, *acc(6')-Ib*, *aph(6)-Id*, *rmtF*, *armA*, *aph(3')-VIb*; *fosA*, *fosA6*, *fosA3*; *blaSHV-11*, *blaSHV-26*, *blaSHV-98*, *blaTEM-1C*, *blaCTX-M-14b*, *blaCTX-M-15*, *blaOXA-9*, *blaOXA-48*, *blaNDM-1*, *blaNDM-5*, and *blaKPC-2* which were responsible for multi-drug resistance mechanisms. The K and O-typing revealed that the 14 isolates were K15, K30, K35, K47, K51, K64, K74, variant type KL114 and O1ab, O2afg, O4, O5, O13, variant type OL104, respectively. The following plasmid and virulence genes were found in all *K. pneumoniae* clones: Col44OI, ColRNAI, IncFIB (Mar) types and *fimH*, *iutA*, *fyuA*, *irp2*, *nlpl*, *terC*, *iucC*, respectively.

Conclusion: Genotyping of clinical MDR-*K. pneumoniae* using WGS has revealed diverse beta-lactamase genes and identified genomic variations between local isolates. The data provide insights into transmission patterns and clonal dissemination within the community.

Keywords: Prevalence, Antimicrobials, UTI, *Klebsiella pneumoniae*, Whole-genome sequencing

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INTRODUCTION

Klebsiella pneumoniae is one of the pathogens that causes opportunistic infection and is among

the major etiologic agents of nosocomial infections such as urinary tract infection and pneumonia [1]. Diseases caused by *K. pneumoniae* generally correlate with high dead

rate, particularly in immunocompromised patients [1]. At a global scale, *K. pneumoniae* is among the most causative agents that frequently show resistance to various classes of antibiotics [2]. These antibiotic resistance traits could be moved from one bacterium to another via vertical and horizontal gene transfer processes using plasmids and transposons [3,4].

The hydrolytic activity of beta-lactamase on extended-spectrum penicillin, cephalosporins, and monobactams has resulted in the use of carbapenem as an antibiotic of last resort in tackling infectious diseases arising from multidrug-resistant bacteria [5]. Nonetheless, the overutilization and misuse of carbapenem antibiotics have induced widespread global resistance in *Klebsiella pneumoniae*. Among CRKP strains, carbapenemase synthesis is the most recognized mechanism of resistance [6]. The β -lactam ring-containing antibiotics can be degraded by carbapenemase. Over the past 20 years, *K. pneumoniae* has been labelled as a vital tool of antibiotics resistance through the production of beta-lactamase, and has been acknowledged as one of the worlds' most important causative agent that needs novel approaches for controlling it [7]. The variability in the genetic make-up of *K. pneumoniae* has been reported previously with the most dominant sequence type (ST) isolated from China [7]. Reports have it that beta-lactamase-producing *K. pneumoniae* found in humans mostly belonged to the same major STs (11, 15, and 307) [8]. Currently, in Nigeria, there is no information on the use of whole genome sequence-based studies for analysis of beta-lactam-resistant determinant in *Klebsiella pneumoniae* of urine origin collected from outpatients visiting medical facilities in Cross River State, Nigeria.

METHODS

Study area and collection of samples

This study was carried out in General Hospital Calabar (GPS: 4.9829° N, 8.3345° E) and General Hospitals in Calabar Municipal and Akampka Local Government Area (GPS: 5.4273° N, 8.5185° E), Cross River State, Nigeria.

Sampling was done from January to June, 2024. A total of 384 urine samples were collected from patients attending medical facilities in these hospitals. Samples were transported in an ice chest and analyzed on arrival at the postgraduate microbiology laboratory of the Department of Microbiology, University of Calabar, Nigeria.

Ethical approval

Ethics Committee of the Cross River State Ministry of Health approved this study (CRSMOH/HRP/REC/2024/537).

Inclusion criteria

Early morning urine samples were collected once from outpatients with symptoms of UTI.

Exclusion criteria

No duplication of isolates from the same samples and patient.

Isolation of bacteria

Standard bacteriological techniques were used to isolate bacteria from urine samples by inoculating them on MacConkey agar as previously described with modification [9].

Bacterial identification

Isolates were used to carry out various biochemical tests, including Gram staining, indole production, citrate utilization, urease activity, methyl red test, and nitrate reduction test for identification using protocols as described previously with slight modification [10].

