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Original Article **Open Access**

Prevalence of extended spectrum β-lactamase genes among multi-drug resistant *Klebsiella pneumoniae* isolates from clinical specimens in Calabar, Nigeria

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Abstract:

Background: The global spread of the extended spectrum beta-lactamases (ESβL) and carbapenemases producing strains of *Klebsiella pneumoniae* constitutes a public health threat to both humans and animals. This study investigated the prevalence of ESβLs genes among multidrug resistant (MDR) *K. pneumoniae* isolates from clinical specimens of patients attending selected hospitals in Calabar, Nigeria.
Methodology: This was a laboratory-based study of 80 *K. pneumoniae* isolates preliminarily identified at the routine microbiology laboratories of four healthcare facilities in Calabar metropolis, with 60 *K pneumoniae* isolates confirmed using Vitek^{R} 2 Compact system (BioMérieux; Marcy L'Étoile, France). Antibiotic susceptibility testing of the confirmed isolates to selected antibiotics was performed by the Kirby Bauer disc diffusion method and result interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines. MDR was defined as resistance to three or more antibiotic classes. ESBL (bla_{CTM-M} and bla_{KPC}) genes were detected among the MDR strains by conventional PCR assay and agarose gel electrophoresis of the gene amplicons. Data of patients from whom the organism were recovered, were extracted from the laboratory records. Pearson's Chi square test was used to measure association between categorical variables, and $p \leq 0.05$ was considered statistically significant.
Results: Of the 60 confirmed *K. pneumoniae* isolates, the highest frequency of the isolate recovery was from patients aged >60 years (25.0%, n=15) and 50-60 years (21.7%, n=13), while the least frequency was from patients aged 1-10 years (5.0%, n=3). Recovery rate was higher in females (n=35, 58.3%) compared to males (n=25, 41.7%). The frequency of resistance of the isolates to meropenem, piperacillin/tazobactam, amoxicillin/clavulanate, ciprofloxacin, gentamicin and ceftriaxone was 25.0% (n=15), 31.7% (n=19), 36.7% (n=22), 48.3% (n=29), 48.3% (n=29) and 76.7% (n=46) respectively. Only 15 (25.0%) of the 60 isolates were MDR strains. Age was significantly associated with MDR strains, with higher frequency in age groups 51-60 years (38.5%, n=5/13) and >60 years (40.0%, n=6/15) compared to other age groups (x^2=13.733, p=0.035). Carriage rate of bla_{CTX-M} gene among the MDR strains was 100% (n=15) while only 26.7% (n=4) carried the bla_{KPC} genes.
Conclusion: The relatively high prevalence of ESβLs encoding genes among *K. pneumoniae* clinical isolates in this study may represent a grim prospect for successful treatment and management of infections caused by this pathogen in the population. Informed and discrete use of antimicrobial drugs, particularly β-lactam antibiotics, for management of bacterial infections will prevent further escalation of drug-resistance in this epidemiological setting.

Keywords: Prevalence; *Klebsiella pneumoniae*; Multidrug resistance, ESBL, bla_{CTX-M}; bla_{KPC}

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Prévalence des gènes de β-lactamases à spectre étendu parmi les isolats de *Klebsiella pneumoniae* multirésistants provenant d'échantillons cliniques à Calabar, au Nigéria

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Résumé:

Contexte: La propagation mondiale des souches de *Klebsiella pneumoniae* productrices de bêta-lactamases à spectre étendu (BLSE) et de carbapénémases constitue une menace pour la santé publique humaine et animale. Cette étude a examiné la prévalence des gènes BLSE parmi les isolats de *K. pneumoniae* multirésistants (MDR) provenant d'échantillons cliniques de patients fréquentant certains hôpitaux de Calabar, au Nigéria.
Méthodologie: Cette étude en laboratoire a porté sur 80 isolats de *K. pneumoniae*, identifiés préliminairement dans les laboratoires de microbiologie de routine de quatre établissements de santé de la métropole de Calabar. Parmi ces isolats, 60 ont été confirmés par le système Vitek® 2 Compact (BioMérieux; Marcy-l'Étoile, France). La sensibilité aux antibiotiques des isolats confirmés a été testée par la méthode de diffusion sur disque de Kirby-Bauer et les résultats interprétés selon les recommandations du CLSI (Institut des Normes Cliniques et de Laboratoire). La multirésistance (MDR) a été définie comme une résistance à trois classes d'antibiotiques ou plus. Les gènes ESBL (bla_{CTM-M} et bla_{KPC}) ont été détectés parmi les souches MDR par PCR conventionnelle et électrophorèse sur gel d'agarose des produits d'amplification. Les données des patients chez lesquels l'organisme a été isolé ont été extraites des dossiers de laboratoire. Le test du χ^2 de Pearson a été utilisé pour évaluer l'association entre les variables catégorielles, et un seuil de signification statistique de $p \leq 0,05$ a été retenu.
Résultats: Parmi les 60 isolats de *K. pneumoniae* confirmés, la fréquence la plus élevée a été observée chez les patients âgés de plus de 60 ans (25,0%, n=15) et de 50 à 60 ans (21,7%, n=13), tandis que la fréquence la plus faible a été constatée chez les patients âgés de 1 à 10 ans (5,0%, n=3). Le taux d'isolement était plus élevé chez les femmes (n=35, 58,3%) que chez les hommes (n=25, 41,7%). La fréquence de résistance des isolats au méropénem, à la pipéracilline/tazobactam, à l'amoxicilline/acide clavulanique, à la ciprofloxacine, à la gentamicine et à la ceftriaxone était respectivement de 25,0% (n=15), 31,7% (n=19), 36,7% (n=22), 48,3% (n=29), 48,3% (n=29) et 76,7% (n=46). Seuls 15 (25,0%) des 60 isolats étaient des souches multirésistantes (MDR). L'âge était significativement associé à la présence de souches MDR, avec une fréquence plus élevée dans les groupes d'âge de 51 à 60 ans (38,5%, n=5/13) et de plus de 60 ans (40,0%, n=6/15) comparativement aux autres groupes d'âge (χ^2 =13,733, p=0,035). Le taux de portage du gène bla_{CTX-M} parmi les souches multirésistantes était de 100.0% (n=15), tandis que seulement 26,7% (n=4) portaient le gène bla_{KPC}.
Conclusion: La prévalence relativement élevée des gènes codant pour des β-lactamines à spectre étendu (BLSE) parmi les isolats cliniques de *K. pneumoniae* dans cette étude pourrait compromettre le succès du traitement et de la prise en charge des infections causées par ce pathogène au sein de la population. Un usage raisonné et judicieux des antibiotiques, en particulier des β-lactamines, pour la prise en charge des infections bactériennes permettra de prévenir l'escalade de la résistance aux antibiotiques dans ce contexte épidémiologique.

Mots-clés: Prévalence; *Klebsiella pneumoniae*; Multirésistance; BLSE; bla_{CTX-M}; bla_{KPC}

Introduction:

Klebsiella pneumoniae is a Gram negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium that is associated with both community-acquired and nosocomial infections including pneumonia, urinary tract infections, septicaemia and wound infections (1). The ability of this bacterium to evade, survive and actively suppress many components of the immune system and grow at many sites in the host, makes it even more life threatening, particularly in patients with underlying medical problems such as alcoholism, diabetes and chronic pulmonary diseases. Most often, *K. pneumoniae* infections occur in association with invasive treatment or long-term care in hospitals and other community settings (1).

Although found as a normal flora of the mouth, skin, and intestines, *K. pneumoniae* can cause destructive changes to human and animal lungs if aspirated, specifically to the alveoli resulting in bloody, brownish or yellow colored jelly-like sputum (2), and it is the most significant member of the genus *Klebsiella* of Enterobacteriaceae family.

The increasing prevalence of multi-drug resistant (MDR) *K. pneumoniae* strains in both hospital and community settings pose serious therapeutic challenges that heighten treatment costs, morbidity and mortality globally. Studies have shown that *K. pneumoniae* can develop resistance to antibiotics faster than other bacteria and is often resistant to multiple antibiotics (3). Current evidence implicates plasmids as the primary source of the resistance genes (4). *K. pneumoniae* is resistant to virtually all beta-lactam antibiotics including carbapenems, due to its ability to produce extended-spectrum beta-lactamases (ESβLs) such as Cefotaximase-Munich (CTX-M). This beta-lactamase, which was initially reported in the late 1980s, became recognized as the most common ESβL group in early 2000, replacing TEM and SHV as the dominant ESβL type (5). Since then, CTX-M variants have been reported among several members of the Enterobacterales and in *Pseudomonas aeruginosa* and *Acinetobacter* (6-8).

Similarly, carbapenems which are the preferred agents for the treatment of serious

infections caused by ESβL-producing *K. pneumoniae*, were considered highly stable to *β*-lactamase hydrolysis, and have retained activity against ESβL producers (9). However, carbapenem resistance in *K. pneumoniae*, which is mainly attributed to the production of *K. pneumoniae* carbapenemase (KPC), has emerged causing substantial clinical challenges, further complicating treatment (10). At present, carbapenem-resistant *K. pneumoniae* (CRKP), listed by the WHO as a critical priority pathogen, has become a global threat to human health due to high morbidity and mortality associated with infections caused by this strain (11). The resistant gene in this strain, which belongs to Class A Ambler classification system, has become the more frequently reported carbapenemase gene in the United States of America (USA) and across the world including Nigeria due to international travels and medical tourism (12).

This study was therefore aimed to determine the prevalence of CTX-M and KPC genes among multidrug resistant strains of *K. pneumoniae* obtained from clinical specimens of patients attending selected healthcare facilities in Calabar, Nigeria.

