Afr. J. Med. Med. Sci. (2020) **49,**

Plasmid profiles of extended spectrum beta lactamase (ESBL) producing multidrug resistant *Klebsiella* species from different clinical sources

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Abstract
Background: Resistance of *Klebsiella* species to conventional antibiotics is often implicated in increasing nosocomial infections, and is due in part to enzymatic hydrolysis either constitutively and/or inductively. Resistance plasmid factors readily spread mostly through Gram-negative bacterial isolates through conjugative plasmids.
This study investigated the presence of extended spectrum beta lactamases (ESBL), profiles of plasmids detected, and resistance to conventional antimicrobial agents among clinical isolates of *Klebsiella* species from three different sources.
*Method***:** Seventy Gram-negative bacterial and lactose fermenters from urine, wounds and sputum specimens from three hospitals in the South West region of Nigeria were studied after identification with microbial identification system. Antibiogram was determined using modified Kirby-Bauer disc diffusion method. Phenotypic detection of ESBL-production was carried out using double-disk synergy tests (DDST). Plasmid DNA were extracted by alkaline lysis method, electrophoresed, viewed by a UV-trans-illuminator, with plasmid size and number determined, following standard protocols
*Results***:** Twenty-nine (29) or 41% of the seventy clinical isolates were confirmed as Klebsiella species distributed as: *Klebsiella pneumoniae* 89.66% (26/29); *Klebsiella oxytoca* 6.89% (2/29) and *Klebsiella ozanae* 3.45% (1/29). Among the *K. pneumoniae* isolates, 13 (50%) were from urine, 8 (30.77%) from wounds and 5 (19%) from sputum. Multidrug resistance was observed with the isolates; as 28 (96.5%) were resistance to at least four (4) different classes of antibiotics. Among the 29 isolates, 14 (48.3%) *Klebsiella* species were ESBL-producers while 15 (51.7%) were non-ESBL producers. The ESBL-producers showed higher antibiotic resistance compared to non-ESBL producers, particularly with respect to β-lactam antibiotics. Plasmid DNA, with sizes range of O.78 - 23 kbp were detected in 17 (58.62%) of the isolates.

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Conclusion: Multidrug resistance (MDR) phenomenon was observed with *Klebsiella* species particularly among the ESBL-producers harbouring high-molecular weight plasmids. There is need for routine ESBL-production surveillance and the rational choice of antibiotics for infection management, reduction and containment of spread of antibiotic resistance in clinical settings.

Keywords: *Klebsiella species, ESBL-producers, plasmids, antibiotic resistance*

Abstrait
Contexte: La résistance des espèces *Klebsiella* aux antibiotiques conventionnels est souvent impliquée dans l'augmentation des infections nosocomiales, et est due en partie à l'hydrolyse enzymatique soit de manière constitutive et / ou inductive. Les facteurs de résistance plasmidiques se propagent facilement principalement à travers des isolats bactériens Gram-négatifs par des plasmides conjugatifs.
Cette étude a examiné la présence de bêta-lactamases à spectre étendu (BLSE), les profils de plasmides détectés et la résistance aux agents antimicrobiens conventionnels parmi les isolats cliniques des espèces *Klebsiella* provenant de trois sources différentes.
Méthode: Soixante-dix fermenteurs de bactéries Gram-négatives et de lactose provenant d'échantillons d'urine, de plaies et d'expectorations provenant de trois hôpitaux de la région du sud-ouest du Nigéria ont été étudiés après identification avec un système d'identification microbienne. L'antibiogramme a été déterminé en utilisant la méthode de diffusion du disque de Kirby-Bauer modifiée. La détection phénotypique de la production de BLSE a été réalisée à l'aide de tests de synergie à double disque (TSDD). L'ADN plasmidique a été extrait par la méthode de lyse alcaline, soumis à l'électrophorèse, vu par un trans-illuminateur-UV, avec la taille et le nombre de plasmides déterminés, en suivant les protocoles standard
Résultats: Vingt-neuf (29) ou 41% des soixante-dix isolats cliniques ont été confirmés comme étant des espèces *Klebsiella* réparties comme: *Klebsiella*

pneumoniae 89,66% (26/29); *Klebsiella oxytoca* 6,89% (2/29) et *Klebsiella ozanae* 3,45% (1/29). Parmi les isolats de *K. pneumoniae*, 13 (50%) provenaient d'urine, 8 (30,77%) de plaies et 5 (19%) d'expectorations. Une résistance aux médicaments multiple a été observée avec les isolats; parce que 28 (96,5%) étaient résistants à au moins quatre (4) classes différentes d'antibiotiques. Parmi les 29 isolats, 14 (48,3%) espèces *Klebsiella* étaient des producteurs-BLSE tandis que 15 (51,7%) étaient des non producteurs-BLSE. Les producteurs-BLSE ont montré une résistance aux antibiotiques plus élevée que les non producteurs-BLSE, en particulier en ce qui concerne les antibiotiques â-lactame. L'ADN plasmidique, avec des tailles allant de 0,78 à 23 kbp, a été détecté dans 17 (58,62%) des isolats.

Conclusion: Un phénomène de résistance aux médicaments multiple (MDR) a été observé avec les espèces *Klebsiella*, en particulier parmi les producteurs-BLSE hébergeant des plasmides de haut poids moléculaire. Il est nécessaire de surveiller systématiquement la production de BLSE et de choisir rationnellement les antibiotiques pour la gestion des infections, la réduction et l'endiguement de la propagation de la résistance aux antibiotiques en milieu clinique.

