World Applied Sciences Journal 21 (3): 371-378, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.21.3.65163

Plasmid Profile of Isolated *Klebsiella* Species in a Tertiary Hospital in Abeokuta, Ogun State, Nigeria

[1]*Olusola A. Akingbade,* [1]*I. J. Ogiogwa,* [2]*Iheanyi O. Okonko,* [2]*Phillip O. Okerentugba,*
[2]*Hope C. Innocent-Adiele,* [3]*Jonathan C. Nwanze and* [3]*Charles C. Onoh*

[1]Department of Microbiology and Parasitology, Federal Medical Centre, Abeokuta, Ogun State, Nigeria
[2]Department of Microbiology, University of Port Harcourt, P.M.B. 5323 Uniport post office, Choba, East-West Road, Port Harcourt, Rivers State, Nigeria
[3]Department of Pharmacology and Therapeutics, Igbinedion University, Okada, Edo State, Nigeria

Abstract: The aim of this study is to determine the antibiotic sensitivity pattern of *Klebsiella sp* isolated clinical samples against commonly used antibiotics in Abeokuta metropolis and to correlate the resistance pattern with their plasmid profile. A total of 100 *Klebsiella sp* were obtained from clinical samples in a tertiary Hospital in Abeokuta. The Isolates were obtained from ear swabs, sputum, wound swabs, eye swabs and catheter and urine samples. Pure cultures of the bacterial isolates were subjected to further standard identification techniques. Commercially available antimicrobial discs were used to determine the drug sensitivity pattern of the isolates. Plasmid isolation was done using a highly pure isolation kit (Plasmid Miniprep Kit). Electrophoresis of the DNA was carried out on a 0.8% agarose gel. Generally, Levoxin, Gentamycin and third generation cephalosporins (Ceftazidime) were the potent antibiotics for treatment of *Klebsiella* infections in this study. Plasmid profile was carried out on 27 selected multi drug resistance (MDR) isolates that were resistant to more than two classes of antibiotics. Ten (37.03%) of the MDR isolates were found to possess plasmid bands. The plasmid molecular weight range was 700-982 bp. Thus, high antibiotic resistance was observed in some *Klebsiella sp* isolates that possessed plasmid bands. The high frequencies with which antibiotics are used empirically to treat various diseases suggest that there might be high rate of failure associated with eradication of microbial infections despite their benefits. To overcome these difficulties and to improve the outcome of serious infections in our institutions, monitoring of resistance patterns in the hospital is needed.

Key words: Antibiotics % Electrophoresis % *Klebsiella sp* % Multi Drug Resistance (MDR) % Plasmid Profile % Nigeria

INTRODUCTION

Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. It is said that evolution of bacteria towards resistance to antimicrobial drugs, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is un-stoppable [1]. *Klebsiella spp* have been reported as opportunistic, worrisome nosocomia and community-associated pathogens [2]. *Klebsiella* isolates have been steadily increasing over the past years and they have been important sources of transferable antibiotic resistance [3]. Indiscriminate use of third generation cephalosporins to treat Gram negative bacterial infections is partly responsible for the emergence of resistance to beta-lactam antibiotics [3].

The epidemiology of nosocomial outbreaks can be more complex when the resistance is mediated by several mechanisms the important one of which is the production of enzymes encoded by several genes that are carried on some bacterial plasmids, $-lactamase and extended spectrum $-lactamases. Plasmids have been found to confer drug resistance to their host bacteria by various

Corresponding Author: Iheanyi O. Okonko, Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Choba, East-West Road, Port Harcourt, Nigeria.
GSM: +234(80)3538-0891.

mating processes such as conjugation, transduction and transformation [4]. Plasmid sizes vary from 1 to over 1,000 Kbp. The number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances [4].

Extended spectrum $-lactamases are mostly plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of $-lactam antibiotics, including different types of penicillins and cephalosporins [5]. *Klebsiella sp.* is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in chronic alcoholics and showing characteristic radiographic abnormalities due to severe pyogenic infection. In recent years, *Klebsiella sp* have become important pathogens in nosocomial infections [6].

Klebsiella species contain many plasmids that differ in numbers and molecular weight, carrying different types of genes including those encoding extended-spectrum $-lactamases (ESBLs), AmpC $-lactamases, inhibitor resistant *TEM* $-lactamases and metallo $-lactamase enzymes. These enzymes confer resistance to various antimicrobial agents including the third and fourth generation cephalosporins, cephamycins, monobactam $-lactamase, $-lactamase/inhibitor combinations and carbapenems [7]. The number of resistance genes carried on the plasmids of multi-resistant *K. pneumoniae* is usually more than one and occasionally as many as five genes are reported [8]. In fact, coexistence of broad-spectrum $-lactamases with ESBLs, ESBLs with *AmpC* $-lactamase, ESBLs or multiple ESBLs with metallo-$-lactamase has become common in multi-resistant *K. pneumoniae* isolates [9]. Extended spectrum $-lactamases are the most prevalent in *K. pneumoniae*, mostly encoded on large plasmids with size between 80 to 160 Kbp [10].