Antibiotic susceptibility profile

Kirby-Bauer disc diffusion technique was used for AST analysis. An overnight culture of test organism adjusted to 0.5 McFarland standard was seeded on Mueller-Hinton agar plates. Different antibiotic discs previously discussed were placed 20 mm apart in the medium, incubated at 37 °C for 24 h [11].

Screening of *K. pneumoniae* isolates for beta-lactamase production

The ESBL production was initially screened using ceftazidime (30 µg) and cefotaxime (30 µg) discs after using an 18-hour-old test bacteria culture to prepare isolates lawn culture on freshly prepared Mueller-Hinton agar. The two-3rd generation cephalosporins were aseptically placed on the MHA and incubated at 37 °C for 18 h, after which measurement of zone of inhibition was taken and compared with CLSI interpretation to ascertain if they were susceptible or resistant [11]. The ESBL-positive (resistant) *K. pneumoniae* isolates were further confirmed phenotypically using the double disk synergy (DDST) test as described previously [12].

AmpC β -lactamase production was phenotypically detected on only isolates that were ESBL positive according to CLSI criteria. ESBL-positive isolates were screened for AmpC β -lactamase production using the method described previously [13]. *Klebsiella pneumoniae* strains were phenotypically screened for their resistance to carbapenem (imipenem, 10 μ g and ertrapenem, 10 μ g; Oxoid, UK) by determining their antimicrobial susceptibility to carbapenem according to CLSI criteria [14].

Whole genome sequencing

Fourteen multidrug-resistant (MDR) *K. pneumoniae* isolates were cultured overnight, and their genomic DNA was purified using the QIAamp® DNA Mini kit, following the manufacturer's instructions [15]. The extracted DNA was assessed for quality and concentration using Qubit® fluorometry and gel electrophoresis [15]. Subsequently, 20 μ L of DNA at a concentration of 30 ng/ μ L per isolate was prepared for Illumina sequencing.

Sequence data quality control

Sequence data quality was evaluated with FastQC toolkit and subsequently trimmed adapters and filtered low-quality reads using the ea-utils package and adapter sequences trimmed [15].

Assembly of sequence

Sequence data were de novo assembled with SPAdes, with optimal assembly parameters; K-mer length, expected coverage, coverage cut-off and insert length selected as previously described [15]. The generated contigs were screened for beta-lactam antibiotic-resistant determinants and associated mechanisms using ResFinder database. Additionally, capsule typing was assessed using Kaptive [16].

Data analysis

The data set was analyzed using Statistical Package for the Social Sciences (SPSS), version 21, as described previously and relevant data were interpreted using simple descriptive statistics.

RESULTS

Distribution of beta-lactamase-producing *K. pneumoniae* phenotypes

The prevalence of ESBL-producing *K. pneumoniae* isolates was the most frequent in

Calabar (21.87 %), Akampka (22.00 %), compared to other β -lactamases (Table 1 a and b).

Genetic features

The commonest among various sequence types (ST) were ST147 (n = 4) and ST11 (n = 4). Other STs (ST219, ST383 and ST6654) had 2 isolates each. Two variants, ST (clones), were recorded: ST6654 (Table 3). Isolates sub-lineage shared some ST as *K. pneumoniae* isolates from other sources; however, their O-locus (Table 3), and virulence gene content vary (Table 4). Isolates' genome length ranges from 5521438 to 6107117 (Table 3).

Among the 14 clones screened for virulence factors, all 13 *K. pneumoniae* isolates harboured one or two virulence genes, with fimH (type 1 fimbriae, n = 13) dominating the genes found in all clones. This was followed by iutA (ferric aerobactin receptor, n = 12), and then by the gene with unknown function (mrKA: ABW83989, n = 11). Other genes found in this study from our clone were terC (tellurim ion resistance protein, n = 9), nlpl (lipoprotein NIPI precursor, n = 8), irp2 (n = 8), iucC (n = 7), fyuA (siderophore receptor, n = 6), ccl (cloacin, n = 2), traT (n = 2), traJ (n = 1) (Table 4).

