

Original Research Article

Phenotypic Identification, Plasmid Profiling and Detection of Resistance Genes in *Klebsiella* sp. from Urine Samples of In- and Out-Patients in Ile-Ife.

Abstract

Uropathogenic *Klebsiella* sp. from urine samples in- and out-patients in Ile-Ife were investigated. Isolation, characterization and identification of the isolates were done using standard microbiological techniques. Antibiotic sensitivity of the *Klebsiella* isolates to 11 different antibiotics was determined by Kirby-Bauer's disc diffusion method on sterile Mueller-Hinton agar plates. Plasmid DNA in representative multiple antibiotic resistant isolates was detected by alkaline lysis (TENS buffer). Resistance (*bla*CTX, *tet*M) genes were detected by Polymerase Chain Reaction (PCR). Statistical analysis of data obtained was done using SPSS-17.0. A total of thirty-two *Klebsiella* sp. (7.02%) were isolated from 456 urine samples of in- and out-patients; 50 urine samples collected from apparently healthy individuals served as control. Antibiotic resistance was high and also varied among the isolates with a range of 37.5% to 100%. All the isolates had multiple antibiotic resistance (MAR) to at least two different classes of antibiotics. There was diversity in the MAR patterns with 21 different antibiotypes which ranged from two to ten different antibiotics. Plasmid DNA of molecular weight of 900 bp to 9160 bp was detected in some representative multiple antibiotic resistant isolates. Three isolates harboured *bla*CTX (543 bp) gene which codes for resistance to beta-lactams. However, *tet*M (974 bp) gene which codes for resistance to tetracyclines was not detected in any of the isolates.

Keywords: *Klebsiella* sp., urine, in- and out-patients, multiple antibiotic resistance, resistance genes

1. Introduction

Klebsiella are usually opportunistic pathogens found as members of the normal intestinal flora of humans and animals and may be isolated from a variety of environmental sources, on the hands of hospital personnels with the principal pathogenic reservoirs being the gastrointestinal tract of humans (Brisse *et al.*, 2006). *Klebsiella* sp. is an important nosocomial microorganism which may cause high rate of fatality in hospital settings, affecting different age groups and sexes (Nordmann *et al.*, 2009). *Klebsiella* sp. was reported as the most prevalent uropathogen causing asymptomatic bacteriuria in some out-patients attending various clinics at the University of Benin teaching hospital in Benin City, Nigeria (Osazuwa *et al.*, 2010). *Klebsiella pneumoniae* is the most common species isolated from hospital patients.

Under normal circumstances the kidney, ureter and the urinary bladder of mammals are sterile. Urine within the urinary bladder is also sterile. However, in both males and females, a few bacteria are usually present in the distal portion of the urethra (Geoffrey *et al.*, 2013). Infections

38 of the urinary tract are one of the most common infectious diseases (Delanghe *et al.*, 2000;
39 Hryniewicz *et al.*, 2001; Kolawale *et al.*, 2009).

40 Nosocomial infections occur worldwide, both in the developed and developing countries. More
41 recently, hospital acquired infections (HAIs) have been shown to be a significant economical
42 burden to patients and public health in developing countries (Nyamogoba and Obala, 2002;
43 Rossello-Urgell, 2004). Colonized patients have a higher risk of nosocomial infection than
44 patients who are not colonized (Koenig and Truwit, 2006). A variety of vehicles have been
45 implicated in the spread of nosocomial pathogens (Samuel *et al.*, 2010). In most of the patients, a
46 variety of sites are colonized, mostly the urinary tract, with or without a serious infection
47 (Dhingra, 2008). In a hospital based patient survey in Saudi Arabia, 38 (33.3%) patients had
48 hospital acquired infection (Hanan *et al.*, 2006), 6.7% was recorded among patients on admission
49 in a study in Ghana (Newman, 2009).

50 Statistical data and evidences from researches prove that multidrug resistant bacteria (bacteria
51 that are resistant to at least two different classes of antibiotics) are emerging worldwide which is
52 a big challenge to healthcare. Multidrug resistant bacteria cause serious nosocomial and
53 community acquired infections that are hard to eradicate using available antibiotics (Archana and
54 Harsh, 2011). Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both
55 increased carriage of *Klebsiella* and the development of multidrug resistant strains that produce
56 extended-spectrum beta-lactamase (ESBL) (Archana and Harsh, 2011; Sachse *et al.*, 2014).
57 *Klebsiella* isolates have steadily increased over the past years and they have been important
58 sources of transferable antibiotic resistance (Shah *et al.*, 2010). For instance, the prevalent
59 pathogens of UTIs have been found to be resistant to most chemotherapeutic agents (Okonko *et al.*, 2009b).
60

61 The epidemiology of nosocomial outbreaks can be more complex when the resistance is
62 mediated by several mechanisms the important one of which is the production of enzymes
63 encoded by several genes that are carried on some bacterial plasmids (Karim *et al.*, 2001).
64 Plasmids that carry several different resistance genes can confer resistance to multiple
65 antibacterial agents (Chen *et al.*, 2014a). *Klebsiella* species contain many plasmids that differ in
66 number and molecular weight, carrying different types of genes including those encoding
67 extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases and inhibitor resistant
68 TEM beta-lactamases. These enzymes confer resistance to various antimicrobial agents including
69 third and fourth generation cephalosporins, cephamycins, beta-lactamase/inhibitor combinations
70 and carbapenems (Essack *et al.*, 2004). The number of resistance genes carried on the plasmids
71 of multidrug resistant *Klebsiella pneumoniae* is usually more than one and occasionally as many
72 as five genes are reported (Poirel *et al.*, 2004).