Despite their widespread distribution, the prevalence of ESBL-producing organisms remains underestimated because a large number of laboratories do not perform routine tests that specifically detect ESBLs [11]. The aim of this study was to determine the antibiotic sensitivity pattern of *Klebsiella sp* isolated from clinical samples against commonly used antibiotics in Abeokuta, Ogun State, Nigeria and to correlate the resistance pattern with their plasmid profile.

MATERIALS AND METHODS

Collection of Samples: A total of 100 *Klebsiella sp* was isolated from clinical samples in a tertiary hospital in Abeokuta. The samples included ear swabs (22), sputum (12), wound swabs (30), High vaginal swabs (12), catheter (10) and urine (14).

Identification of Isolates: One hundred isolates of *Klebsiella* species were identified based on standard microbiological methods [12, 13].

Antimicrobial Sensitivity Testing: Fifteen commercially available antimicrobial discs (Abtek Biological Ltd UK) were used to determine the drug sensitivity and resistance pattern of the isolates including Gentamycin (Gen, 10mg), Erythromycin (Ery, 10mg), Ampicillin (Amp, 25mg), Augmentin (Aug, 25mg), Cotrimoxazole (Cot, 25mg), Tetracycline (Tet, 25mg), Streptomycin (Str), Ciprofloxacin (Cip, 10mg), Cloxacillin (Cxc, 5mg), Amoxicillin (Amx, 25mg), Cefuroxime (Cxm, 30mg), Ceftriaxone (Cef, 30mg), Levoxin (Lev, 25mg) Ceftazidime (Caz, 30mg) and Ofloxacin (OFL, 30mg). The antimicrobial sensitivity test of the isolates were carried out as described by the Kirby – Bauer disc diffusion method [14] as recommended by the National Committee for Clinical Laboratory Standards [15]. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard. The standardized bacterial suspension was then swabbed and inoculated on to Muller Hinton Agar (Lab Limited UK) and left to dry for 10minutes, before placing the antimicrobial discs. Antibiotic impregnated discs of 8mm diameter were used for the test. After incubation, the diameter of the zone of inhibition were measured and compared with zone diameter interpretative chart [15, 16] to determine the sensitivity of the isolates to antibiotics.

Plasmid Isolation and Profiling: Plasmid isolation was done using a commercial plasmid isolation kit (Plasmid Miniprep Kit, Zymogen Co. Ltd. UK) according to the manufacturer instructions.

Gel Electrophoresis: Electrophoresis of the DNA was carried out on a 0.8% agarose gel according to Bikandi [17] procedures.

RESULTS

Antimicrobial Sensitivity Testing: Table 1 shows that *Klebsiella sp* isolates obtained from ear swabs had highest susceptibility to levoxin followed by gentamycin and ceftazidime and highest resistance to tetracycline, cotrimoxazole and cloxacillin. *Klebsiella sp* isolates from sputum sample showed the highest susceptibility to ceftazidime, ciprofloxacin and levoxin and high resistant

Table 1: Antibiotic susceptibility pattern of 100 *Klebsiella sp* isolates obtained from different clinical samples