Materials and method:

Study area:

This study was carried out in four health facilities in Calabar metropolis including University of Calabar Teaching Hospital (UCTH), Nigerian Navy Hospital Calabar (NNHC), General Hospital Calabar (GHC) and Arubah Specialist Clinic Calabar (ASCC) within the Calabar metropolis, Cross River State, Nigeria.

Ethical consideration:

Ethical approval for this research work was fully granted by the University of Calabar Teaching Hospital Health Research Ethics Committee (UCTH NHREC NO: NHREC/07/10/2012) and Cross River State Health Research Ethics Committee (REG. NO: CRSMOH/HRP/REC/2023/401).

Collection of isolates:

A total of 80 microbial isolates suspected to be *K. pneumoniae* were obtained from medical laboratories across four healthcare facilities in Calabar. Isolates were initially subcultured on both MacConkey agar and Cysteine-Lactose-Electrolyte-Deficient (CLED) agar media. Vitek® 2 Compact system (BioMérieux; Marcy L'Étoile, France) was used to confirm 60 *K. pneumoniae* isolates.

Antibiotic susceptibility testing (AST):

Antimicrobial susceptibility of confirmed *K. pneumoniae* isolates was performed to six classes of antibiotics including gentamicin (10µg), ciprofloxacin (5µg), meropenem (10 µg), amoxicillin/clavulanate (30µg), piperacillin/tazobactam (30µg) and ceftriaxone (10µg), using the Kirby-Bauer disc diffusion technique. Sterile swabs were used to inoculate Mueller Hinton (MH) agar plates with inoculum of isolates grown in tryptone soy broth, with comparable turbidity with 0.5 McFarland standard. Antibiotic discs were applied onto inoculated MH agar plates and the plates incubated at 37^0C for 24 hours. The zone diameters of growth inhibition were measured with a ruler and results were recorded as sensitive (S) or resistant (R) using the interpretative criteria of the Clinical and Laboratory Standard Institute (13).

Bacterial genomic DNA extraction:

Genomic DNA of each confirmed bacterial isolate was extracted using the boiling method. Five millilitres of an overnight broth culture of the confirmed bacterial isolate in Luria Bertani (LB) were spun at 12000rpm for 3 min. The cells were re-suspended in 500µl of normal saline and heated at 95^0C for 15 min. The heated bacterial suspension was cooled on ice for 10 minutes and spun for 3 min at 12000 rpm, 200µl of the supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20°C for other downstream reactions.

PCR amplification and agarose gel electrophoresis:

The bla_{CTX-M} gene in the extracted DNA from the isolates was amplified using the CTX-M F: 5- CGCTTTGCGATGTGCAG -3' and CTX-M R: 5'-ACCGCGATATCGTTGGT -3' primers (Inqaba, South Africa) in an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles, and annealing temperature of 52^0C. Similarly, bla_{KPC} gene was amplified using the KPC F: 5-GCTCAGGC GCAACTGTAAG-3' and KPC R: 5'-AGCACAGCG GCAGCAAGAAAG-3' primers (Inqaba, South Africa) at annealing temperature of 58^0C. The products were resolved on a 1% agarose gel at 200V for 15 minutes and visualized on blue light imaging system for a 550 bp product size.

Data analysis:

Data were presented as frequency and percentages. Pearson's Chi square test was used to measure association between categorical variables, while *p*-value less than or equal to 0.05 ($p \leq 0.05$) was considered statistically significant.

Results:

Of the 60 *K. pneumoniae* isolates confirmed by VITEK® 2 COMPACT system, 38 (63.3%) were hospital-acquired strain while 22 (36.7%) were community-acquired strain.

Fifteen (25.0%) of the 60 *K. pneumoniae* isolates were MDR (resistant to at least 3 different class of antibiotics tested), 11 (28.9%) were hospital-acquired strain while 4 (18.2%) were community-acquired strain (OR=1.833, 95% CI=0.5043-6.665, p=0.5373) (Table 2). Age was significantly associated with MDR *K. pneumoniae* strains, with significantly higher frequency of isolation in age groups 51-60 years (38.5%, n=5/13) and >60 years (40%, n=6/15) compared to other age groups (x^2= 13.733, p=0.035). However, there was no significant association with respect to gender (x^2=1.667, p=0.197) (Table 1).

Antimicrobial susceptibility testing of the isolates against selected antibiotics showed that hospital-acquired *K. pneumoniae* strains (n=38) exhibited low resistance to meropenem (n=9, 23.7%), followed by piperacillin/tazobactam (n=12, 31.6%), amoxicillin/clavulanate (n=13, 34.2%), ciprofloxacin (n= 17, 44.7%), and gentamicin (n=17, 44.7%), but high resistance to ceftriaxone (n=30, 78.9 %). Similarly, community-acquired *K. pneumoniae* strains (n=22) exhibited low resistance to meropenem (n=6, 18.2%), piperacillin/tazobactam (n=7, 31.8%), amoxicillin/clavulanate (n=9, 40.9%), ciprofloxacin (n=12, 54.5%), and gentamicin (n=12, 54.5%) while the strains showed high resistance to ceftriaxone (n=16, 72.7%) (Table 3).