73 Indiscriminate use of third generation cephalosporins to treat Gram-negative bacterial infections
74 is partly responsible for the emergence of resistance to beta-lactams (Shah *et al.*, 2010).
75 *Klebsiella pneumoniae* is at the forefront of antimicrobial resistance for Gram-negative
76 pathogenic bacteria, as strains resistant to third-generation cephalosporins and carbapenems are
77 widely reported. The worldwide diffusion of these strains is of great concern, due to the high

morbidity and mortality often associated with *Klebsiella pneumoniae* infections in hospital environments (Gaiarsa *et al.*, 2015). In a study, resistance was observed to aminoglycosides, fluoroquinolones, chloramphenicol, and co-trimoxazole, genes encoding TEM, SHV, CTX-M, OXA-2, and AmpC types of beta-lactamases in the *Escherichia coli*, *Klebsiella* sp. and *Enterobacter* sp. isolates were also detected (Priamchuk *et al.*, 2010).

Antibiotic resistance is a large and growing problem in the microbial world that accounts for most of Africa's disease burden (Okeke *et al.*, 2007). Many factors play in the emergence of antibiotic resistant bacteria (WHO, 2012) from poor utilization of antimicrobial agents, to the transmission of resistant bacteria from patient to patient, from healthcare workers to patients and vice versa, to a lack of guidelines for appropriate and judicious use of antimicrobial agents.

This study reports the prevalence of uropathogenic *Klebsiella* sp. from in- and out-patients attending Obafemi Awolowo University Teaching Hospitals Complex and Obafemi Awolowo University Health Centre, Ile-Ife, Nigeria. The aim of the study was to determine the antibiotic sensitivity pattern of the isolates, detect plasmid DNA and resistance genes in multiple antibiotic resistant isolates.

2. Materials and Methods

2.1 Study population, sampling and bacteriological analysis

In- and out-patients were recruited from Obafemi Awolowo University Teaching Hospitals Complex and Obafemi Awolowo University Health Centre, Ile-Ife, Nigeria. The subjects were educated to collect midstream urine specimen in sterile wide-mouthed capped universal bottles. The samples were transported in ice pack (4°C) to the laboratory for analysis. Preliminary identification of the isolates was based on phenotypic characteristics on sterile MacConkey agar plates. The identity of the isolates was further confirmed by various conventional biochemical tests following Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1957).

2.2 Antibiotic susceptibility testing

Antibiotic susceptibility of the isolates was tested by Kirby-Bauer's disc diffusion method using the following antibiotics: cotrimoxazole (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), amoxicillin (30 µg), augmentin (30 µg), gentamycin (10 µg), pefloxacin (30 µg), ofloxacin (10 µg), streptomycin (30 µg), ceftriaxone (30 µg), nitrofurantoin (200 µg) and tetracycline (30 µg) (Fondos, Nigeria). Broth culture of the isolates was standardized by comparing its turbidity to that of 0.5 McFarland standard and subsequently seeded on sterile Mueller-Hinton agar plates. The antibiotic discs were aseptically placed on the inoculated Mueller-Hinton agar plates using sterile forceps. The plates were incubated in an inverted position at 37°C for 18-24 h. The diameter of the zones of inhibition were measured to the nearest millimetre and compared to the zone diameter interpretative chart of the Clinical and Laboratory Standards Institute (CLSI, 2013).

2.3 Plasmid Profiling

Plasmid DNA of the representative multiple antibiotic resistant *Klebsiella* isolates were extracted using TENS (25 mM Tris, 10 mM EDTA, 0.1 N NaOH and 0.5% SDS- Sigma products) buffer. The plasmid DNA was separated by electrophoresis using 0.8% agarose gel (Oxoid, England) in Tris-acetate-EDTA buffer (10 ml of 50X TAE per 1000 ml of sterile distilled water) and ethidium bromide (2 ml of 10 mg/ml), a Lambda DNA/HindIII Marker, 2 (Fermentas, USA) was used as standard. The plasmid DNA bands were visualized under ultraviolet transilluminator and compared to the reference marker.

2.4 Detection of resistance genes

Deoxyribonucleic acid of representative multiple antibiotic resistant *Klebsiella* isolates was extracted by boiling. The sequences of the forward and reverse primers used are represented in Table 1. Polymerase chain reaction (PCR) was performed in 20 µl reaction mixtures which contained 3.0 µl of the DNA template (genomic DNA of the bacteria), 4.0 µl of the master mix, 0.2 µl each of the forward and reverse primers, and sterile distilled water was added to make a final volume of 20 µl. The mixture was vortexed and amplified in a thermocycler. The thermocycler was programmed as follows: an initial denaturation step at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for *bla*CTX, 57°C for *tet*M for 1 minute, extension at 72°C for 90 seconds, a final extension step at 72°C for 10 minutes and a final hold at 4°C. The amplified PCR products were separated by electrophoresis using 1.5% agarose gel (Oxoid, England) in Tris-acetate-EDTA (10 ml of 50X TAE per 1000 ml of sterile distilled water) buffer and ethidium bromide (2 ml of 10 mg/ml). A 100 bp DNA marker (Solis Biodyne, Estonia) was used as a standard. The gel was visualised under ultraviolet transilluminator and the fragment size compared to that of the standard.