Mots clés: Espèces *Klebsiella*, producteurs-BLSE, plasmides, résistance aux antibiotiques

Introduction

Klebsiella bacterial species are widely recognized as important opportunistic pathogens in hospital patients, representing 3–8% of all nosocomial bacterial infections and ranking second behind *Escherichia coli* as a cause of nosocomial Gram-negative infections [1, 2]. Infections with *Klebsiella* are caused mainly by *K. pneumoniae* and *K. oxytoca* in a proportion estimated at 2 to 1 [3], and to a much lesser degree, *K. oxytoca* has been isolated from human clinical specimens. *Klebsiella pneumoniae* accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemia and soft tissue infections [4-6]. This is because *K. pneumoniae* has the ability to spread quickly from the gastrointestinal tract of the patients and further, by hands of health care personnel to colonize other patients, and thereby lead to nosocomial clonal outbreaks [2]. Hospital outbreaks of multidrug-resistant (MDR) *Klebsiella* spp., especially those in neonatal wards, are often caused by new strains, the so-called extended-spectrum-â-lactamase (ESBL) producers.

ESBLs mediated resistance to extended spectrum â-lactams, including third generation cephalosporins and monobactams such as aztreonam are common [7]. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and subsequently, the development of multidrug-resistant strains that produce ESBLs [8]. ESBLs have been reported worldwide in many different genera of Enterobacteriaceae and *Pseudomonas aeruginosa* [9]. Outbreak of *Klebsiella pneumonia*e carrying ESBLs genes has been documented [10, 11]. The prevalence of ESBLs varies among different geographical location and from country to country [12].

The genetic expression of antibiotic resistance may be plasmid or chromosomally-mediated. Plasmid - mediated ESBLs is often accompanied with resistance that is restructured to other classes of antibiotics [13, 14). These transferable plasmids enabled *K. pneumoniae* to rapidly acquire antibiotic resistance [15, 16]. There are reports of infections with ESBL-producing Enterobacteria isolates with evidence of multi-resistant plasmids, particularly among *E. coli* and *Klebsiella* species in Nigeria [17, 18]. This study investigated ESBL-production, plasmid content and their possible role in antibiotic resistance of clinical isolates of *Klebsiella* species and highlighted the public health implications.

Materials and methods

Sample collection and identification

A total of seventy (70) isolates were collected for this study and were distributed as follows. Fifty-five isolates of lactose fermenting bacteria from urine, wounds and sputum were collected from the Microbiology Departments of two hospitals in South West Nigeria: 35 isolates from the University College Hospital (UCH) Ibadan and 20 isolates from Obafemi Awolowo University Teaching Hospital (OAUTH) Ife while 15 isolates were collected from the University of Ilorin Teaching Hospital (UITH) Ilorin over a period of seven months (January – July 2014). *Klebsiella pneumoniae* ATCC 13883 used as a reference organism was obtained from the Department of Pharmaceutical microbiology, University of Ibadan, Nigeria. The isolates were identified and confirmed as *Klebsiella* spp. with the use of the Microbact® Gram-negative identification kit (Oxoid, UK).

Antibiotic susceptibility testing

Antibiotic susceptibility test was carried out by the modified Kirby-Bauer technique [19]. Antibiotic disc used were ciprofloxacin (5µg), nitrofurantoin (300µg), ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), cefotaxime (30µg), augmentin® (30µg), ofloxacin (5µg), imipenem (10µg) and ceftriaxone (30µg). The diameters of zone of inhibition were measured and interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI guidelines [20].

Determination of ESBL production

Isolates were subjected to initial screen test according to CLSI guidelines using ceftazidime (30µg) and ceftriaxone (30µg) disc diffusion; isolates showing d" 22mm with ceftazidime (30µg), d" 25mm with cefotaxime (30µg) and d" 27mm with aztreonam and d" 22mm with cefpodoxime (10µg) were identified as potential ESBL producers. Phenotypic detection of ESBL was done using the double disk synergy test (DDST). Mueller Hinton agar plates were inoculated with strains of *Klebsiella* spp. recovered from wound, urine and sputum specimens. Amoxicillin-clavulanate disc (20µg/10µg) was then placed at the centre of each of the inoculated plates. Discs containing ceftazidime (30µg), cefotaxime (30µg) and cefepime (30µg) (Oxoid, UK) respectively were placed 20mm (centre to centre of the discs) from the amoxicillin-clavulanate disc. The plates were incubated aerobically at 37°C overnight. After overnight incubation, a clear extension of the edges of the zones of inhibition of any of the cephalosporin antibiotics towards the disc containing clavulanic acid is described as synergy indicating the presence of an ESBL [21].

Plasmid profiling

Plasmid DNA was extracted from the fourteen (14) multidrug resistant isolates using alkaline lysis method [22]. Extracted plasmid DNA was electrophoresed at 60-100v on 0.8% agarose gel and stained with 1mg/ml of ethidium bromide. The gel was photographed with a Polaroid camera under the view of a UV trans-illuminator. DNA molecular weights and distance migrated were determined according to Kim *et al*. [23].

Determination of minimum inhibitory concentration (MIC)

Agar dilution method according to CLSI [20] was used for the determination of the MIC of ceftriaxone and ciprofloxacin on clinical isolates (n=11) of ESBL-producing *Klebsiella pneumoniae* and the reference strain. Various concentrations of the antibiotics ranged 128-0.03µg/ml were prepared in a decreasing order, and 2mls of the varying concentrations were seeded into 18mls of Mueller Hinton agar and allowed to set. Organisms from overnight broth culture with turbidity equivalent to the 0.5 McFarland standard (1.0 x 10^8 cfu/ml) was then streaked on each plate. All plates were incubated at 37^0C for 24 hours. The MIC was taken as the minimum concentration of the antimicrobial drug where no visible growth was detected.

Statistical analysis

Pearson's chi-square and correlation coefficient was analysed using MATLAB and R-Package software. Statistical determination of ESBL production and plasmid presence in relation to clinical sources, as well as determination of correlation between percentage resistance and plasmid positive isolates were done.