Overall, meropenem showed high antimicrobial activity against *K. pneumoniae* strains with low resistant rate of 25.0% (n=15), followed by piperacillin/tazobactam (n=19, 31.7%), amoxicillin/clavulanate (n=22, 36.7 %), ciprofloxacin and gentamicin (n=29, 48.3 %), while the isolates showed high resistance to ceftriaxone (n=46, 76.7%) (Table 3). A high frequency of the isolates (n=15, 25.0%) were resistant to 4 antibiotics with multiple antibiotic resistance index (MARI) of 0.7, followed by MARI of 0.5 (n=13, 21.7%), MARI of 0.8 (20.0%), MARI of 0.3 (16.7%), MARI of 1.0 (8.3%) and MARI of 0.2 (8.3%) (Table 4), while the AST of the MDR-*K. pneumoniae* strains identified R3 (resistance to 3 antibiotics) as the most frequent profile (n=9, 60%) followed by R4 (n=3, 20.0%) and R5 (n=3, 20.0%) (Table 5).

Agarose gel electrophoresis of $bla_{\text{CTX-M}}$ and bla_{KPC} genes (Plates 1 and 2) showed that all the 15 MDR *K. pneumoniae* strains carried $bla_{\text{CTX-M}}$ gene (25.0% carriage rate prevalence in the study) while only 4 isolates carried bla_{KPC} gene (6.7% carriage rate in the study).

Table 1: Frequency distribution of multidrug-resistant *Klebsiella pneumoniae* by age and gender of patients from whom they were isolated

Variables	No of isolates (%)	No of MDR isolates (%)	x^2	p value
Age group (years)				
1-10	3 (5.0)	0	13.733	0.035*
11-20	4 (6.7)	1 (25.0)		
21-30	7 (11.7)	0		
31-40	8 (13.3)	1 (12.5)		
41-50	10 (16.7)	2 (20.0)		
51-60	13 (21.7)	5 (38.5)		
>60	15 (25.0)	6 (40.0)		
Sex				
Male	25 (41.7)	9 (36.0)	1.667	0.197
Female	35 (58.3)	6 (17.1)		
Total	**60 (100.0)**	**15 (25.0)**		

*=statistically significant at p<0.05; MDR=Multidrug resistant

Table 2: Distribution of *Klebsiella pneumoniae* and multidrug-resistant strains by setting

Strain	Number of isolates	Number of MDR isolates	x^2	OR (95% CI)	p value
HA-KP	38 (63.3)	11 (28.9)	0.3828	1.833 (0.5043-6.665)	0.5373
CA-HP	22 (36.7)	4 (18.2)			
Total	**60 (100.0)**	**15 (25.0)**			

HA-Hospital acquired; CA-Community acquired; MDR-Multi-drug resistant; x^2-Chi square; OR-Odd ratio; CI-Confidence interval

Table 3: Antibiotic susceptibility testing of *Klebsiella pneumoniae* isolates to routinely used antibiotics drugs according to setting

Isolates	Status	TZP	CIP	CRO	CN	AMC	MEM
HA-KP (n=38)	S	26 (68.4)	21 (55.3)	8 (21.1)	21 (55.3)	25 (65.8)	29 (76.3)
	R	12 (31.6)	17 (44.7)	30 (78.9)	17 (44.7)	13 (34.2)	9 (23.7)
CA-KP (n=22)	S	15 (68.2)	12 (54.5)	6 (27.3)	12 (54.5)	13 (59.1)	16 (72.7)
	R	7 (31.8)	10 (45.5)	16 (72.7)	10 (45.5)	9 (40.9)	6 (18.3)
Total (n=60)	S	41 (68.3)	31 (51.7)	14 (23.3)	31 (51.7)	38 (63.3)	45 (75.0)
	R	19 (31.7)	29 (48.3)	46 (76.7)	29 (48.3)	22 (36.7)	15 (25.0)

HA-KP: Hospital-acquired *Klebsiella pneumoniae*; CA-KP: Community-acquired *Klebsiella pneumoniae*; TZP: Piperacillin-tazobactam; CIP: Ciprofloxacin; CRO: Ceftriaxone; CN: Gentamicin; AMC: Amoxicillin-clavulanic acid; MEM: Meropenem

Table 4: Multiple antibiotic resistance index of *Klebsiella pneumoniae* isolates