Table 1. Primers used in the detection of resistance (*bla*CTX (543 bp), *tet*M (974 bp) genes.

Primer	Sequence	Reference
<i>bla</i> CTX (forward)	5'-ATGTGCAGYACCAGTAARGTKATGGC-3'	Jemima and
<i>bla</i> CTX (reverse)	5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3'	Verghese, 2008.
<i>tet</i> M (forward)	5'-CGAACAAGAGGAAAGCATAAG-3'	Li and Wang,
<i>tet</i> M (reverse)	5'-CAATACAATAGGAGCAAGC-3'	2010.

2.5 Statistical analysis

Significant difference in antibiotic resistance among the *Klebsiella* isolates was detected using T-test (SPSS-17.0).

147

148 3. Results

149 A total of thirty-two *Klebsiella* sp. were isolated from 456 urine samples of in- and out-patients
 150 collected. These include nineteen and thirteen *Klebsiella* isolates from in- and out-patients
 151 respectively. These isolates comprised *Klebsiella pneumoniae* (59.4%), *Klebsiella ozaenae*
 152 (28.1%) and *Klebsiella oxytoca* (12.5%). Antibiotic resistance was high and also varied among
 153 the isolates. Most of the isolates were resistant to nitrofurantoin (100%), augmentin (93.75%)
 154 and tetracycline (71.88%). The least resistance was observed towards ciprofloxacin and
 155 ofloxacin (31.25% each). Among the isolates, resistance to augmentin was found in *Klebsiella*
 156 *pneumoniae* (94.7%), *Klebsiella oxytoca* (75.0%) and *Klebsiella ozaenae* (88.9%). Resistance to
 157 tetracycline was observed in *Klebsiella pneumoniae* (63.2%), *Klebsiella oxytoca* (100.00%) and
 158 *Klebsiella ozaenae* (77.8%). There was no significant difference in antibiotic resistance among
 159 the *Klebsiella* isolates at $p > 0.05$ ($t_{\alpha 0.05}$ 3.01, $p = 0.71$ for tetracycline, $t_{\alpha 0.05}$ 2.21, $p = 0.71$ for
 160 augmentin) (Table 2).

161 The multiple antibiotic resistant (MAR) isolates were described as being resistant to two or more
 162 different classes of antibiotics. There was diversity in the MAR pattern with 21 different
 163 antibiotypes which ranged from two to eleven different antibiotics. Virtually all the isolates
 164 (100%) showed multiple antibiotic resistance to the antibiotics used. This consists of *Klebsiella*
 165 *pneumoniae* (59.4%), *Klebsiella ozaenae* (28.1%) and *Klebsiella oxytoca* (12.5%). The most
 166 prevalent antibiotype (CH CPX AUG GEN COT TET NIT) was expressed in *Klebsiella*
 167 *pneumoniae*, *Klebsiella ozaenae* and *Klebsiella oxytoca* isolated from urine samples of both in-
 168 and out-patients; they were resistant to all the antibiotics tested. The MAR patterns of the isolates
 169 are represented in Tables 3a and 3b.

170

171 **Table 2. Percentage antibiotic susceptibilities of *Klebsiella* sp. from urine samples of in- and**
 172 **out-patients.**

173	174 Antibiotics (µg)	Number of		Percentage (%)	
		isolates	Susceptibility	Intermediate	Resistant
175	Chloramphenicol (30 µg)	32	37.50	18.75	43.75
176	Ciprofloxacin (10 µg)	32	53.13	15.63	31.25
177	Amoxicillin (30 µg)	32	12.50	25.00	62.50
178	Augmentin (30 µg)	32	0.00	6.25	93.75
179	Gentamycin (10 µg)	32	62.50	3.13	34.38
180	Ofloxacin (10 µg)	32	62.50	6.25	31.25

181	Streptomycin (30 µg)	32	46.88	12.50	40.63
182	Nitrofurantoin (200 µg)	32	0.00	0.00	100.00
183	Cotrimoxazole (25 µg)	32	34.38	18.75	46.88
184	Tetracycline (30 µg)	32	6.25	21.88	71.88
185	Ceftriaxone (30 µg)	32	3.13	28.13	68.75

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189 **Table 3a. Multiple antibiotic resistance profile of *Klebsiella* sp. from urine samples of in-**
190 **patients.**

191	Isolates	Number of	MAR patterns	Frequency
192	antibiotic classes			
193	<i>Klebsiella</i>	2	AUG NIT	2
194	<i>pneumoniae</i>		CRO NIT	1
195		3	AUG GEN NIT	1
196			AUG TET NIT	1
197			CRO TET NIT	1
198		4	AUG GEN TET NIT	1
199			CH AUG TET NIT	1
200		5	CH AUG GEN TET NIT	1
201		7	CH CPX AUG GEN COT TET NIT	1
202			CH CPX CRO GEN COT TET NIT	1
203	<i>Klebsiella ozaenae</i>	3	AUG TET NIT	1
204		4	CH AUG COT NIT	1
205		6	CH AUG GEN COT TET NIT	1