Table 1. Antibiotic resistance profile of ESBL producers and non-ESBL producers

Antibiotics	ESBL positive (n = 14)			ESBL negative (n=15)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
CRX	100	0.00	0.00	86.6	6.7	6.7
CAZ	85.7	0.00	14.2	66.7	0.00	33.3
CPR	64.3	7.1	28.6	100	0.00	0.00
NIT	85.7	7.1	7.1	60	0.00	40.0
AUG	100	0.00	0.00	93.3	0.00	6.7
OFL	64.3	21.4	14.3	73.3	6.7	20.0
CXM	92.9	0.00	7.1	66.6	6.7	26.7
GEN	42.9	7.1	50.0	73.3	0.00	26.7
CTR	57.1	21.4	21.4	46.7	53.3	0.00
IPM	21.4	0.00	78.6	6.7	20.0	73.3

Key: CRX= Cefuroxime, CAZ = Ceftazidme, CPR = Ciprofloxacin NIT=Nitrofurantoin, AUG = Augmentin, OFL = Ofloxacin, CXM = Cefotaxime GEN = Gentamicin, CTR = Ceftriaxone, IPM = Imipenem.

Results

A total of 29 (41.40%) of the 70 isolates were confirmed to be *Klebsiella* spp., out of which were *Klebsiella pneumoniae* 89.66% (26/29), *Klebsiella oxytoca* 6.89% (2/29) and *Klebsiella ozanae* 3.45% (1/29). Among *the K. pneumoniae* isolates, 13 (50%) were from urine, 8 (30.8%) from wounds and 5 (19.2%) from sputum (Tables 2, 3 and 4).

Antibiotic susceptibility showed that 28 (96.6%) of *Klebsiella* isolates were resistant to Augmentin®, followed by cefuroxime 26 (89.6%), cefotaxime 23 (79.3%), ceftazidime 22 (75.8%), ciprofloxacin 21 (72.4%), nitrofurantoin 21 (72.4%), ofloxacin 20 (68.9%), gentamicin 17 (58.6), ceftriaxone 15 (51.7%), and imipenem 4 (13.7%). The multiple

Table 2. Plasmid profile of sputum isolates (n=5) in relation to ESBL production

Isolates	ESBL	No of Plasmid	Plasmid size (Kbp)
S03 Kpn	+	1	23
S04 Kpn	-	-	
S05 Kpn	-	1	1.2
S06 Kpn	+	2	22, 1.2
S07 Kpn	+	-	-

Key: Isolates from sputum (S03-S07); Kpn= Klebsiella pneumoniae; + = present; - = absent

Table 3. Plasmid profile of wound isolates(n=9) in relation to ESBL production

Isolates	ESBL	No of Plasmid	Plasmid size (Kbp)
W02 Kpn	-	-	-
W03 Kpn	+	2	19.8, 1.1
W04 Kpn	-	-	-
W05 Kpn	+	2	19.8, 0.78
W06 Kpn	+	-	-
W08 Kpn	+	1	23
W14 Kox	-	-	-
W16 Kpn	-	1	23
W18 Kpn	+	1	23

Key: *Isolates from wound (W02 - W06, W08, W14, W16, W18); Kpn= Klebsiella pneumoniae; Kox= Klebsiella oxytoca; + = present; - = absent*

Table 4. Plasmid profile of urine isolates (n=15) in relation to ESBL production

Isolates	ESBL	No of Plasmid	Plasmid size (Kbp)
U01 Kpn	-	1	23
U03 Koz	-	-	-
U06 Kpn	-	2	7.8, 2.5
U09 Kpn	+	2	6.5, 2.1
U16 Kpn	+	1	7.8
U18 Kpn	+	-	-
U19 Kox	-	-	-
U20 Kpn	-	1	1.97
U23 Kpn	-	-	-
U26 Kpn	+	1	23
U30 Kpn	-	1	23
U31 Kpn	-	-	-
U34 Kpn	+	1	23
U35 Kpn	+	1	23

Key: *Isolates from urine (U01, U03, U06, U09, U16, U18, U19, U20, U23, U26, U30, U31, U34, U35); Kpn= Klebsiella pneumoniae; Kox= Klebsiella oxytoca; Koz= Klebsiella ozanae; + = present; - = absent*

Table 5: Multiple antibiotic resistance (MAR) index of the *Klebsiella* isolates

Isolate name/ number	Resistant pattern	MAR index	Resistant category
Kpn S06	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR, IPM	1	MDR
Kpn W18	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR, IPM	1	MDR
Kpn S04	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR	0.9	MDR
Kpn S05	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR	0.9	MDR
Kpn S07	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR	0.9	MDR
*Kpn*W16	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR	0.9	MDR
Kpn U01	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR	0.9	MDR
Kpn U26	CRX, CAZ, CPR, NIT, AUG, CXM, GEN, CTR, OFL	0.9	MDR
Kpn U31	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN, CTR	0.9	MDR
Kpn U34	CRX, CAZ, NIT, AUG, CXM, GEN, IPM, CPR, OFL	0.9	MDR
Kpn U30	CRX, CAZ, CPR, NIT, AUG, OFL, CXM, GEN	0.8	MDR
Kpn U17	CRX, CAZ, NIT, OFL, CXM, CTR, CPR, GEN	0.8	MDR
Kpn U18	CRX, CAZ, OFL, CXM, GEN, CTR, CPR, NIT	0.8	MDR
Kpn W03	CRX, CAZ, CPR, AUG, OFL, NIT, GEN, CXM	0.8	MDR
Kpn U35	CAZ, CPR, OFL, AUG, NIT, CXM, CRX,	0.7	MDR
Kpn U23	CRX, CAZ, AUG, CXM, CTR, OFL, GEN	0.7	MDR
Kpn U16	CRX, CPR, AUG, OFL, NIT, CXM, CAZ	0.7	MDR
Kox W08	CAZ, CXM, AUG, OFL, CPR, CRX, GEN	0.7	MDR
Kpn W02	CRX, CXM, AUG, CPR, GEN, NIT, CAZ	0.7	MDR
Kpn W05	CRX, CAZ, NIT, AUG, CXM, CTR	0.6	MDR
Kpn U20	CRX, CAZ, CPR, AUG, OFL, GEN	0.6	MDR
Kpn U09	CRX, CAZ, AUG, CXM, NIT, CTR	0.6	MDR
Koz U03	CRX, CPR, NIT, AUG, OFL, CXM	0.6	MDR
Kpn U06	CRX, CPR, AUG, OFL, IPM	0.5	MDR
Kpn W14	CRX, NIT, AUG, CXM, CTR	0.5	MDR
Kpn W06	CRX, CAZ, AUG, CXM, CTR	0.5	MDR
Kpn S03	CRX, NIT, AUG, CXM, CTR	0.5	MDR
Kox U19	CPR, AUG, OFL, GEN	0.4	MDR
Kpn W04	CRX	0.1	