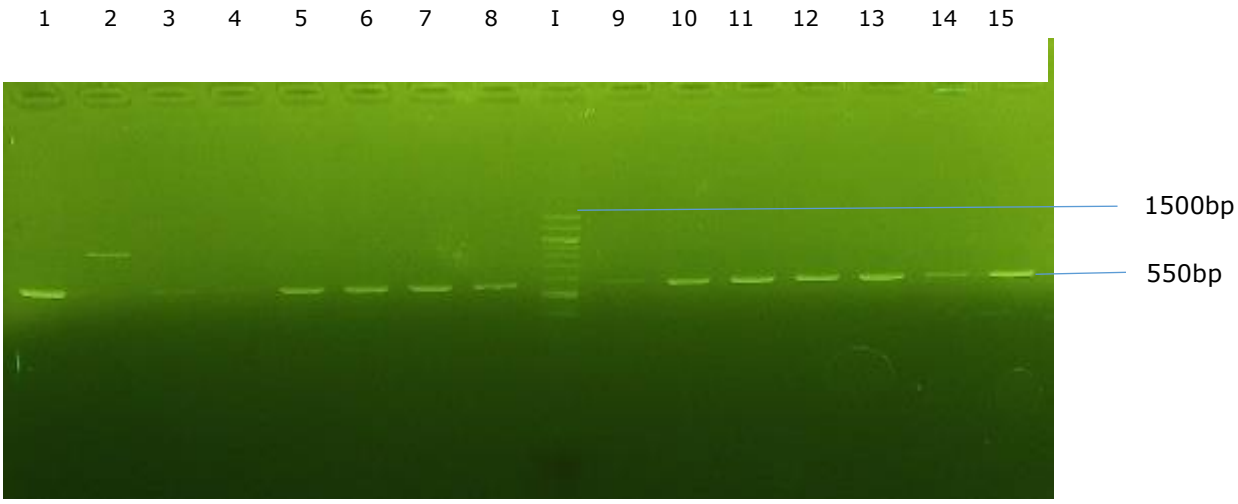
No of antibiotics	No of isolates (%)	HA-KP (%)	CA-KP (%)	MARI
1	5 (8.3)	3 (5.0)	2 (3.3)	0.2
2	10 (16.7)	6 (10.0)	3 (6.7)	0.3
3	13 (21.7)	8 (13.3)	5 (8.3)	0.5
4	15 (25.0)	9 (15.0)	6 (10.0)	0.7
5	12 (20.0)	7 (11.5)	5 (8.3)	0.8
6	5 (8.3)	2 (3.3)	3 (5.0)	1.0

HA-KP: Hospital-acquired *Klebsiella pneumoniae*; CA-KP: Community-acquired *Klebsiella pneumoniae*

Table 5: Resistance pattern of MDR *Klebsiella pneumoniae* strains to the classes of antibiotics tested

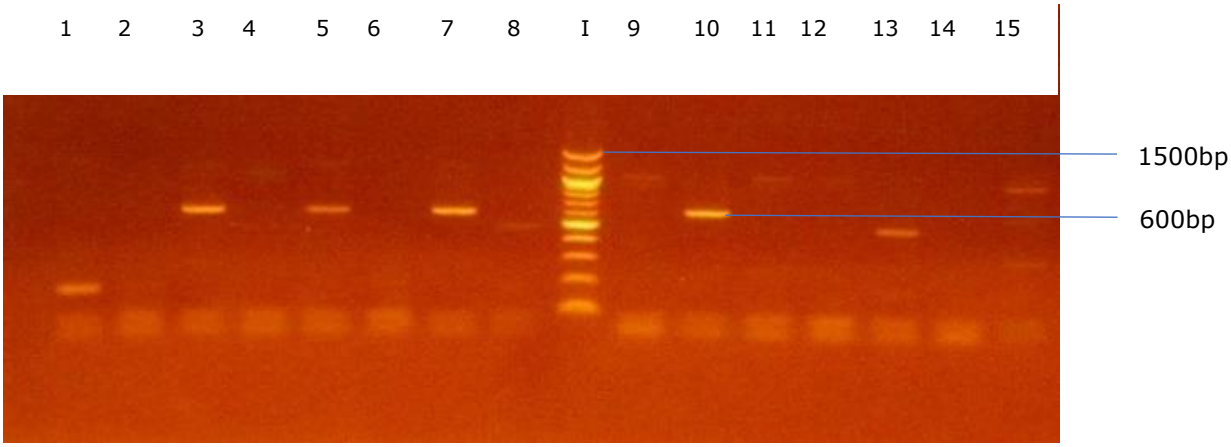
Class of antibiotics	HA-KP (%)	CA-KP (%)	Total	Prevalence (%)
R5	2 (13.3)	1 (6.7)	3	20.0
R4	1 (6.7)	2 (13.3)	3	20.0
R3	5 (33.3)	4 (26.7)	9	60.0
Total	**8 (53.3)**	**7 (46.7)**	**15**	

HA-KP: Hospital-acquired *Klebsiella pneumoniae*; CA-KP: Community-acquired *Klebsiella pneumoniae*; R=Resistance



Lanes 1-15 represent CTX-M gene bands (550bp); Lane I represents 100bp molecular ladder of 1500bp

Plate 1: Agarose gel electrophoresis of CTX-M gene of multidrug-resistant *Klebsiella pneumoniae* strains



Lane 3, 5, 7 and 10 represent KPC gene band (600bp); Lane I represents 100bp molecular ladder of 1500bp