206			CPX AUG GEN COT TET NIT	1
207	7		CH CPX AUG GEN COT TET NIT	1
208			CH CPX AUG STR COT TET NIT	1
209			CH CPX CRO STR COT TET NIT	1
210	<i>Klebsiella oxytoca</i>	3	AUG TET NIT	1
211			Total	19
212				

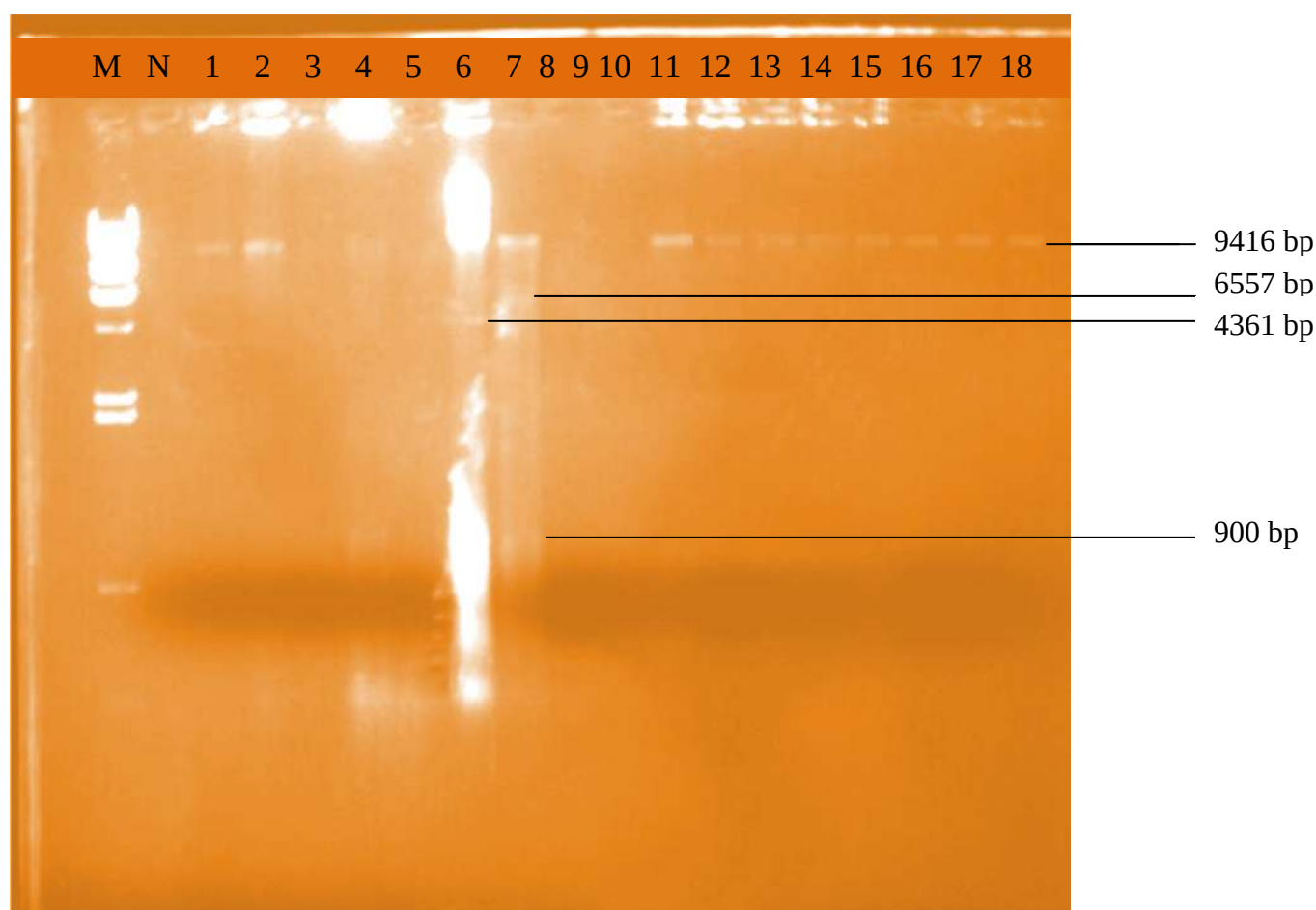
213 **Key:** CH-Chloramphenicol, AUG-Augmentin, AMX-Amoxicillin, CRO-Ceftriaxone, CPX-
 214 Ciprofloxacin, GEN-Gentamycin, STR-Streptomycin, TET-Tetracycline, COT-Cotrimoxazole,
 215 NIT-Nitrofurantoin.