Key: *Kpn= Klebsiella pneumoniae; Kox= Klebsiella oxytoca; Koz= Klebsiella ozanae*
Isolates numbers between 01 to 35, S= sputum, W= wound, U= urine

Table 6: MIC of Ceftriaxone and Ciprofloxacin on ESBL Producers

MIC (µg/ml) on ESBL Producing *Klebsiella* isolates		
Isolates	Ceftriaxone	Ciprofloxacin
Kpn S03	16.0	0.5
Kpn S06	≥128	≥128
Kpn S07	16.0	16.0
Kpn W03	8.0	2.0
Kpn W06	128	1.0
Kpn W08	2.0	1.0
Kpn W18	2.0	1.0
Kpn U16	16.0	64.0
Kpn U26	8.0	2.0
Kpn U34	64.0	8.0
Kpn U35	8.0	2.0
Kpn ATCC 13883	0.12	0.02

Key: Kpn= Klebsiella pneumoniae S= Sputum isolates, W= wound isolates, U= Urine isolates

antibiotic resistance (MAR) index (Table 5) shows that 28 (96.5%) of the isolates had a MAR index of ≥0.4.

MAR index was calculated as a/b, where:

a= Number of antibiotics to which test isolates depicted resistance.

b= Total number of antibiotics to which test isolates were. evaluated for susceptibility.

Minimum inhibitory concentrations (MICs) of ceftriaxone and ciprofloxacin were determined on ESBL producing *Klebsiella* isolates (and on the reference strain *K. pneumoniae* ATCC 13883) with the result shown in Table 6. The MICs of ceftriaxone were from 2.0 to ≥128.0 while that of ciprofloxacin were from 0.5 to >128.0 on the isolates. According

to CLSI equivalent MIC breakpoint [20], out of the 11 *K. pneumoniae* isolates tested, 4 (58.1%) demonstrated ciprofloxacin resistance and 6 (5.45%) showed resistance to ceftriaxone (MIC, ≥ 16 µg/ml), while 4 (3.64%) were resistant to ciprofloxacin (MIC, ≥4). The isolates from wounds were more susceptible to ciprofloxacin than urine and sputum isolates.

ESBL production indicated on plate 1 showed that 14 (48.3%) were ESBL-producers while 15 (51.7%) were non-ESBL-producers. Antibiotic susceptibility pattern of ESBL and non-ESBL-producing isolates (Table 1) showed a higher percentage resistance in ESBL-producing isolates compared to the non-ESBL – producers, particularly with respect to beta-lactam antibiotics.

Plasmid DNA was detected in 17 (58.62%) of the isolates with plasmid size range 0.78 - 23 kbp. Twelve (12) isolates had one plasmid each, of which seven, three and two were urine, wounds and sputum isolates, respectively. Nine (9) of these one-plasmid containing isolates were 23.0 kbp in size. The other three (3) had DNA sizes of 19.8 kbp, 7.8 kbp and 1.2 kbp respectively.

Five (5) isolates had two plasmid DNA, two each from urine and wounds while the remaining one from sputum specimens, with DNA sizes range 0.78 - 22 kbp (Tables 2, 3 and 4). One urine isolate with two plasmids was ESBL-negative while three (3) isolates without plasmid was positive for ESBL-production (plate 2 and plate 3).

Statistical analysis

Using the Pearson's chi-squared test; at P= 0.05 level, result shows that the P e" 0.05 (P= 0.975), hence there was no significant difference among the clinical sources of ESBL producing *Klebsiella* isolates and presence of plasmids. However, using Pearson's correlation coefficient (R^2), result shows that P= 0.000 and R^2= 1.000, a high correlation was found between percentage resistance and plasmid positive isolates of *Klebsiella.*

Discussion

Klebsiella species are important pathogens in nosocomial infections [24], with increasing resistance to multiple antibiotics [25]. In recent years following extensive use of the expanded spectrum cephalosporins, outbreaks of infection caused by extended spectrum beta lactamase producing *K. pneumoniae* has been widely reported throughout the world [26]. The clinical isolates of *Klebsiella* spp. in this study were found to be multidrug resistant (MDR) with 28 (96.5%) resistant to at least 3 different antibiotics with a MAR index of e"0.4. It was noted that MAR index values greater than 0.2 indicates high risk source of contamination where antibiotics are often used [42,43], showing that a greater proportion of the isolates, having MAR index e"0.4 are from a high risk source. The high resistance observed in this study has been corroborated by previous studies [27, 28].