Plate 2: Agarose gel electrophoresis of KPC gene of some multidrug-resistant *Klebsiella pneumoniae* strains

Table 6: Prevalence of ESβL genes among multidrug-resistant *Klebsiella pneumoniae* strains

Genes	No of MDR strain (%)	Prevalence (%)
bla_{CTX-M}	15 (100.0)	25.0
bla_{KPC}	4 (26.7)	6.7
bla_{CTX-M} & bla_{KPC}	4 (26.7)	6.7

MDR=Multidrug resistant

Discussion:

The frequency distribution of the *K. pneumoniae* isolates by age showed that patients in the age group >60 years had the highest number of *K. pneumoniae* recovered (25.0%, n=15), compared to patients in the age group 1-10 years (5.0%, n=3) in the study. This agrees with the study of Wang et al., (14) who reported prevalence of 53.6% among the elderly compared to 4.8% among minors in their region of study. The significant difference in frequency of *K. pneumoniae* recovery from focus of infection by age suggest a growing health need of the elderly population due to underlying ailments and changes in immune responses to invading pathogens. The frequency of *K. pneumoniae* isolation/infection was higher among patients on hospital admission (n=35, 58.3%) compared to those acquired from community (n=25, 41.7%). Although there was no significant difference in the frequency of *K. pneumoniae* isolation/infection between hospitalized and non-hospitalized patients (p=0.197), there are sufficient evidence that linked *K. pneumoniae* infection to hospital environments (15-17), however, the study revealed the extent to which the infection cause by this pathogen is fast spreading in community setting within the study population.

Antibiotic sensitivity testing results showed highest sensitivity of the isolates to meropenem (n=45, 75.0%), with 25.0% resistance rate. Resistance of these *K. pneumoniae* isolates to the carbapenems was lower compared to the finding from Ebonyi State, where Ejikeugwu et al., (18) reported 43.0% meropenem-resistant *K. pneumoniae* isolates (18). Similarly, high antimicrobial activity was observed with piperacillin/tazobactam against the bacterium. Previous research by Muhammed et al., (19) and Rodrıguez-Bano et al., (20) corroborates our finding. The use of piperacillin/tazobactam combination therapy as a choice drug for treatment of MDR *K. pneumoniae* could spare the carbapenems as the last line of drug against MDR-Enterobacteriaceae. Ceftriaxone on the other hand, showed the least antimicrobial activity against the isolates suggesting overuse/misuse of this antibiotic due to over-the-counter access of the drug in the population. There was however, no significant difference in the antimicrobial susceptibility pattern of the *K. pneumoniae* isolates with respect to hospital or community setting, underscoring the antibiotic 'selection pressure' present in both settings.

Fifteen (25.0%) of *K. pneumoniae* isolates were multidrug resistant. Majority of the MDR isolates were recovered among hospitalized individuals, however, the MDR rate was relatively low compared to the finding of Akinyemi et al., (21) in Lagos, Nigeria who reported 62.8% prevalence, but agrees with the finding of Tesfa et al., (22) who put the global prevalence of multidrug-resistant *K. pneumoniae* colonization in the range of 0.13% to 22%.Infections caused by MDR *K. pneumoniae* is associated with high mortality, often linked to inadequate or inappropriate antibiotic treatment.

The bla_{CTX-M} gene was detected in all the MDR *K. pneumoniae* strains, however, only 4 isolates carried the bla_{KPC} gene and were detected in the isolates recovered from hospitalized patients above 50 years of age in UCTH

and NNHC. Four of the strains co-habored *bla*$_{CTX-M}$ and *bla*$_{KPC}$ genes while the other 11 MDR strains harbored only the *bla*$_{CTX-M}$ gene. Although, the predominance of CTX-M gene in the population is unclear, however, it is thought that this resistant gene in the population, may have a link to frequent international travels of patients to high-prone areas for advance medical services. The report by Asir et al., (23) in India, corroborates the 100% distribution of the CTX-M gene in the population. Furthermore, in other part of Nigeria, low prevalence of the ESβL gene was reported (24) but in others such as Port Harcourt, Sokoto State, and in Southwestern Nigeria the prevalence of *bla*$_{CTX-M}$ gene is put at 41.67%, 35.71% and 32% respectively (25-27).

Additionally, 4 *K. pneumoniae* isolates recovered from sputum and urine samples of adult males and females habored *bla*$_{KPC}$ gene, and medical records showed that one of patients had visited the USA for medical treatment while the other three individuals received medical care in Nigeria, suggesting that this rare type of carbapenemase in Calabar, may also have a link to international travels for medical treatments. The study by Kabiru et al., (28) in four medical centres in Lagos Nigeria, identified 7.0% of carbapenemase-producing *K. pneumoniae* strains from similar sample types, while Oshun and Ogunsola (29) reported only 2.6% carbapenemase producing *K. pneumoniae* in one of the teaching hospitals in Lagos (29). In other parts of Africa, specifically in Sudanese and Egyptian University Hospitals, low prevalence of *bla*$_{KPC}$ gene was also detected in the study population (30-31).