Source/No.		Amp	Amx	Aug	Cef	Caz	Cxm	Cip	Cxc ??	Cot	Ery	Gen	Lev	Ofl	Str	Tet
(Ear swabs = 22)	S(%)	5(22.7)	8(36.4)	13(59.1)	12(54.6)	14(63.6)	10(45.5)	13(59.1)	4(18.2)	4(18.2)	5(22.7)	14(63.6)	16(72.7)	10(45.5)	11(50.0)	4(18.2)
	R(%)	17(77.3)	14(63.6)	9(40.9)	10(45.5)	8(36.4)	12(54.6)	9(40.9)	18(81.8)	18(81.8)	17(77.3)	8(36.4)	6(27.3)	12(54.6)	11(50.0)	18(81.8)
(Sputum = 12)	S(%)	3(25.0)	4(33.3)	8(66.7)	9(75.0)	10(83.3)	7(58.3)	10(83.3)	2(16.7)	3(25.0)	6(50.0)	9(75.0)	10(83.3)	8(66.7)	7(58.3)	5(41.7)
	R(%)	9(75.0)	8(66.7)	4(33.3)	3(25.0)	2(16.7)	5(41.7)	2(16.7)	10(83.3)	9(75.0)	6(50.0)	3(25.0)	2(16.7)	4(33.3)	5(41.7)	7(58.3)
(Wound swabs = 30)	S(%)	12(40.0)	10(33.3)	18(60.0)	21(70.0)	21(70.0)	19(63.3)	23(76.7)	6(20.0)	5(16.7)	13(43.3)	28(93.3)	24(80.0)	20(66.7)	12(40.0)	9(30.0)
	R(%)	18(60.0)	20(66.7)	12(40.0)	9(30.0)	9(30.0)	11(36.7)	7(23.3)	24(80.0)	25(83.3)	17(56.7)	2(6.7)	6(20.0)	10(33.3)	18(60.0)	21(70.0)
(HVS swabs = 12)	S(%)	5(41.7)	4(33.3)	9(75.0)	9(75.0)	9(75.0)	7(58.3)	10(83.3)	4(33.3)	3(25.0)	8(66.67)	10(83.3)	11(91.7)	8(66.67)	8(66.67)	5(41.7)
	R(%)	7(58.3)	8(66.67)	3(25.0)	3(25.0)	3(25.0)	5(41.7)	2(16.7)	8(66.67)	9(75.0)	4(33.3)	2(16.7)	1(8.3)	4(33.3)	4(33.3)	7(58.3)
(Catheter = 10)	S(%)	2(20.0)	3(30.0)	6(60.0)	7(70.0)	8(80.0)	6(60.0)	7(70.0)	2(20.0)	3(30.0)	5(50.0)	8(80.0)	9(90.0)	7(70.0)	6(60.0)	4(40.0)
	R(%)	8(80.0)	7(70.0)	4(40.0)	3(30.0)	2(20.0)	4(40.0)	3(30.0)	8(80.0)	7(70.0)	5(50.0)	2(20.0)	1(10.0)	3(30.0)	4(40.0)	6(60.0)
(Urine = 14)	S(%)	6(42.9)	2(14.3)	12(85.7)	10(71.4)	11(78.6)	10(71.4)	12(85.7)	4(28.6)	4(28.6)	6(42.9)	12(85.7)	13(92.9)	10(71.4)	9(64.3)	6(42.9)
	R(%)	8(57.1)	12(85.7)	2(14.3)	4(28.6)	3(21.4)	4(28.6)	2(14.3)	10(71.4)	10(71.4)	8(57.1)	2(14.3)	1(7.1)	4(28.6)	5(35.7)	8(57.1)
Overall Sensitivity (%)		33(33.0)	31(31.0)	66(66.0)	68(68.0)	73(73.0)	59(59.0)	75(75.0)	22(22.0)	22(22.0)	43(43.0)	81(81.0)	83(83.0)	63(63.0)	53(53.0)	33(33.3)
Overall Resistance (%)		67(67.0)	69(69.0)	34(34.0)	32(32.0)	27(27.0)	41(41.0)	25(25.0)	78(78.0)	78(78.0)	57(57.0)	19(19.0)	17(17.0)	37(37.0)	47(47.0)	67(67.0)

Gen = Gentamycin, Ery = Erythromycin, Lev = Levoxin, Amp = Ampicillin, Aug = Augmentin,
Cef = Ceftriaxone, Cot = Cotrimoxazole, Ofl = Ofloxacin, Tet = Tetracycline, Str = Streptomycin,
Cip = Ciprofloxacin, Cxc = Cloxacillin, Amx = Amoxicillin, Cxm = Cefuroxime, Caz =
Ceftazidime, S – Sensitive, R – Resistant

Table 2: Antibiotic resistance profile of *Klebsiella sp* detected from the selected clinical samples