Plasmid replicon typing

The distribution of the replicon types amongst 14 of the *K. pneumoniae* isolates revealed 7 STs to harbour ColRNAI plasmid, which encodes colistin resistance gene, Col44OI (n=7), Col44OII (n = 4). The incompatibility plasmid replicon IncL/M(Poxa-48) was found in 6 isolates, this plasmid carries gene that encodes carbapenemase production, IncHI1B (n = 9), IncF1B (Mar; n = 9), IncR (n = 5), IncFIB (K; n = 5), IncF1B (pKPHS1; n = 2), IncFII (K; n = 2), IncFII (pHN7A8; n = 1; Table 5).

The 14 clinical MDR-*K. pneumoniae* clones in this study showed widespread ESBL phenotype. These genes included blaSHV-11, blaSHV-26, blaTEM-1B, blaTEM-1C, blaCTX-M-14b, blaCTX-M-15. It was observed in this study that some clones simultaneously carried multiple beta-lactam resistance mechanisms. Carbapenemase phenotypes were also observed in all the clones, including blaNDM-1, blaNDM-2, blaKPC-2, blaOXA-1, blaOXA-9 and blaOXA-48. Three isolates were observed to carry gene resistance for rifampicin, ST147 and the two ST6654. All the clones harboured the fosA gene responsible for resistance against fosfomycin, similar to that reported earlier [17].

Clones also carried the following genes, including aph (3')-Ia, aadA1, aph (3'')-Ib, armA, rmtf, catA1, catA2; these genes have been previously reported [18], trimethoprim (dfrA1 and dfrA5) and macrolide (mph(A) and mph(E)). Plasmid-mediated resistance to quinolone/efflux pump was present in all isolates, including the

following genes: qnr1, qnrS1, OqxA and OqxB [19].

DISCUSSION

The choice of antibiotics for the treatment of community-acquired infection is important, especially those caused by *K. pneumoniae*, as they usually develop resistance [20].

Table 1a: Proportion of antibiotic resistance profile among beta-lactamase-producing clinical *K. pneumoniae* isolates from General Hospital, Calabar (n = 31)

Antibiotics class/Antibiotics	ESBL (n=21)	AmpC (n=6)	ESBL+AmpC (n=1)	Carbapenemase (n=3)
β-lactam	R (n; %) S (n; %)	R (n; %) S (n; %)	R (n; %) S (n; %)	R (n; %) S (n; %)
Piperacillin	21(100) 0(00)	6(100) 0(00)	1(100) 0(00)	3(100) 0(00)
Amoxicillin+clavulanic acid	21(100) 0(00)	6(100) 0(00)	1(100) 0(00)	3(100) 0(00)
Cefoxitin	8(38.09) 13(61.90)	5(83.33) 1(16.66)	1(100) 0(00)	3(100) 0(00)
Cefotaxime	18(85.71) 3(14.28)	1(16.66) 5(83.33)	1(100) 0(00)	3(100) 0(00)
Ceftazidime	17(80.95) 4(19.05)	4(66.66) 2(33.33)	1(100) 0(00)	3(100) 0(00)
Imipenem	0(00) 21(100)	1(16.66) 5(83.33)	0(00) 1(100)	3(100) 0(00)
Monobactam				
Aztreonam	19(90.47) 2(9.52)	2(33.33) 4(66.66)	1(100) 0(00)	1(33.33) 2(66.66)
Aminoglycoside				
Streptomycin	18(85.71) 3(14.29)	1(16.66) 5(83.33)	1(100) 0(00)	1(33.33) 2(66.66)
Amphenicol				
Chloramphenicol	8(38.09) 13(61.90)	4(66.66) 2(33.33)	0(00) 1(100)	3(100) 0(00)
Tetracycline	11(52.38) 10(47.61)	3(50) 3(50)	1(100) 0(00)	3(100) 0(00)
Folic acid inhibitors				
Sulfonamide	17(80.95) 4(19.04)	5(83.33) 1(16.66)	1(100) 0(00)	1(33.33) 2(66.66)
Quinolones				
Nalidixic acids	14(66.66) 7(33.33)	4(66.66) 2(33.33)	1(100) 0(00)	1(33.33) 2(66.66)
Fluoroquinolones				
Ofloxacin	15(71.42) 6(28.57)	5(83.33) 1(33.33)	1(100) 0(00)	1(33.33) 2(66.66)

Note: R = resistant, S = sensitive

Table 1b: Proportion of antibiotic resistance profile among beta-lactamase-producing clinical *K. pneumoniae* isolates from General Hospital, Akampka