216 **Table 3b. Multiple antibiotic resistance profile of *Klebsiella* sp. from urine samples of out-**
 217 **patients.**

218	Isolates	Number of	MAR patterns	Frequency
219		antibiotic classes		
220	<i>Klebsiella</i>	2	AUG NIT	1
221	<i>pneumoniae</i>		AMX NIT	1
222			CRO NIT	1
223		3	AUG TET NIT	2
224		5	CPX AUG COT TET NIT	1
225		6	CH AUG STR COT TET NIT	1
226		7	CH CPX AUG GEN COT TET NIT	1
227	<i>Klebsiella ozaenae</i>	5	CH AUG GEN COT NIT	1
228		7	CH CPX AUG STR COT TET NIT	1
229	<i>Klebsiella oxytoca</i>	3	CRO TET NIT	1
230		5	AUG STR COT TET NIT	1
231		7	CH CPX AUG GEN COT TET NIT	1
232			Total	13
233				

234 **Key:** CH-Chloramphenicol, AUG-Augmentin, AMX-Amoxicillin, CRO-Ceftriaxone, CPX-
235 Ciprofloxacin, GEN-Gentamycin, STR-Streptomycin, TET-Tetracycline, COT-Cotrimoxazole,
236 NIT-Nitrofurantoin.

237
238 Plasmid DNA was detected in *Klebsiella pneumoniae* (8), *Klebsiella oxytoca* (1) and *Klebsiella*
239 *ozaenae* (4) among the representative multiple antibiotic resistant isolates. The molecular weight
240 of the plasmid DNA ranged from 500 to 9416 bp. However, an isolate (*Klebsiella pneumoniae*)
241 from urine samples of in-patients had a multiple plasmid DNA with molecular weight of 900,
242 6557 and 9416 bp. Two isolates; *Klebsiella ozaenae* and *Klebsiella pneumoniae* from urine
243 samples of out-patients also harboured multiple plasmid DNA of molecular weight ranging from
244 900 to 9416 bp. The agarose gel electrophoresis of plasmid DNA in the MAR *Klebsiella* isolates
245 is represented in Plate 1. Tables 4a and 4b show the molecular weight of the plasmid DNA.



246
247 **Plate 1. Plasmid profile of MAR *Klebsiella* sp. from urine samples of in- and out-patients.**

248 Lane M = Lambda DNA/HindIII Marker, 2, Lane N = Negative control, Lane 1-5 (K402, K391-
249 UTI, K95-Fibroid in pregnancy, K84, K108-?UTI) = *Klebsiella pneumoniae*, Lane 6 (K85) =

250 *Klebsiella oxytoca*, Lane 7 (K151) = *Klebsiella ozaenae*, Lane 8, 9 (K326-?UTI, K37) =
 251 *Klebsiella pneumoniae*, Lane 10, 11 (K14, K339-Bladder outlet obstruction) = *Klebsiella*
 252 *ozaenae*, Lane 12, 13 (K152, K88-Hypertension) = *Klebsiella pneumoniae*, Lane 14 (K272-
 253 Hypertension) = *Klebsiella ozaenae*, Lane 15 (K301-Benign prostate enlargement) = *Klebsiella*
 254 *pneumoniae*, Lane 16 (K105-UTI) = *Klebsiella ozaenae*, Lane 17 (K158) = *Klebsiella*
 255 *pneumoniae*, Lane 18 (K255-Urethral stricture) = *Klebsiella oxytoca*.

256

257 **Table 4a. Molecular weight of plasmid DNA in MAR *Klebsiella* sp. from urine samples of**
 258 **in-patients.**

259	Lanes/Isolates	Number of Plasmids	Molecular Weight (bp)	MAR Patterns
260	L1=402	-	-	AUG TET NIT
261	L2=391	1	9416	AUG GEN NIT
262	L3=95	1	9416	CRO TET NIT
263	L4=84	-	-	CH CPX AUG GEN
264				COT TET NIT
265	L8=326	3	9416, 6557, 900	AUG GEN TET NIT
266	L11=339	1	9416	CPX AUG GEN COT
267				TET NIT
268	L13=88	1	9416	CH AUG TET NIT
269	L14=272	1	9416	CH CPX AUG STR COT
270				TET NIT
271	L15=301	1	9416	CH AUG GEN TET NIT
272	L16=105	1	9416	CH CPX CRO STR COT
273				TET NIT

274 L18=255 1 9416 AUG TET NIT

275 Key: LI-L4, L8, L12, L13, L15 = *Klebsiella pneumoniae*, L11, L14, L16 = *Klebsiella ozaenae*,
 276 L18 = *Klebsiella oxytoca*, CH-Chloramphenicol, AUG-Augmentin, CRO-Ceftriaxone, CPX-
 277 Ciprofloxacin, GEN-Gentamycin, STR-Streptomycin, TET-Tetracycline, COT-Cotrimoxazole,
 278 NIT-Nitrofurantoin.