Bacteria Isolates (n= 27)	Code of the isolates	Antibiotic
Klebsiella sp	*E4*	Amp, Amx, Aug, Cxm, Cip, Cxc, Cot, Ery,Ofl, Str, Tet
Klebsiella sp	*E7*	Amp, Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Gen, Lev,
Klebsiella sp	*E9*	Amp, Amx, Aug, Cxc, Cot, Ery, Gen, Lev, Ofl, Str, Tet
Klebsiella sp	*E13*	Amp, Amx, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Str, Tet
Klebsiella sp	*E16*	Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Gen, Str, Tet
Klebsiella sp	*E21*	Amp, Amx, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Ofl, Str, Tet
Klebsiella sp	*S4*	Amp, Amx, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery,Ofl, Str,
Klebsiella sp	*S6*	Amp, Amx, Cxm, Cxc, Cot, Ery, Gen, Ofl, Str, Tet
Klebsiella sp	*S10*	Amp, Amx, Cxm, Cip, Cxc, Cot, Ery, Lev, Ofl, Str, Tet
Klebsiella sp	*W2*	Amp, Amx, Cxm, Cip, Cxc, Cot, Ery, Ofl, Str, Tet
Klebsiella sp	*W6*	Amp, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery, Gen, Lev, Ofl, Str,
Klebsiella sp	*W11*	Amp, Amx, Aug, Cef, Caz, Cxm,Cxc, Cot, Ery, Gen, Str,
Klebsiella sp	*W12*	Amp, Amx, Aug,Cxm, Cip, Cxc, Cot, Ery, Lev, Ofl, Str, Tet
Klebsiella sp	*W16*	Amp, Amx,Cef, Caz, Cxm, Cip, Cxc, Cot, Ery,Ofl, Str,
Klebsiella sp	*W19*	Amx, Cxm, Cip, Cxc, Cot, Ery,Ofl, Str, Tet
Klebsiella sp	*W23*	Amp, Amx, Cef, Cxm, Cxc, Cot, Ery, Ofl, Str, Tet
Klebsiella sp	*W25*	Amp, Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery,
Klebsiella sp	*H5*	Amp, Amx, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery, Gen, Ofl,
Klebsiella sp	*C1*	Amp, Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery,Str, Tet
Klebsiella sp	*C4*	Amp, Amx, Caz, Cxm, Cip, Cxc, Cot, Ery, Gen, Ofl,
Klebsiella sp	*C6*	Amp, Amx, Cef, Cxm, Cip, Cxc, Cot, Ery, Lev, Ofl, Str,
Klebsiella sp	*C7*	Amp, Amx, Cxm, Cip, Cxc, Cot, Ery, Gen, Str, Tet
Klebsiella sp	*C8*	Amp, Amx, Aug, Cef, Cxm, Cip, Cxc, Cot, Ery, Gen, Lev, Str, Tet
Klebsiella sp	*C10*	Amp, Amx, Aug, Cef, Caz, Cxc, Cot, Ery, Str, Tet
Klebsiella sp	*U2*	Amp, Amx, Cef, Cxc, Cot, Ery, Lev, Ofl, Str, Tet
Klebsiella sp	*U3*	Amp, Amx, Cxm, Cip, Cxc, Cot, Ery, Gen, Str, Tet
Klebsiella sp	*U7*	Amp, Amx, Aug, Cef, Caz, Cxm, Cip, Cxc, Cot, Ery, Gen, Tet

to cloxacillin, cotrimoxazole, ampicillin, amoxicillin and tetracycline in that order. *Klebsiella sp* isolates from wound swabs were susceptible to gentamycin, levoxin, ciprofloxacin, ceftazidime and ofloxacin and had high resistance to cotrimoxazole and cloxacillin in that order. *Klebsiella sp* from high vaginal swabs had highest susceptibility to Levoxin, gentamycin and ciprofloxacin in that order and were resistant to cotrimoxazole, cloxacillin and amoxicillin. *Klebsiella sp* obtained from catheter samples had highest susceptibility to levoxin, gentamycin

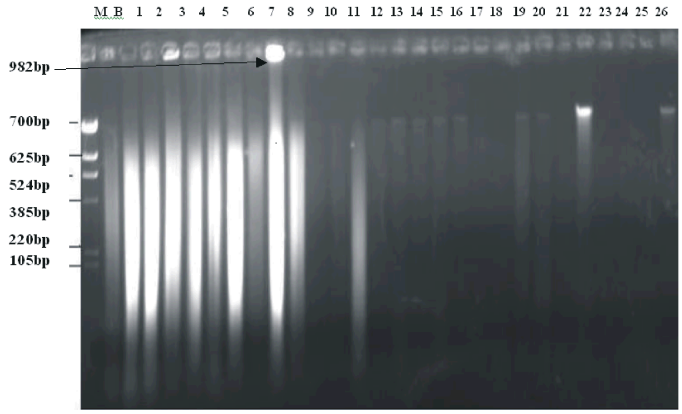

Fig. 1: Plasmid profiles of *Klebsiella sp isolates. Lane* M, 1000bp ladder; Lane B is a negative control, Lanes 1-27 are the clinical isolates (Lanes 8, 13-17, 20, 21, 23 and 27 are positive)

and ceftazidime and high resistant rate to cloxacillin, ampicillin, amoxicillin and cotrimoxazole. *Klebsiella sp* isolated from urine samples had high susceptibility rate to levoxin, gentamycin, ciprofloxacin and augmentin and were resistant to amoxicillin, cloxacillin, cotrimoxazole, erythromycin and tetracycline.

The antibiotic susceptibility pattern of the 100 *Klebsiella sp* isolates obtained from different clinical samples showed that twenty seven isolates were resistant to more than two classes of antibiotics(MDR); Six (E4, E7, E9, E13, E16 and E21) from ear swab, three (S4, S6 and S10) from sputum, eight (W2, W6, W11, W12, W16, W19, W23 and W25) from wound swab, one (H5) HVS, six (C1, C4, C6, C7, C8 and C10) from catheter and three (U2, U3 and U7) from urine samples (Table 2).

Plasmid Isolation and Profiling: Plasmid profile was carried out on the 27 MDR *Klebsiella sp* isolates. Ten (37.03%) were found to possess plasmid bands. Three of the plasmids were detected from isolates obtained from ear swab samples, one from isolate obtained from sputum samples and three plasmids from isolates obtained from wound swab samples. Two plasmids were detected from isolates obtained from catheter samples while one plasmid was detected from isolate obtained from urine samples. The plasmid sizes of the isolates were 982, 700, 732, 748, 756, 765, 815, 815, 837 and 842bp found in lanes 8, 13-17, 20, 21, 23 and 27 respectively (Fig. 1).