Antibiotics class/Antibiotics	ESBL (n=11)	AmpC (n=2)
β-lactam	R (n; %) S (n; %)	R (n; %) S (n; %)
Piperacillin	10(90.90) 1(9.09)	2(100) 0(00)
Amoxicillin+clavulanic acid	9(81.81) 2(18.18)	2(100) 0(00)
Cefoxitin	8(72.72) 3(27.27)	2(100) 0(00)
Cefotaxime	10(90.90) 1(9.09)	2(100) 0(00)
Ceftazidime	9(81.81) 2(18.18)	2(100) 0(00)
Imipenem	0(00) 11(100)	0(00) 2(100)
Monobactam		
Aztreonam	9(81.81) 2(18.18)	1(50) 1(50)
Aminoglycoside		
Streptomycin	6(54.54) 5(45.45)	2(100) 0(00)
Amphenicol		
Chloramphenicol	11(100) 0(00)	2(100) 0(00)
Tetracycline	11(100) 0(00)	2(100) 0(00)
Folic acid inhibitors		
Sulfonamide	4(36.36) 7(63.63)	0(00) 2(100)
Quinolones		
Nalidixic acids	5(45.45) 6(54.54)	0(00) 2(100)
Fluoroquinolones		
Ofloxacin	3(27.27) 8(72.72)	0(00) 2(100)

Note: R = resistant, S = sensitive

Table 2: Age-wise distribution of beta-lactamase-producing clinical *K. pneumoniae* isolates from General Hospital, Calabar and Akampka

Age group	No of +ve	ESBL +ve	AmpC +ve	ESBL+ AmpC+ve	Carb +ve	Total	Non Producers	No of +ve	ESBL +ve	AmpC +ve	ESBL+ AmpC	Carb +ve	Non producer
10-20	9	2(22.22)	0(00.00)	0(00.00)	0(00)	2(22.22)	7(77.77)	3	0(00.00)	0(00.00)	0(00.00)	0(00)	3(100.00)
21-30	15	3(20.00)	1(6.66)	0(00.00)	0(00)	4(26.66)	11(73.33)	3	0(00.00)	0(00.00)	0(00.00)	0(00)	3(100.00)
31-40	16	3(18.75)	1(6.25)	0(00.00)	1(6.25)	5(31.25)	11(68.75)	9	2(22.22)	0(00.00)	0(00.00)	0(00)	7(77.77)
41-50	14	4((28.57)	2(14.28)	0(00.00)	0(00)	6(42.85)	8(57.14)	8	2(25.00)	0(00.00)	0(00.00)	0(00)	6(75.00)
51-60	17	3(17.64)	0(00.00)	1(5.88)	1(5.88)	5(29.41)	12(70.58)	10	2(20.00)	0(00.00)	0(00.00)	0(00)	8(80.00)
>60	25	6(24.00)	2(8.00)	0(00.00)	1(4.00)	9(36.00)	16(64.00)	17	5(29.41)	2(11.76)	0(00.00)	0(00)	10(58.82)
	96	21(21.87)	6(6.25)	1(1.04)	3(3.13)				11(22.0)	2(4.00)			37(74.00)

Table 3: Genetic features, capsule and O-polysaccharide typing of clinical *K. pneumoniae* isolates from urine

Site	Sequence type (ST)	KL-type	O-type	O-locus	Genome length
Urine	11	K15	O4	O4	5702599
Urine	11	K47	O13	OL13	6097479
Urine	11	K47	O13	OL13	6088960
Urine	11	K15	O4	O4	5697384
Urine	147	K64	O29	O1/O2v1	5947215
Urine	147	K51	O2afg	O1/O2v2	5739379
Urine	147	K74	OL104	OL104	6107117
Urine	219	KL114	O1ab	O1/O2v1	5526847
Urine	219	KL114	O1ab	O1/O2v1	5521438
Urine	383	K30	O2afg	O1/O2v2	5840077
Urine	383	K30	O2afg	O1/O2v2	5840077
Urine	6654	K35	O5	O5	5594821
Urine	6654	K35	O5	O5	5515457

Table 4: Virulence factors found in clinical MDR-*K. pneumoniae* clones in this study