279 **Table 4b. Molecular weight of plasmid DNA in MAR *Klebsiella* sp. from urine samples of**
 280 **out-patients.**

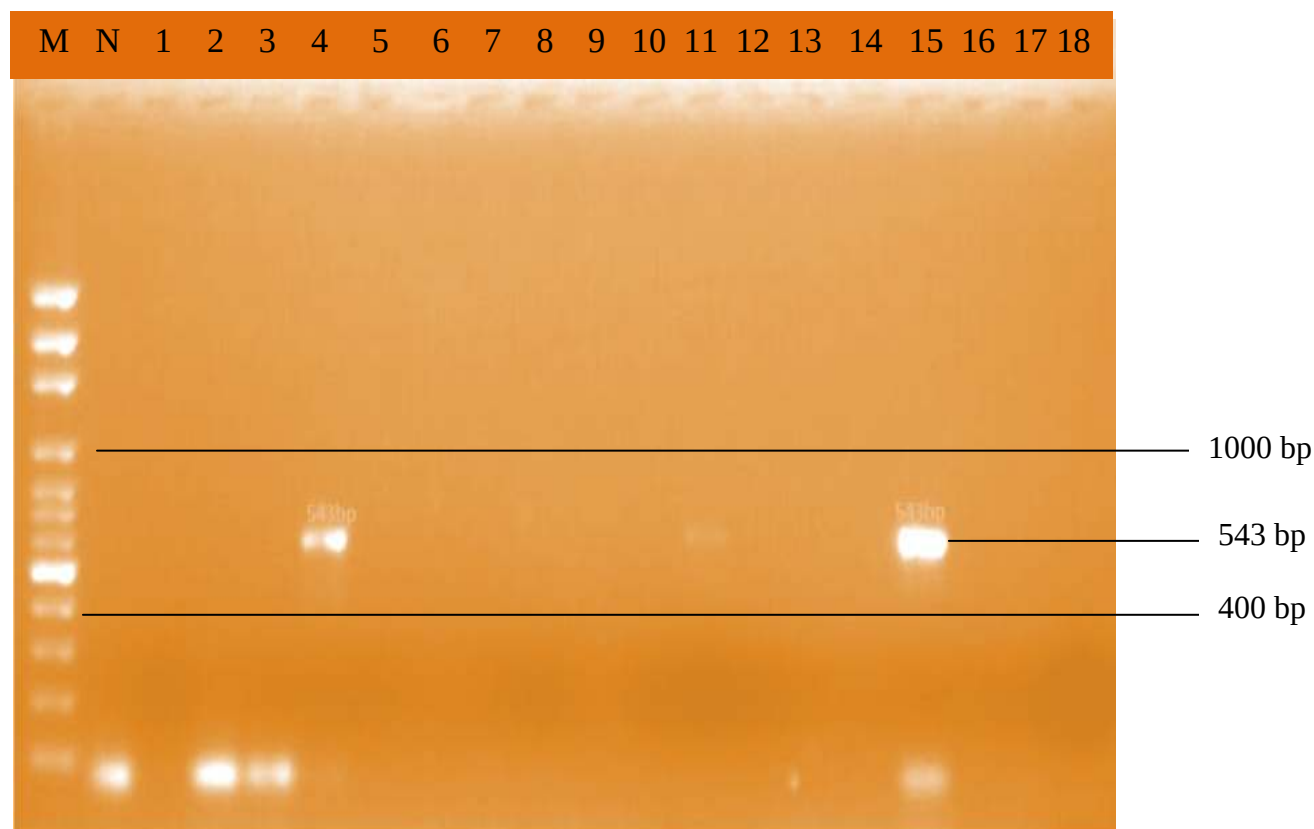
281	Lanes/Isolates	Number of Plasmids	Molecular Weight (bp)	MAR Patterns
282	L5=108	2	9416, 900	AUG TET NIT
283	L6=85	-	-	AUG STR COT TET
284				NIT
285	L7=151	2	9416, 4361	CH CPX AUG STR
286				COT TET NIT
287	L9=37	-	-	CH AUG STR COT
288				TET NIT
289	L10=14	-	-	CH AUG GEN COT
290				NIT
291	L12=152	1	9416	CPX AUG COT TET
292				NIT
293	L17=158	1	9416	AUG TET NIT

294 Key: L5, L9, L12, L17 = *Klebsiella pneumoniae*, L6= *Klebsiella oxytoca*, L7, L10 = *Klebsiella*
 295 *ozaenae*, CH-Chloramphenicol, AUG-Augmentin, CRO-Ceftriaxone, CPX-Ciprofloxacin, GEN-
 296 Gentamycin, STR-Streptomycin, TET-Tetracycline, COT-Cotrimoxazole, NIT-Nitrofurantoin.

297

298 Among the MAR *Klebsiella* isolates selected, there was amplification of *bla*CTX (543 bp) gene
 299 in Lanes 4, 11 and 15 representing *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Klebsiella*
 300 *pneumoniae* respectively from urine samples of in-patients. This implies that the isolates
 301 harboured *bla*CTX (543 bp) gene which codes for resistance to beta-lactams. However, none of
 302 the selected MAR *Klebsiella* isolates harboured *tetM* (974) gene. These results are shown in
 303 Plates 2a and 2b.