DISCUSSION

ESBL-producing strains of *Enterobacteriaceae* have been reported to cause outbreaks of infections [11, 18-19] leading to serious antibiotic management concerns [11, 20]. Risk factors that have been associated with the acquisition of ESBL producing organisms are usage of central venous or arterial catheters, emergency intra-abdominal surgery and lung or gastrointestinal tract pathologies [11, 21]. *K. pneumoniae* have the ability to transfer plasmids containing resistance genes to an indigenous *E. coli* during therapy period [7].

The present study showed high resistance of the *Klebsiella* isolates to the tested antibiotics. This high resistant rate of strains to various antimicrobial agents corresponds with findings by Akiyoshi *et al*. [22]. A high level of resistance to most antibiotics by *Klebsiella sp*. was also reported by Okonko *et al*. [23]. Most of the *Klebsiella sp* obtained showed resistance to first line antibiotics that are commonly prescribed, such as Ampicillin, Amoxicillin, Cloxacillin and Cotrimoxazole. There are reports covering high levels of resistance of *Klebsiella sp* towards these antibiotics in many countries [24].

Subha and Ananthan [24] revealed that *K. pneumoniae* contains a common large plasmid (89 kb) which confers resistance to ampicillin, kanamycin and chloramphenicol and intermediately resistant to amikacin. According to Eisen *et al*. [25], *Klebsiella sp* is an opportunistic pathogen and is a causative agent of several kinds of infections in human. It is one of the major pathogens in nurseries, intensive care units and hospital wards in spite of many effective available antibiotics. This study revealed that streptomycin is not effective (47.0%) for infection caused by *Klebsiella sp* in Abeokuta.

Antibiotics such Levoxin, Gentamycin and Ceftazidime recorded high effectiveness across all tested *Klebsiella*. These antibiotics can be considered as a drug of choice for treatment of *Klebsiella* infections in

Abeokuta. The results of this study support the recommendation of the aminoglycoside (gentamycin) and the third generation cephalosporins (Ceftazidime) as suitable antibiotics for treating *Klebsiella* infections [26].

The overall sensitivity reported for Ceftriaxone (68.0%), Cefuroxime (59.0%) and Ceftazidime (73.0%) was higher than the values reported in previous studies. In a study by Shah *et al.* [3], Cefoxitin (31.66 %) and cefotaxime (30.00 %) were the other antibiotics which were found to be sensitive against *Klebsiella* isolates. The values reported in this study were marginally higher than that reported in studies by Datta *et al.* [27] and Shivaprakash *et al.* [28].

The antibiogram revealed that all isolates were resistant to ampicillin, amoxicillin, cloxacillin, cotrimoxazole and tetracycline in almost equal magnitude. Unless the antibiotic susceptibility test-result is known, ampicillin, amoxicillin, tetracycline, cloxacillin and co-trimoxazole is not advised to be used for treatment of *Klebsiella* infection in Abeokuta. Although some of the *Klebsiella* isolates were susceptible to tetracycline (33.3%), the *Klebsiella sp* clone presented in this study was multi-drug resistant, with resistance to most of the antibiotics used, thereby compromising the use of extended spectrum cephalosporins, quinolones and aminoglycosides as therapeutic options. Similarly, Jain *et al*. [29] showed that ESBL-producing organisms were resistant to ampicillin, cotrimoxazole, tetracycline and gentamicin. Resistance to ceftazidime was also reported by Enwuru *et al.* [2].

Some of the strains obtained in this study harboured resistance plasmids with high-level of multi-drug resistance ability to more than two classes of antimicrobial agents. This could pose a dangerous threat to effective therapy. It is documented that bacteria harbour series of antibiotic resistant genes which can be transferred to others horizontally [30]. Plasmids were detected in 10(37.03%) out of the 27 selected multi-drug resistant isolates obtained from the clinical samples. This plasmid rate was lower to 67% of isolates showing multidrug resistance to more than two antibiotics in Lagos, Nigeria [31].

In deed, the epidemiology of ESBLs has undergone dramatic changes in recent years with the emergence and spread of the CTXM-type enzyme worldwide [11, 32, 33]. ESBL-producing members of the *Enterobacteriaceae* have been reported in Saudi Arabia [11, 34-37], Kuwait [11, 38-41], Oman [42], Bahrain [11] and the United Arab Emirates [11, 43, 44].