Clone	Vir gene
ST147	FimH
	mrkA: ABW83989
	iutA, fyuA, irp2, nlpl, terC, iucC
ST383	fimH, mrkA; ABW83989, terC, iutA, nlpl, irp2, fyuA
ST6654	fimH, mrkA; ABW83989, terC, nlpl, ccl, iutA
ST147	mrkA; ABW83989, fimH, iutA, terC, fyuA, irp2, ccl
ST11	terC, iucC, iutA, fimH, mrkA; ABW83989, ccl, irp2, fyuA
ST147	traT, iucC, iutA, mrkA; ABW83989, fimH, iutA, irp2, fyuA, nlpl
ST383	mrkA; ABW83989, fimH, irp2, fyuA, iucC, iutA, terC, nlpl
ST219	terC, mrkA; ABW83989, iutA, fimH
ST6654	fimH, mrkA; ABW83989, ccl, nlpl, iutA, traT, terC
ST11	traJ, fimH, mrkA; ABW83989, ccl, iucC, iutA, irp2, flyuA
ST11	iucC, iutA, ccl, fimH, irp2, fyuA,
ST219	fimH, mrkA;83989, terC, iutA
ST147	Nlpl, fimH, iutA, iucC, ccl, irp2, fyuA

Table 5: Plasmid replicons found in clinical MDR-*K. pneumoniae* clones in this study

Clone	Col	Inc(other)	IncR
ST147	Col44OI	IncHI1B, :IncFIB (Mar)	-
ST383	Col44OI	IncHI1B, IncFIB (Mar)	-
ST6654	ColRNAI, Col44OI	IncFIB(K)	IncR
ST147	ColRNAI, Col44OII	IncHI1B, IncFIB (Mar)	IncR
ST11	ColRNAI, Col44OII, Col44OI	IncL/M(pOXA-48), IncHI1B, IncFIB (Mar)	-
ST147	-	IncL/M(pOXA-48), IncHI1B, IncFIB (Mar), IncFII(K), IncFIB (Pqi1), IncF1B(Pkphs1)	-
ST383	Col44OI	IncHI1B, IncFIB (Mar)	-
ST219	-	IncFIB(K), IncL/M(pOXA-48),	
ST6654	ColRNAI, Col44OI	IncFII(K), IncFIB (Pqi1)	IncR
ST11	ColRNAI	IncHI1B, IncFIB (Mar), IncFII(K), IncFIB(K), IncFII(pHN7A8)	IncR
ST11	ColRNAI, Col44OI, Col44OII	IncL/M(pOXA-48), IncHI1B, IncFIB (Mar)	-
ST219	-	IncFIB(K), IncL/M(pOXA-48),	-
ST147	ColRNAI, Col44OI	IncHI1B, IncFIB (Mar)"	IncR

Table 6: Acquired antimicrobial resistance determinant and their mechanism from *in silico* analysis of sequence data of clones of clinical MDR-*Klebsiella pneumoniae* isolated