Conclusion:

The MDR prevalence of 25.0% was seen among *K. pneumoniae* isolates with high resistance to the third-generation cephalosporin antibiotic tested in this study. The commonest ESβLs-encoding gene detected among the MDR strains was *bla*$_{CTX-M}$ gene. Generally, an informed and appropriate use of antimicrobial drugs, particularly the beta-lactam antibiotics, for management of bacterial infections, will prevent further escalation of drug-resistant *K. pneumoniae* strains in this epidemiological setting.

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Contributions of authors:

IIU and NOU were involved in the conceptualization, design and writing of the manuscript; IIU and SOI were involved in data collection and analysis of results; Ethical approval of the study was facilitated by BCO and VUA. NOU supervised the research work. AAU, FBU and JIU edited the manuscript. All authors read and approved the manuscript for publication.

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References:

1. Dao, T. T., Liebenthal, D., Tran, T. K., et al. *Klebsiella pneumoniae* oropharyngeal carriage in rural and urban Vietnam and the effect of alcohol consumption. PLoS One. 2014; 9 (3): E91999. https://doi.org/10.1371/journal.pone.0091999
2. Ryan, K. J., and Ray, C. G. (eds.) Sherris Medical Microbiology (4th ed.). McGraw Hill. 2004: 370.
3. Munita, J. M., and Arias, C. A. Mechanism of antibiotic resistance. Microbiol. Spectr. 2016; 4: 2. https://doi.org/10.1128/microbiolspec.AM-00 16-2015
4. Hudson, C., Bent, Z., Meagher, R., and Williams, K. "Resistance Determinants and Mobile Genetic Elements of an NDM-1-Encoding *Klebsiella pneumoniae* Strain". PLoS One. 2014; 9 (6): E99209. https://doi.org/10.1371/journal.pone.0099260
5. Bradford, P. A. Extended-spectrum-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001; 14: 933-951. https://doi.org/10.1128/CMR.14.4.933-951.2001
6. Celenza, G., Pellegrini, C., and Caccamo, M. Spread of bla(CTX-M-type) and bla(PER-2) b-lactamase genes in clinical isolates from Bolivian hospitals. J Antimicrob Chemother. 2006; 57: 975–978. https://doi.org/10.1093/jac/dkI265
7. Picao, R. C., Poirel, L., and Gales, A. C. Further identification of CTX-M-2 extended-spectrum b-lactamase in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2009; 53: 2225–2226. https://doi.org/10.1128/AAC.00436-09
8. Walther-Rasmussen, J., and Høiby, N. Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum β-lactamases. Can J Microbiol 2004; 50: 137–165. https://doi.org/10.1139/w04-076
9. Colodner, R., Raz, R., and Chazan, B. Susceptibility pattern of ESβL-producing bacteria isolated from inpatients to five antimicrobial drugs in a community hospital in northern Israel. Int J Antimicrob Agents. 2004; 24: 409–410. https://doi.org/10.1016/j.ijantimicag.2004.08.005
10. Nordmann, P., Cuzon, G., and Naas, T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis. 2009; 9: 228–236. https://doi.org/10.1016/S1473-3099(09)70104-3
11. World Health Organization. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics.