304



305

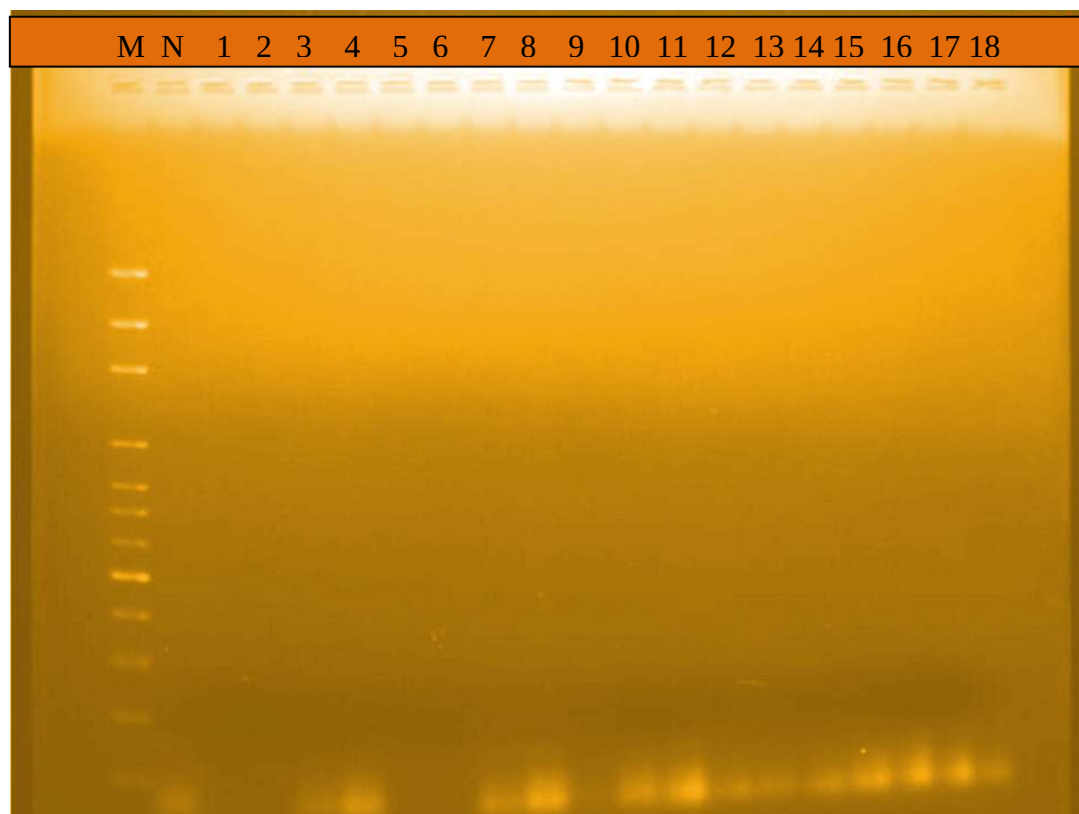
306 **Plate 2a. Agarose gel electrophoresis of the amplification products coding *bla*CTX (543 bp)**
 307 **gene in MAR *Klebsiella* sp. from urine samples of in- and out-patients.**

308 Lane M = 100 bp DNA Marker, Lane N = Negative control, Lane 1-5 (K402, K391-UTI, K95-
 309 Fibroid in pregnancy, K84, K108-?UTI) = *Klebsiella pneumoniae*, Lane 6 (K85) = *Klebsiella*
 310 *oxytoca*, Lane 7 (K151) = *Klebsiella ozaenae*, Lane 8, 9 (K326-?UTI, K37) = *Klebsiella*
 311 *pneumoniae*, Lane 10, 11 (K14, K339-Bladder outlet obstruction) = *Klebsiella ozaenae*, Lane 12,
 312 13 (K152, K88-Hypertension) = *Klebsiella pneumoniae*, Lane 14 (K272-Hypertension) =
 313 *Klebsiella ozaenae*, Lane 15 (K301-Benign prostate enlargement) = *Klebsiella pneumoniae*,

314 Lane 16 (K105-UTI) = *Klebsiella ozaenae*, Lane 17 (K158) = *Klebsiella pneumoniae*, Lane 18
315 (K255-Urethral stricture) = *Klebsiella oxytoca*.

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317



318

319 **Plate 2b. Agarose gel electrophoresis of the amplification products showing the absence of**
320 ***tetM* (974 bp) gene in MAR *Klebsiella* sp. from urine samples of in- and out-patients.**

321 Lane M = 100 bp DNA Marker, Lane N = Negative control, Lane 1-5 (K402, K391-UTI, K95-
322 Fibroid in pregnancy, K84, K108-?UTI) = *Klebsiella pneumoniae*, Lane 6 (K85) = *Klebsiella*
323 *oxytoca*, Lane 7 (K151) = *Klebsiella ozaenae*, Lane 8, 9 (K326-?UTI, K37) = *Klebsiella*
324 *pneumoniae*, Lane 10, 11 (K14, K339-Bladder outlet obstruction) = *Klebsiella ozaenae*, Lane 12,
325 13 (K152, K88-Hypertension) = *Klebsiella pneumoniae*, Lane 14 (K272-Hypertension) =
326 *Klebsiella ozaenae*, Lane 15 (K301-Benign prostate enlargement) = *Klebsiella pneumoniae*,
327 Lane 16 (K105-UTI) = *Klebsiella ozaenae*, Lane 17 (K158) = *Klebsiella pneumoniae*, Lane 18
328 (K255-Urethral stricture) = *Klebsiella oxytoca*.