From this study, it was observed that the ten *Klebsiella sp* isolates obtained from the clinical samples had plasmid molecular weight of 982, 700, 732, 748, 756, 765, 815, 815, 837 and 842bp. High antibiotic resistance was observed in some *Klebsiella sp* isolates that possessed plasmid bands. The study by Woodford *et al*. [45] pointed out that *Klebsiella pneumoniae* contains two plasmids of molecular weight approximately 95 and 200 Kbp size encoding beta-lactamase enzymes represented by *blaTEM-1*, *blaSHV-12*, *blaCTX-M-3* and *blaDHA-1*. Also, Ensor *et al*. [46] revealed in their study that *K. pneumoniae* contains a common large plasmid 89 Kbp which confers resistance to ampicillin, kanamycin, chloramphenicol and amikacin. The *Klebsiella* spp isolates were more susceptible to the quinolones and more resistant to the penicillin as evident in this study. This is similar to the findings reported by Orhue *et al.* [47]. Resistance to third-generation cephalosporins in these isolates could be largely due to the production of extended-spectrum b-lactamase (ESBL) enzymes, which hydrolyze oxyimino-cephalosporins that are inhibited by clavulanic acid [11].

Resistance genes may either develop from modifications of existing resistance genes or from cryptic resistance genes that hide undetected in the genomes of antibiotic sensitive bacteria [48-49]. The development of resistance to newly introduced antimicrobial drugs is difficult to predict and depends on many factors, including antimicrobial use policies [48-49]. Every antibiotic so far discovered has conferred resistance. Most threatening is the development of new resistance mechanisms that involve resistance genes that are on mobile genetic elements and facilitates spread within and between species [49].

The study showed that 10(37.03%) of the MDR isolates were found to possess plasmid bands. However, the plasmid molecular weight ranged from 700-982 bp. The high antibiotic resistance was observed in some *Klebsiella sp* isolates used in this study. This suggests that there might be high rate of failure associated with eradication of microbial infections despite their benefits. In order to overcome these difficulties and to improve the outcome of serious infections in our institutions, monitoring of resistance patterns in the hospital is recommended.

ACKNOWLEDGEMENT

We are grateful to the staff of the Medical Microbiology and Parasitiology Laboratory, FMC, Abeokuta for providing technical guidance and most of the logistics for this work.

REFERENCES

1. Courvalin, P., 2005. Antimicrobial Drug Resistance: "Prediction Is Very Difficult, especially about the Future". Emerging Infectious Diseases, 11: 1503-1506. Make references like this style.
2. Enwuru, N.V., C.A. Enwuru, S.O. Ogbonnia and A.A. Adepoju-Bello, 2011. Metallo-#-Lactamase Production by Escherichia Coli and Klebsiella Species Isolated from Hospital and Community Subjects in Lagos, Nigeria. Nature and Science, 9: 1-5. Make references like this style.
3. Shah, R.K., Y.I. Singh, R.K. Sanjana, N. Chaudhary and D. Saldanha, 2010. Study of Extended spectrum beta-lactamases (ESBLs) producing Klebsiella species in various clinical specimens: A preliminary report. Journal of College of Medical Sciences-Nepal, 6(3): 19-23.
4. Lipps, G., 2008. Plasmids, Current Research and Future Trends. Horizon Scientific Press, UK, pp: 263.
5. Lautenbach, E., B.L. Strom, W.B. Bilker, J.B. Patel, P.H. Edelstein and N.O. Fishman, 2004. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum $-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Clinical Infectious Diseases, 33: 1288-1294.
6. Nordmann, P., G. Cuzon and T. Naas, 2009. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis., 9(4): 228-236.
7. Essack, S.Y., L.M.C. Hall and D.M. Livermore, 2004. Klebsiella pneumoniaeisolate from South Africa with multiple TEM, SHV and AmpC beta-lactamases. Int. J. Antimicrob Agents, 23: 398-400.
8. Poirel, L., D. Girlich, T. Naas and P. Nordmann, 2004. OXA-28, an extended- spectrum variant of OXA-10 $-lactamase from pseudomonas aeruginosaand its plasmid and integron-located gene. Antimicrobial Agents and Chemotherapy, 45(2): 447-453.
9. Yan, J.J., W.C. Ko and J.J. Wu, 2001. Identification of a plasmid encoding SHV- 12, TEM-1 and a variant of IMP-2 metallo-$-lactamase, IMP-8, from a clinical isolate of Klebsiella pneumoniae. Antimicrobial Agents and Chemotherapy, 45: 2368-2371.
10. Hanson, N.D., K.S. Thomson, E.S. Moland, C.C. Sanders, G. Berthold and R.G. Penn, 2003. Molecular characterization of a multiply resistant Klebsiella pneumonia encoding ESBLs and a plasmid-mediated-AmpC. J. Antimicrobial Chemotherapy, 44: 377-380.
11. Dashti, A.A., M.M. Jadaon, H.H. Gomaa, B. Noronha and E.E. Udo, 2010. Transmission of a Klebsiella pneumoniae clone harbouring genes for CTX-M-15-like and SHV-112 enzymes in a neonatal intensive care unit of a Kuwaiti hospital. Journal of Medical Microbiology, 59: 687-692.
12. Kumar, M.S., V. Laxmi and R. Rajagopalan, 2006. Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care hospital. Indian Journal of Medical Microbiology, 24: 208-11.
13. MacFaddin, J.F., 2000. Biochemical tests for identification of medical bacteria. The Williams and Wiknis Co. Baltimore.
14. Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standard single disc method. American Journal Clinical Pathology, 45: 493-496.
15. National Committee for Clinical Laboratory Standards (NCCLS), 2007. Performance standards for antimicrobial susceptibility testing: twelfth informational supplement. NCCLS document M100-S12. PA, USA.
16. Clinical and Laboratory Standards Institute (CLSI), 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved standard-Third edition, CLSI document M31-A3, Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne Pennsylvania, USA, 28(8): 1-99.
17. Bikandi, J., R. San Millán, A. Rementeria and J. Garaizar, 2004. In silico analysis of complete bacterial genomes: PCR AFLP-PCR and endonuclease restriction. Bioinformatics, 20: 798-799.
18. Naseer, U., O.B. Nata°s, B.C. Haldorsen, B. Bue, H. Grundt, T. R. Walsh and A. Sundsfjord, 2007. Nosocomial outbreak of CTX-M-15-producing *E. coli* in Norway. APMIS, 115: 120-126.
19. Shenoy, S., A. Hegde, S. R. Dominic, S. Kamath and N. Arvind, 2007. An outbreak of extended spectrum b-lactamase producing Klebsiella pneumoniae in a neonatal intensive care unit. Indian J. Pathol. Microbiol., 50: 669-670.
20. Jonathan, N., 2005. Screening for extended-spectrum b-lactamase producing pathogenic enterobacteria in district general hospitals. J. Clin Microbiol., 43: 1488-1490.