Clone	Aminoglycosides	Beta-lactam		Fosfomycin	Macrolides, Linomastine	Phenicol	Fluoroquinolone	Sulphonamide	
		ESBL	Carbapenem						
ST147	<i>aph(3')-Ia, aadA1, aac(6')-Ib, aph(6)-Id, aph(3'')-Ib, aph(3')-VI</i>	<i>blaSHV-11, blaSHV-67, blaTEM-1C, blaTEM-1B, blaCTX-M-14b, blaCTX-M-15</i>	<i>blaOXA-9, blaNDM-5, blaOXA-48</i>	<i>fosA</i>	<i>mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1</i>
ST11	<i>armA, aph(3')-VI, aph(3')-Ia</i>	<i>blaSHV-182, blaSHV-159, blaSHV-158, blaCTX-M-65</i>	<i>blaKPC-2, blaNDM-1</i>	<i>fosA6</i>	<i>mph(A), msr(E), mph(E)</i>	-	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST11	<i>aph(3')-VI, armA, aph(3')-Ia</i>	<i>blaSHV-182, blaSHV-159, blaSHV-158, blaCTX-M-15</i>	<i>blaKPC-2, blaNDM-1</i>	<i>fosA6, fosA3</i>	<i>mph(A), msr(E), mph(E)</i>	<i>catA2</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST147	<i>aph(3')-VI, aph(3')-Ia, aad1, aa(6')-Ib</i>	<i>blaSHV-11, blaSHV-67, blaCTX-M-14b</i>	<i>blaNDM-5, blaOXA-9</i>	<i>fosA</i>	<i>Mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1</i>
ST147	<i>aadA1, aac(6')-Ib, aph(3')-Ia, rmtF, aac(6')-Ib Hangzhou, aph(6)-Id, aph(3'')-Ib, aph(3')-VIb, aph(3')-VI</i>	<i>BlaSHV-11, blaSHV-67, blaTEM-1C, blaTEM-1B, blaCTX-M-14b, blaCTX-M-15</i>	<i>blaOXA-9, blaNDM-5, blaOXA-48</i>	<i>fosA</i>	<i>mph(A)</i>	<i>catA1</i>	<i>OqxB, qnr1, qnrS1</i>	<i>OqxA,</i>	<i>Sul1</i>
ST11	<i>aph(3')-VI, armA</i>	<i>blaSHV-182, blaSHV-159, blaSHV-158, blaTEM-1C, blaTEM-1B, blaCTX-M-15</i>	<i>blaNDM-5, blaOXA-48</i>	<i>fosA6</i>	<i>mph(A), msr(E), mph(E)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST6654	<i>aadA1, aph(3')-VI, aac(6')-Ib, aph(3')-Ia, rmtF</i>	<i>blaSHV-11, blaSHV-67, blaCTX-M-15, blaTEM-1A</i>	<i>blaOXA-9, blaNDM-1</i>	<i>fosA</i>	<i>mph(A)</i>	-	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1</i>
ST383	<i>aph(3')-Ia, aph(6)-Ia, aph(3'')-Ib, aph(3')-VI, armA, aadA1, aac(6')-Ib, aph(3')-VIb</i>	<i>blaSHV-199, blaSHV-194, blaSHV-179, blaSHV-145, blaSHV-98, blaSHV-78, blaSHV-26, blaCTX-M-14b, blaTEM-1C, blaTEM-1B, blaCTX-M-15</i>	<i>blaNDM-5, blaOXA-9, blaOXA-48</i>	<i>fosA</i>	<i>msr(E), mph(E), mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST383	<i>aph(3')-VI, aadA1, aac(6')-Ib, armA, aph(3')-Ia</i>	<i>blaSHV-199, blaSHV-194, blaSHV-179, blaSHV-145, blaSHV-98, blaSHV-78, blaSHV-26, blaCTX-M-14b, blaTEM-1C, blaTEM-1B, blaCTX-M-15</i>	<i>blaNDM-5, blaOXA-9</i>	<i>fosA</i>	<i>msr(E), mph(E), mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST11	<i>aadA1, aac(6')-Ib, aph(3')-VI, armA</i>	<i>blaSHV-182, blaSHV-159, blaSHV-158, blaTEM-1C, blaTEM-1B, blaCTX-M-15</i>	<i>blaNDM-5, blaOXA-9</i>	<i>fosA</i>	<i>msr(E), mph(E), mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST6654	<i>aadA1, aph(3')-VI, aac(6')-Ib, rmtF, aac(6')-Ib Hangzhou, aph(3')-Ia</i>	<i>blaSHV-11, blaSHV-67, blaCTX-M-15, blaTEM-1A</i>	<i>blaOXA-1, blaNDM-1</i>	<i>fosA</i>	<i>mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1</i>
ST147	<i>aph(3')-VI, aph(3'')-Ia, aadA1, aac(6')-Ib</i>	<i>blaSHV-11, blaSHV-67, blaCTX-M-15, blaTEM-1A</i>	<i>blaOXA-9, blaNDM-5</i>	<i>fosA</i>	<i>mph(A)</i>	<i>catA1</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1</i>
ST219	<i>aph(3')-Id, aph(3'')-Ib, aadA2, aph(3')-Ia</i>	<i>blaCTX-M-15, blaSHV-199, blaSHV-194, blaSHV179, blaSHV-145, blaSHV-98, blaSHV-78, blaSHV-26</i>	<i>blaOXA-48</i>	<i>fosA</i>	<i>mph(A)</i>	<i>catA2</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>
ST219	<i>aph(6)-Id, aph(3'')-Ib, aadA2, aph(3')-Ia</i>	<i>blaCTX-M-15, blaSHV-199, blaSHV-194, blaSHV-179, blaSHV-145, blaSHV-98"</i>	<i>blaOXA-48</i>	<i>fosA</i>	<i>mph(A)</i>	<i>catA2</i>	<i>OqxB, qnrS1</i>	<i>OqxA,</i>	<i>Sul1, Sul2</i>

In this study, findings indicate a high prevalence of *K. pneumoniae* resistance, with the highest rates observed against Nalidixic acid (89.23 % in Calabar) and Streptomycin (78.37 % in Akamkpa). Higher resistance levels to nalidixic acid and streptomycin have been reported previously [21].