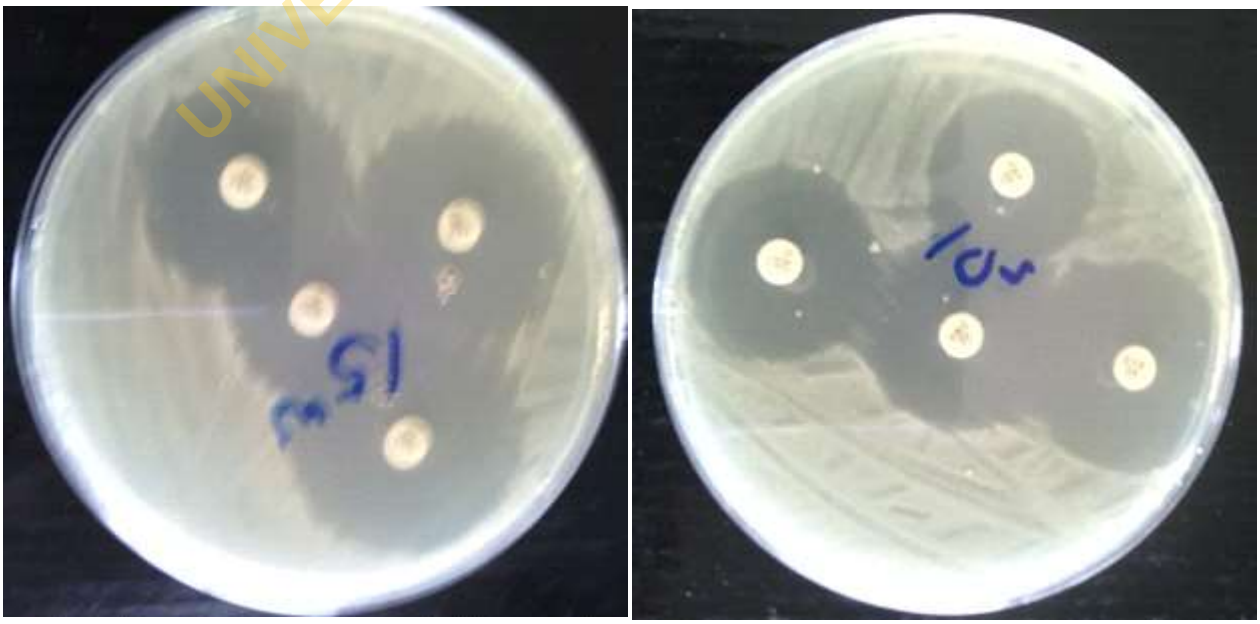

Plate 1: Characteristic (key shaped) ESBL pattern of the *Klebsiella* isolates observed on plates.

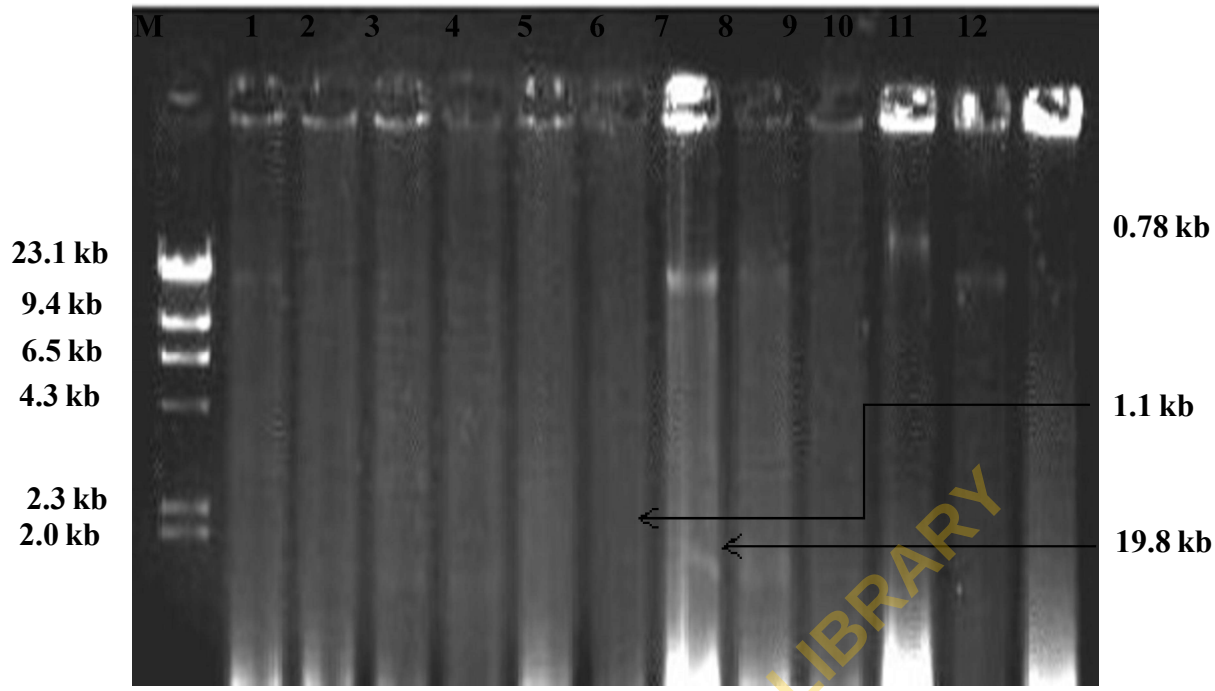

Plate 2: Agarose gel electrophoresis of plasmid DNA recovered from clinical isolates of *Klebsiella* spp. Lane M: Lambda DNA/ *Hind* III marker: Lanes 1 and 11 shows different isolates carrying 23.0 kbp plasmid; Lane 7 carried both 19.8 and 1.1 kbp; Lane 8 carried both 19.8 and 0.78 kbp Lanes 2, 3, 4, 5, 6, 9, 10 and 12 lacked plasmids