21. Paterson, D.L., K.M. Hujer, A.M. Hujer, B. Yeiser, M.D. Bonomo, L.B. Rice and R.A. Bonomo and the International Klebsiella Study Group, 2003. Extended-spectrum b-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type b-lactamases. Antimicrob Agents Chemother, 47: 3554-3560.
22. Akiyoshi, T.K., O. Intentsu, I. Toyoko and G. Sachiko, 2005. An epidemiological study of the susceptibility and frequency of multiple- drug resistant strains of Pseudomonas aeruginosa. J. Infect. Chemother., 11(2): 64.
23. Okonko, I.O., F.A. Soleye, T.A. Amusan, A.A. Ogun, T.A. Ogunnusi and J. Ejembi, 2009. Incidence of Multi-Drug Resistance (MDR) Organisms in Abeokuta, Southwestern Nigeria. Global Journal of Pharmacology, 3(2): 69-80.
24. Subha, A. and S. Ananthan, 2002. Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among Klebsiella pneumoniae in Chennai. Indian Journal of Medical Microbiology, 20(2): 92-95.
25. Eisen, D., E.G. Russell, E.J. Roper, M.L. Grayson and J. Turnidge, 1995. Random amplified polymorphic DNA and plasmid analyses used in investigation of an outbreak of multi-resistant Klebsiella pneumoniae. J. Clin. Microbiol., 33: 713-717.
26. Abe-Aibinu, I.E., V. Ohaegbulam and T.O. Odugbemi, 2000. A comparative study on the antimicrobial susceptibility patterns of Klebisella and Enterobacter species from the Lagos University Teaching Hospital. Journal of the Nigerian Infection Control Association, 3(2): 14-17.
27. Datta, P., A. Thakur and B. Mishra, 2004. Prevalence of Clinical strains resistant to various beta-lactams in a tertiary care hospital in India. Ind. J. Med. Microbiol., 57: 146-149.
28. Shivaprakasha, S., K. Radhakrishnan and A.R. Gireesh, 2007. Routine screening for ESBL production, a necessity of today. Internet Journal of Microbiol., 3: 1.
29. Jain, A., I. Roy, M.K. Gupta, M. Kumar and S.K. Agarwal, 2003. Prevalence of extended-spectrum b-lactamase-producing Gram-CTX-M-15 and SHV-112 in *K. pneumoniae* from a negative bacteria in septicaemic neonates in a tertiary care hospital. J. Med. Microbiol., 52: 421-425.
30. Piddock, L.J.V., 2006. Clinically relevant chromosomally encoded multi drug resistance efflux pumps in bacteria. Clin. Microbiol. Rev., 19(2): 382-402.
31. Umolu, P., O. Omigie, Y. Tatfeng, F.I. Omorogbe, F. Aisabokhale and O.P. Ugbodagah, 2006. Antimicrobial susceptibility and plasmid profiles of Escherichia coli isolates obtained from different human clinical specimens in Lagos-Nigeria. J. American Science, 2(4): 70-75.
32. Paterson, D.L. and R. A. Bonomo, 2005. Extended-spectrum blactamases: a clinical update. Clin Microbiol. Rev., 18: 657-686.
33. Bonnet, R., 2004. Growing group of extended-spectrum blactamases: the CTX-M enzymes. Antimicrob Agents Chemother, 48: 1-14.
34. Panhotra, B.R., A. K. Saxena and A.M. Al-Ghamdi, 2004. Extended spectrum b-lactamase-producing Klebsiella pneumoniae acquired bacteremia. Risk factors and clinical outcome. Saudi Med. J., 25: 1871-1876.
35. Kader, A.A. and K. Angamuthu, 2005. Extended-spectrum blactamases in urinary isolates of Escherichia coli, Klebsiella pneumoniae and other Gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia. Saudi Med. J., 26: 956-959.
36. El-Khizzi, N.A. and S.M. Bakheshwain, 2006. Prevalence of extended-spectrum b-lactamases among Enterobacteriaceae isolated from blood culture in a tertiary care hospital. Saudi Med. J., 27: 37-40.
37. Kader, A.A., A. Kumar and K.A. Kamath, 2007. Faecal carriage of extended-spectrum b-lactamase-producing Escherichia coli and Klebsiella pneumoniae in patients and asymptomatic healthy individuals. Infect. Control Hosp. Epidemiol., 28: 1114-1116. Make references like this style.
38. Dimitrov, T.S., E.E. Udo, M. Emara, F. Awni and R. Passadilla, 2004. Etiology and antibiotic susceptibility patterns of community-acquired urinary tract infection in a Kuwait hospital. Med. Princ Pract, 13: 334-339.
39. Jamal, W., V.O. Rotimi, F. Khodakhast, R. Saleem, A. Pazhoor and G. Al Hashim, 2005. Prevalence of extended-spectrum b-lactamase in Enterobacteriaceae, Pseudomonas and Stenotrophomonas as determined by the VITEK 2 and Etest systems in a Kuwait teaching hospital. Med. Princ Pract, 14: 325-331.