http://remed.org/wp-content/uploads/2017/03/lobal-priority-list-of-antibiotic-resistant-2017.pdf (Accessed on 20 February 2021).
12. Asuquo, A. E., Ulom, U. I., Ibeneme, E. O., and Utsalo, S. J. Detection of Plasmid-Borne NDM-1 Gene in Clinical Isolates of Enterobacteriaceae and Their Carbapenem Antibiogram in Cross River State, Nigeria, BJMLS. 2022; 7 (1): 118-127.
13. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. (31st ed) CLSI Suppl. M100. 2021.
14. Wang, N., Zhan, M., Liu, J., et al. W. Prevalence of Carbapenem-Resistant *Klebsiella pneumoniae* Infection in a Northern Province in China: Clinical Characteristics, Drug Resistance, and Geographic Distribution. Infect Drug Resist. 2022; 15: 569–579. https://doi.org/10.2147/IDR.S347343
15. Magill, S. S., Edwards. J. R., Bamberg, W., et al. Multistate point-prevalence survey of health care-associated infections. N Engl J Med. 2014; 370:1198–1208. https://doi.org/10.1056/NEJMoa1306801
16. Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A. "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis". Lancet. 2022; 399 (10325):629–655. https://doi.org/10.1016/S0140-6736(21)02724-0
17. Wen-Chien, K. O., David, L., Paterson, A. J., et al. Community-Acquired *Klebsiella pneumoniae* Bacteremia: Global Differences in Clinical Patterns. Emerg Infect Dis. 2002; 8(2), 160-166. https://doi.org/10.3201/eid0802.010025
18. Ejikeugwu, C., Nworie, O., Saki, M., Al-Dahmoshi, H. O. M., Al-Khafaji, N. S. K., and Ezeador, C. Metallo-β-lactamase and Amp C genes in *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. BMC Microbiol. 2021; 21: 124. https://doi.org/10.1186/s12866-021-02208-0
19. Muhammed, M., Flokas, M. E., and Detsis, M. Comparison between carbapenems and β-lactam/β-lactamase inhibitors in the treatment for bloodstream infections caused by extended-spectrum β-lactamase-producing Enterobacteria-ceae: a systematic review and meta-analysis. Open Forum Infect Dis. 2017; 4: 99. https://doi.org/10.1093/ofid/ofw220
20. Rodriguez-Bano, J., Gutierrez-Gutie´rrez, B., and Machuca, I. Treatment of infections caused by extended-spectrum-b-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clin Microbiol Rev. 2018; 31: 79–17. https://doi.org/10.1128/CMR.00079-17
21. Akinyemi, K.O., Abegunrin, R.O., Iwalokun, B. A., et al. The Emergence of *Klebsiella pneumoniae* with Reduced Susceptibility against Third Generation Cephalosporins and Carbapenems in Lagos Hospitals, Nigeria. Antibiotics. 2021; 10: 142. https://doi.org/10.3390/antibiotics10080962
22. Tesfa T., Mitiku H., Edae M., and Assefa, N. Prevalence and incidence of carbapenem-resistant *K. pneumoniae* colonization: systematic review and meta-analysis. Syst Rev. 2022; 11(1). https://doi.org/10.1186/s13643-022-02110-3
23. Asir, J., Nair, S., Devi, S., Prashanth, K., Saranathan, R., and Kanungo, R. Simultaneous gut colonisation and infection by ESBL producing *Escherichia coli* in hospitalised patients. AMJ. 2015; 8: 200–207. https://doi.org/10.21767/AMJ.2015.2351
24. Odewale, G., Jibola-Shittu, M. Y., Ojurongbe, O., Olowe, R. A., and Olowe, O. A. Genotypic Determination of Extended Spectrum β-Lactamases and Carbapenemase Production in Clinical Isolates of *Klebsiella pneumoniae* in Southwest Nigeria. Infect Dis Rep. 2023; 15: 339–353. https://doi.org/10.3390/idr15030034
25. Akinbami, O. R., Olofinsae, S., and Ayeni, F. A. Prevalence of extended spectrum beta lactamase and plasmid mediated quinolone resistant genes in strains of *Klebsiella pneumoniae, Morganella morganii, Leclercia adecarboxylata* and *Citrobacter freundii* isolated from poultry in South Western Nigeria. Peer J. 2018; 6: E5053. https://doi.org/10.7717/peerj.5053
26. Okogeri, E. I., Amala, S. E., Nwokah, E. G., and Monsi, T. P. Cross-sectional study of extended spectrum β-lactamase genes (SHV, TEM, CTX-M and OXA) in *Klebsiella* species from clinical specimens in Port Harcourt, Nigeria. Am J Med Sci. 2020; 8: 180–186. https://doi.org/10.5923/j.ajmms.20201006.08
27. Olowo-okere, A., Ibrahim, Y. K. E., Olayinka, B. O., et al. Phenotypic and genotypic characteriza-tion of clinical carbapenem-resistant Enterobact-eriaceae isolates from Sokoto, Northwest Nigeria. New Microbes New Infect. 2020; 37: 100727. https://doi.org/10.1016/j.nmni.2020.100696
28. Kabiru, O. A., Rebecca, O. A., Bamidele, A. I., Christopher, O. F., and Oliwia, M. The Emergence of *Klebsiella pneumoniae* with Reduced Susceptibility against Third Generation Cephalosporins and Carbapenems in Lagos Hospitals, Nigeria. Antibiotics. 2021; 10(2): 142. https://doi.org/10.3390/antibiotics10101213
29. Oshun, P. O., and Ogunsola, F. T. Prevalence of Carbapenem resistant *Klebsiella pneumoniae* infections in a Nigerian Teaching Hospitals. J Hosp Med. 2018; 28: 2. https://doi.org/10.4172/2167-1079.1000392
30. Albasha, A. M., Osman, E. H., Abd-Alhalim, S., Alshaib, E. F., Al-Hassan, L., and Altay, H. N. Detection of Several Carbapenems Resistant and Virulence Genes in Classical and Hyper-Virulent Strains of *Klebsiella pneumoniae* Isolated from Hospitalized Neonates and Adults in Khartoum. BMC Res Notes. 2020; 13: 312. https://doi.org/10.1186/s13104-020-05319-w
31. Taha, M. S., Hagras, M. M., Shalaby, M. M., et al. Genotypic Characterization of Carbapenem-Resistant *Klebsiella pneumoniae* Isolated from an Egyptian University Hospital. Pathogens. 2023; 12: 121. https://doi.org/10.3390/pathogens12101045