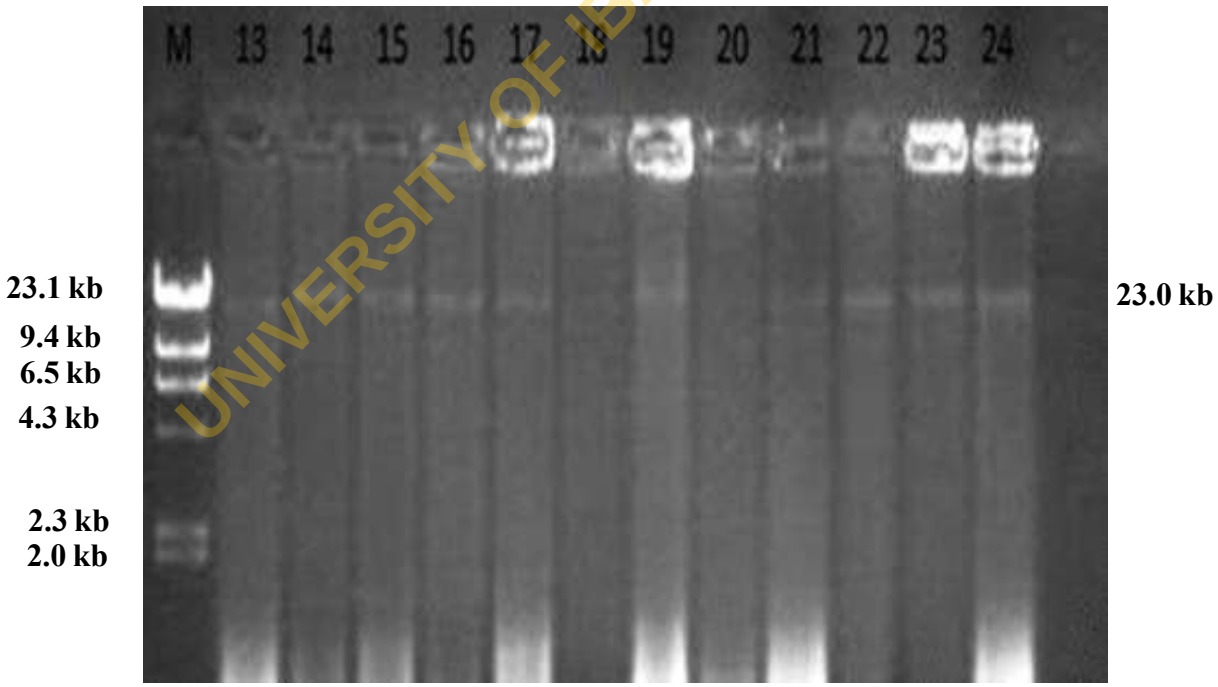

Plate 3: Agarose gel electrophoresis plasmid DNA recovered from the hospital clinical of *Klebsiella* species. Lane M: Lambda DNA/*Hind* III marker: Lane 13 shows isolate carrying 19.8 kbp plasmid; Lanes 14, 15, 16, 17, 21, 22, 23 and 24 shows different isolates carrying 23.0 kbp plasmid; Lanes 18 and 20 isolates lacked plasmids

Although significant bacteriuria has been ascribed to *K. ozanae* [29], *K. pneumoniae* (subsp. *pneumoniae*) was the most significant specie in this study. Occurrence of *Klebsiella* spp. in relation to anatomical site in this study showed that urine (51.7%) was the site where most of the isolates were isolated followed by wound (31.0%) and least is sputum (17.2%). This shows that *Klebsiella* is a significant

factor in the aetiology of urinary tract infection. Previous works had documented the isolation of *Klebsiella* species as the main causative agents of urinary tract infection [28].

Antibiotic susceptibility profile of ESBL positive isolates revealed 100% resistance to cefuroxime, cefotaxime (92.9%), ceftazidime (85.7%) and ceftriaxone (57.1%). This similar trend of resistance by ESBL producers to third generation cephalosporins have earlier been reported [30]. Cephalosporins, particularly second and third generation cephalosporins have been used for *Klebsiella* infections [31]. The isolates were most sensitive to imipenem, with 25 (86.2%) showing susceptibility; this finding is closely related to the report of the study conducted in LUTH [43]. However, considering the MIC values of ceftriaxone and ciprofloxacin on the ESBL producing isolates, according to CLSI equivalent breakpoint interpretation, the isolates were more susceptible (63.6%) to ciprofloxacin than to ceftriaxone (45.5%). Similar observations, and decreased susceptibility of ESBL producing *Klebsiella* to fluoroquinolones (ciprofloxacin) and third generation cephalosporins (ceftriaxone) have been documented [31-34]. Fluoroquinolones are the next drugs of choice to the imipenems, therefore high level resistance of ESBL producing *Klebsiella* isolates in this study and as previously reported is worrisome because of limited availability of antimicrobial alternatives.

In this study, 14 (48.3%) isolates were ESBL-producers, producing a characteristic key or T-shaped inhibition zone pattern (plate 1). This is similar to earlier study conducted by Okesola and Oni [32], where ESBL prevalence rate was 43.2%, and 40% prevalence rate was observed by Babypadmini and Appalarafu [33]. A study in Abeokuta, Nigeria reported a lower prevalence rate of 21.6% [18]. ESBL-producing organisms have been isolated with prevalence rates of 44.6% in Enugu and 6.7% in Ebonyi, Eastern Nigeria [34]. This result emphasized the need for application of ESBL-standard confirmatory tests, and even ESBL-plasmid detection assays, in hospital laboratories.

Plasmid profiles showed that seventeen isolates possessed plasmid bands of various sizes, range 0.78 - 23 kbp (Tables 2-4). The result from this study is higher than a report from a study conducted in Lagos University Teaching Hospital [35], plasmid sizes ranged from 3.0kbp to 4.9kbp but lower than those reported by other workers that revealed a very large plasmid DNA range 11.8kbp to 35.5kbp [18]. High resistance rate was observed with plasmid-bearing isolates. Five (20.8%) of MDR isolates subjected to plasmid profiling revealed the absence of plasmids. Two wound isolates were susceptible to two antibiotics each (ofloxacin and imipenem; gentamicin and imipenem), while two sputum isolates were susceptible to only imipenem, indicative of extremely resistance phenomenon (XDR) [36].