40. Dashti, A.A. and P.W. West, 2007. Extended-spectrum b-lactamaseproducing Escherichia coli isolated in the Al-Amiri hospital in 2003 and compared with isolates from the Farwania hospital outbreak in 1994-96 in Kuwait. J. Chemother, 19: 271-276.
41. Dashti, A.A., P. West, R. Paton and S.G. Amyes, 2006. Characterization of extended-spectrum b-lactamase (ESBL)-producing Kuwait and UK strains identified by the VITEK system and subsequent comparison of the VITEK system with other commercial ESBL-testing systems using these strains. J. Med. Microbiol., 55: 417-421.
42. Rafay, A.M., Z. Al-Muharrmi and R. Toki, 2007. Prevalence of extended-spectrum b-lactamases producing isolates over a 1-year period at a university hospital in Oman. Saudi Med. J., 28: 22-27.
43. Sonnevend, A., K. Al Dhaheri, T. Mag, M. Herpay, J. Kolodziejek, N. Nowotny, A. Usmani, F.A. Sheikh and T. Pal, 2006. CTX-M-15-producing multidrug-resistant enteroaggregative *Escherichia coli* in the United Arab Emirates. Clin Microbiol. Infect, 12: 582-585.
44. Al-Zarouni, M., A. Senok, F. Rashid, S.M. Al-Jesmi and D. Panigrahi, 2008. Prevalence and antimicrobial susceptibility pattern of extended-spectrum-b-lactamase-producing Enterobacteriaceae in the United Arab Emirates. Med. Princ Pract, 17: 32-36.
45. Woodford, N., J. Zhang, M. Warner, M.E. Kaufmann, J. Matos, A. MacDonald, D. Brudney, D. Sompolinsky, Navon S-Venezia and D.M. Livermore, 2008. Arrival of Klebsiella pneumoniae producing KPC carbapenemase in the United Kingdom. Journal of Antimicrobial Chemotherapy, 62(6): 1261-1264.
46. Ensor, V.M., M. Shahid, J.T. Evans and P.M. Hawkey, 2006. Occurrence, prevalence and genetic environment of CTX-M $-lactamases in Enterobacteriaceaefrom Indian hospitals. Journal of Antimicrobial Chemotherapy, 58: 1260-1263.
47. Orhue, P.O., E.O. Igumbor, I.B. Enweani, F.I. Esumeh, R.E. Ohenhen and E.O. Igbinosa, 2012. Antibiogram types of Klebsiella aerogenes isolated from urinary tract infection (UTI). African Journal of Microbiology Research, 6(20): 4336-4339.
48. Hall, B.G., 2004. Predicting the evolution of antibiotic resistance genes. Nature Reviews Microbiology, 2: 430-435.
49. Geenen, P.L., M.G.J. Koene, H. Blaak, A.H. Havelaar and A.W. Van De Giessen, 2010. Risk profile on antimicrobial resistance transmissible from food animals to humans. National Institute of Public Health and the Environment (RIVM)-rapport 330334001, pp: 121.