This could be attributed to constitutively expressed chromosomal class β -lactamases [21]. Lower resistance to tetracycline (Calabar, 7.69 %) and aztreonam (Akamkpa, 29.72 %) was observed. Similar observations were made by Celestin where *Klebsiella* species showed 7% resistance rate to aztreonam [5].

The resistance by *K. pneumoniae* to carbapenem (imipenem) was 100 %. Similar findings were made were resistance to cephalosporin by *K. pneumoniae* was close to 100 % [22]. Report has it that *Klebsiella* susceptibility in outpatients showed high variability, ranging from 29 – 82 % for amoxicillin/clavulanic, 74 – 84 % for cefotaxime and 54 – 94 % for ciprofloxacin [22]. These differences could be a result of the sampling size pattern, method, the types of antibiotics routinely administered, and the prescription rate in those settings [23].

The ESBLs production was 21.87 % for Calabar and 22 % for Akampka isolates. There has been report from previous studies indicating significant variability in the prevalence of ESBL-producing *K. pneumoniae* clinical isolates with reported rates ranging from 3 to 74.5 % [22]. Carbapenemase was produced in 3.13 % of isolates. Co-production of ESBL and AmpC was recorded at 1.04 %. A higher rate of co-production was observed [23,24]. The highest antimicrobial resistance was recorded among ESBL producers for Piperacillin, Amoxicillin-clavulanic acids, cefotaxime and ceftazidime in Calabar and in Akampka.

In Nigeria, this is the first report on analysis based on whole-genome sequence for clinical isolates of *K. pneumoniae* from urine. This study revealed two variant sequence types, ST 6654 along with STs 219, 383, 11, and 147. A 2016 study identified an MDR ST11 clone in *Ovis orientalis musimon* [25], reflecting a broader trend where BlaOXA-48 and blaCTX-M-15 are frequently reported in clinical *K. pneumoniae* [25]. While blaOXA-48 and blaCTX-M-15 have been common in European *K. pneumoniae* for over a century, this study found both blaOXA-48 and blaCTX-M-15 in all 14 isolated clones. All the clones had presence of fosA, fosA3 and fosA6 genes. Chromosomal fosA genes enable Gram-negative pathogens to develop resistance

against fosfomycin [17]. Four vital proteins enable *K. pneumoniae* to cause infection [26]. One of these is adherence-related genes(fimH) belonging to type 1 fimbriae and was found in all clones. This fimbria helps in the attachment to host cells and subsequent spreading [26].

Phenolate siderophore yersiniabactin-related gene and citrate-hydroxamate siderophore aerobactins [26] were found in clones. Plasmids help in the spreading of resistance determinants and were investigated. IncFIB and IncFII plasmids were found in 10 out of 14 clones in this study. Three Inc-type plasmids were found: IncHI1B, IncL/M and IncR. The IncR plasmid confers the ability to transfer blaKPC to other Enterobacteriaceae via conjugation mechanism. Seven of the clones harboured the Col440I-type plasmid replicon, while another seven carried ColRNAI. The prevalence of blaNDM-1 mediated carbapenem resistance in *K. pneumoniae* is driven by multi-replicon plasmids including ColRNAI, IncFIB Col440I, IncFII and IncFIB [27], which often co-occur and were all found in the clones recovered from urine in the current study. The findings of this study should be interpreted within the context of the following limitations: small sample size restricts geographical representation; cross-sectional nature of the analysis precludes longitudinal data analysis and reliance on short-read sequencing constrains complex genomic analysis.

CONCLUSION

Genotyping of clinical MDR-*K. pneumoniae* using WGS has revealed diverse beta-lactamase genes and identified genomic variations between local isolates. This study provides insight into the molecular features of the antibiotic resistance mechanism, virulome in these clones and the need for surveillance of these resistant strains in these flash areas. The data provide insights into transmission patterns and clonal dissemination within the community.

DECLARATIONS

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Ethical approval

The Ethics Committee of the Cross River State Ministry of Health approved this study (CRSMOH/HRP/REC/2024/537).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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