This is not also un-expected since the same antimicrobial resistance pattern can be encoded by unrelated plasmids, transposons, phages and chromosomal genes [37]. The observed differences in the plasmid sizes of clinical bacterial isolates in this study were in agreement with that reported earlier [38]. They showed that plasmids of *K. pneumoniae* isolated from human patients were distributed widely and showed great diversity.

ESBL production is encoded by genes that are prevalently located on large conjugative plasmids [39], which are easily transmitted among different members in the Enterobacteriaceae. Accumulation of these genes could be responsible for the observed multidrug resistant phenomenon, as previously reported [40, 41]. This study has revealed clinical isolates of *Klebsiella* from different specimens, with majority of them harbouring high molecular weight plasmids, with multidrug resistance.

However, this study is limited by the non-determination of minimum inhibitory concentrations of some of the antibiotics, and plasmid-curing which could have given more insight into the roles played by the plasmids or otherwise in the observed multi drug phenomenon.

Conclusion

The study revealed high multidrug resistance of *Klebsiella* species carrying plasmids which could aid the transfer of multidrug resistance to other bacterial species. The fact that all the ESBL-producers were MDR is decidedly worrisome, considering the public health implications like treatment failures and the associated morbidity and mortality. The data further showed the imperatives of rational antibiotic usage to reduce and contain the scourge of antibiotics resistance.

Reference

1. Podschun R and Ullmann U. Klebsiella spp. As Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clin. Microbiol. Rev. 1998; 11(4): 589-603.
2. Frandsen TH and Andersen LP. Spread/Outbreak of multidrug-resistant Klebsiella pneumonia in tertiary hospitals. Microbial pathogens and

strategies for combating them: science, technology and education (A. Méndez-Vilas, Ed.), 2013; 1905-1910.
3. Bauernfeind A, Petermüller C and Schneider R. Bacteriocins as tools in analysis of nosocomial Klebsiella pneumonia infections. J. Clin. Microbiol. 1981; 14: 15-19.
4. Hill HR, Hunt CE and Matsen JM. Nosocomial colonization with Klebsiella,type 26, in a neonatal intensive-care unit associated with an outbreak of sepsis, menigitism and necrotizing enterocolitis. J. Peds. 1974; 85(3): 415-419.
5. Araque M, Nieves B, Lauretti L and Rossiolini GM. Molecular basis of extended-spectrum beta-lactamases production in nosocomial isolates of Klebsiella pneumonia from Mérida, Venezuela. Int. J. Antimicrob. Agents. 2000; 15: 37-42.
6. Gray J and Omar N. Nosocomial infections in neonatal intensive care units in developed and developing countries: how can we narrow the gap? J Hospi Infect. 2012; 83: 193-195
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement update. CLSI document M100-S10. Clinical and Laboratory Standards Institute, Wayne, PA. 2010.
8. Paterson DL. Resistance in Gram-negative bacteria:Enterobacteriaceae. Am. J. Infect. Control. 2006; 34: 20-28.
9. Friedman C, Callery S, Jeanes A, Piaskowski P and Scott L. Best Infection Control Practices for patients with Extended Spectrum ß-Lactamase Enterobacteriacae, Intl. Infect. Cont. Council. 2005.
10. Parasakthi N, Vadivelu J, Ariffin H, Iyer L, Selvi P and Arasu A. Epidemiology and molecular characterization of nosocomially transmitted multidrug-resistant Klebsiella pneumonia. Intl. J. Infect. Dis. 2000; 4(3): 123-128.
11. Fillipa N,Carricajo A, Grattard F et al. Outbreak of multidrug-resistant Klebsiella pneumoniae carrying qnrB1 and blaCTX-M15in a French intensive care unit. Annals of Intensive Care. 2013; 3: 18-21.
12. Winokur PL, Canton R, Casellas JM and Legakis N. Clin. Inf. Dis. 2001; 15 (2): 94-103.
13. Rapp RP and Urban C. Kleibsiella pneumoniae carbapenemases in enterobacteriaceae: History, evolution, and microbiology concerns. Pharmacother. 2012; 32(5): 399-407.
14. Snitkin ES, Zelazny AM, Thomas PJ and Stock F. NISC comparative sequencing program, Henderson D K, Palmore TN, Segre JA. Tracking a hospital outbreak of carbapenem-resistant Kleibsiella pneumonia with whole-genome sequencing. Sci Transl. Med. 2012;4: 148-116.
15. Maritn CM, Ikari NS, Zimmerman J and Waitz A. A virulent nosocomial Klebsiella with a transferable R factor for gentamicin: emergence and suppression. J. Infec. Dis . 1971; 124: S24-s29.
16. Asensio A, González-Diego P, Baquero F et al. Outbreak of a multiresistant Klebsiella pneumonia strain in an intensive care unit: Antibiotic use as risk factor for colonization and infection. Clin. Infect. Dis. 2000; 30: 55-60.
17. Akinduti PA, Oluwaseun E, Motayo BO and Adeyakinu AF. Emerging Multidrug resistant Ampc Beta-Lactamase and Carbapenemase enteric Isolates in Abeokuta. Nature and Science. 2012; 10(7): 70-74.
18. Motayo BO,Akinduti PA, Adeyakinu AF et al. Antibiogram and plasmid profiling of carbapenemase and extended spectrum Beta-lactamase (ESBL) producing Eschrrichia coli and Klebsiella pneumoniae in Abeokuta, South western, Nigeria. Afr Health Sci. 2013; 13(4): 1091-1097
19. Bauer AW, Kirby WM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 1966;45(4): 493-496.
20. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement update. Wayne, PA. 2014, CLSI document M100-S24.
21. Therrien C and Levesque RC. Molecular basis of antibiotic resistance and ß-lactamase inhibition by mechanism-based inactivators: perspectives and future directions; FEMS Microbiol. Rev. 2000; 24(3): 251-262.
22. Sambrook J, Maniatis T and Fritsch ET. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 1982.
23. Kim YK, Pai H, Lee HJ, Park SE and Choi EH. Bloodstream infections by ESBL producing Escherichia coli and Klebsiella pneumonia in Children: Epidemiology and Clinical outcome. Antimicrob Agents Chemother. 2002; 46: 1481-1491.
24. Nordmann P,Cuzon G, Naas T. The real threat of Klebsiella pneumonia carbapenemase

producing bacteria. Lancet Infectious Dis. 2009; 9(4) 228-236.
25. Tonkic M and Goic-Barisic I. Prevalence and antimicrobial resistance of extended spectrum ß-lactamases producing Escherichia coli and Klebsiella pneumoniae strains isolated in a university hospital in Spliit, Croatia. Int. Microbiol. 2005; 8(2): 119-124.
26. El-Khizzi NA and Bakheshuain SM. Prevalence of Extended-Spectrum Beta-lactamase among Enterobacteriaceae isolated from blood culture in a Tertiary Care Hospital. Saudi Med. J. 2006; 27(1): 37-40.
27. Tsuji A, Kobayashi I,Oguri T, Inoue M, Yabuuchi E and Goto S. An epidemiological study of the susceptibility and Frequency of multiple-drug-resistant strains of Pseudomonas aeruginosa isolated at medical institutes nationwide in Japan. J. Infect. Chemother. 2005; 11: 64.
28. Okonko IO, Soleye FA, Amusan TA, Ogun AA, Ogunnusi TA and Ejembi J.Incidence of Multi-Drug Resistance (MDR) Organisms in Abeokuta, South western Nigeria. Global J. Pharmacol. 2009; 3(2): 69-80.
29. Janda JM and ABBOTT SL. The Genera Klebsiella and Raoultella. The Enterobacteria. Washington, USA: ASM Press. 2006; (2nd ed., pp. 115-129).
30. Olowe OA, Oladipo GO, Makanjuola OA and Olaitan JO. Prevalence of extended spectrum ß-lactamases (ESBLs) carrying genes in Klebsiella spp. From clinical samples at Ile Ife, South Western Nigeria. Int. J. Pharm. Med. & Bio. Sc. 2012; 1(2): 12.
31. Jett BD and Ritchie DJ In vitro activities of various ß-lactam antimicrobial agents against clinical isolates of Escherichia coli and Klebsiella spp resistant to oxyimino cephalosporins. Antimicrob. Agents Chemother. 1995: 39 (5) 1187-1190.
32. Okesola OA and Oni AA. Prevalence of extended-spectrum ß-lactamase producing Klebsiella in a tertiary care hospital in South West Nigeria. Int. J. Pharm. Biomed. Sci. 2012; 3(4): 148-151.
33. Babypadmini S and Appalarafu B. ESBLs in urinary isolates of E. coli and Klebsiella pneumonia – prevalence and susceptibility pattern in a tertiary care hospital. Ind. J. Med. Microbiol. 2004; 22:172.
34. Iroha IR, Egwu OA, Ngozi AT, Chidiebube NA and Chika EP. Extended Spectrum Beta-Lactamase (ESBL) Mediated Resistance to Antibiotics among Klebsiella pneumonia in Enugu Metropolis. Maced. J. Med. Sci. 2009; 2: 196-199.
35. Adenipekun EO, Aibinu IE, Daini OA *et al.* Occurrence of ß-lactamase resistance among Isolates from Cancer patients in Lagos, Nigeria. Researcher. 2009; 1(6):1-6.
36. Effah CY, Sun, T, Liu S. et al. Klebsiella pneumonia: an increasing threat to public health. Ann Clin Microbiol Antimicrob. 2020; 19(1). https://doi.org/10.1186/s12941-019-0343-8.
37. Nester EW, Anderson DG, Roberts CE, Roberts CE, Pearsall NN and Nester MT. Microbiology: A human perspective. Fourth edition. M$_c$Graw Hill Companies Inc., New York,USA. 2004; pp 691-698.
38. Karbasizaed V, Badami N and Emtiazi G.Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan. Iran. Afr. J. Biotechnol. 2003; 2(10): 379-383.
39. Podschun R, Heineken P, Ullmann U and Sonntag HG. Comparative Investigations of Klebsiella Species of Clinical Origin: Plasmid Patterns, Biochemical Reactions, Antibiotic Resistance and Serotypes. Medical Microbiology, Infectious Diseases, Virology, Parasitology. 1986; 263(3): 335-345
40. Sirot D. Extended- spectrum plasmid mediated ß-lactamases in the 21st Century:Antimicrob. Agents and Chemother. 2001; 32: 2227-2238.
41. Bradford PA. Extended-spectrum Beta-Lactamases in the 21st Century: Antimicrob. Agents and Chemother. 2001; 32: 2227-2238.
42. Mthembu, MS. The usefulness of multiple antibiotic resistance (MAR) indexing technique in differentiating faecal coliform bacteria from different sources. Thesis (Msc) University of Zululand; 2008.
43. Krumperman, PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of faecal contamination of foods. Applied Environ. Microbiol. 1983; 46: 165-170.
44. Osundiya OO, Oladele RO, Oduyebo OO. Multiple antibiotic resistance (MAR) indices of Pseudomonas and Klebsiella isolates in Lagos University Teaching Hospital Afr. J. Clin. Exper. Microbiol. 2013; 14(3): 164-168.