329 4. Discussion

330 The recovery of *Klebsiella* sp. (7.02%) from urine samples of in- and out-patients in the study
 331 area establishes the fact that *Klebsiella* sp. can be found in the urine of in- and out-patients
 332 presenting different clinical conditions, with *Klebsiella pneumoniae* (59.4%) predominating
 333 among the isolates. This is supported by other studies, though with higher prevalence such as
 334 19.1% by Osundiya *et al.* (2013), 10.8% by Feglo *et al.* (2010) and 19.72% by Akter *et al.*
 335 (2014) on the investigation of *Klebsiella* sp. in urine samples from in- and out-patients in
 336 Nigeria, Ghana and India respectively. Hospital acquired infections (HAIs) have been shown to
 337 be of serious concern in patients and public health in developing countries. They are a major
 338 cause of death and increased morbidity in hospitalized patients. They may cause increased
 339 functional disability and emotional stress and may lead to conditions that reduce quality of life.
 340 Not only do they affect the general health of patients, but they are also a huge burden financially.
 341 The greatest contributors to these costs are the increased stays that patients with nosocomial
 342 infections require (WHO, 2002; Gastmeier, 2004). *Klebsiella* species commonly cause infections
 343 following intravenous and urinary catheterization, and infections complicating burns (Deirdre *et al.*,
 344 2006). Individuals who are immunosuppressed by therapy (e.g. cancer patients or transplant
 345 recipients) or by congenital defects of the immune system may develop *Klebsiella* infections.
 346 Such infections often have lethal course (Abbas and Lichtman, 2010). The use of antibiotics can
 347 be a factor that increases the risk of nosocomial infection with *Klebsiella* sp. (Weinserberg *et al.*,
 348 2009). Sepsis and septic shock can follow entry of the bacteria into the blood, and may lead to
 349 death (Dellinger *et al.*, 2008; Patel and Balk, 2012). High resistance to antibiotics was observed
 350 in most of the *Klebsiella* isolates. *Klebsiella* sp. has been reported to show high resistance rates
 351 to various antimicrobial agents including fluoroquinolones, tetracycline and beta-lactams
 352 (Okonko *et al.*, 2009a; Dashti *et al.*, 2010; Archana and Harsh, 2011; Orhue *et al.*, 2012; Olusola
 353 *et al.*, 2013; Nwosu *et al.*, 2014). *Klebsiella* isolates have been steadily increasing over the past
 354 years and they have been important sources of transferable antibiotic resistance (Shah *et al.*,
 355 2010). Reports from multiple studies from different parts of Nigeria though have observed
 356 temporal trends in the prevalence of resistance among enteric organisms; they have shown
 357 increasing prevalence in the last fifteen years (Okeke *et al.*, 2000; Nwosu *et al.*, 2014). The
 358 misuse of antimicrobial agents accelerates this natural phenomenon that is antibiotic resistance
 359 (Nwosu *et al.*, 2014). Poor infection control practices also encourage the spread of antimicrobial
 360 resistance (AMR) (WHO, 2013). The percentage multiple antibiotic resistances are higher in
 361 isolates from urine samples of in-patients than out-patients, implying a high risk of nosocomial
 362 infection. These isolates were observed to have resistance to commonly used antibiotics in
 363 treating *Klebsiella* related infections. Extensive use of broad-spectrum antibiotics in hospitalized
 364 patients has led to both increased carriage of *Klebsiella* and the development of multidrug
 365 resistant strains that produce extended-spectrum beta-lactamase (ESBL) (Archana and Harsh,
 366 2011; Sachse *et al.*, 2014).

367 The presence of plasmid DNA among the multiple antibiotic resistant *Klebsiella* isolates might
 368 have conferred antibiotic resistance in them. In hospital-acquired infections, for example, the
 369 same plasmid type may suggest single source of infection. The finding of multiple plasmid

profiles may also suggest multiple sources of infection or endogenous infections. Plasmids that carry several different resistance genes can confer resistance to multiple antibacterial agents. Antibacterial resistance genes can be exchanged between different bacterial strains or species via plasmids that carry these resistance genes (Baker-Austin *et al.*, 2006; Alekshun and Levy, 2007; Chen *et al.*, 2014a). Plasmids have been found to confer drug resistance to their host bacteria by various mating processes such as conjugation (Lipps, 2008).

The presence of *bla*CTX (543 bp) gene in the isolates might have mediated resistance to the beta-lactam antibiotics. Increasing resistance to broad spectrum cephalosporins in *Klebsiella* species predominantly due to the production of ESBLs were reported from different countries (Khanfar *et al.*, 2009). The beta-lactamases produced by bacteria are known to protect against the lethal effect of penicillins, cephalosporins and monobactams on their cell wall synthesis. The ESBL producing bacteria are increasingly causing urinary tract infections both in hospitalized and out-patients. This could make therapy of UTI difficult and promote greater use of expensive broad spectrum antibiotics, such as carbapenems (Mehrgan and Rahbar, 2008). Although *TetM* (974 bp) gene may be found in Gram-negative organisms, it was not detected in any of the MAR *Klebsiella* isolates represented. The *Klebsiella* isolates in this study may have other mechanisms of resistance or resistance genes (*tetK*, *tetA*, *tetO* e.t.c.) through which resistance to tetracycline was conferred; there may be a need for further investigation. The MAR *Klebsiella* isolates harbouring plasmid DNA and resistance gene were from in- and out-patients suffering from UTI, fibroid in pregnancy, diabetes, hypertension, urethral stricture, bladder outlet obstruction and benign prostate enlargement. This implies a high risk of nosocomial infection that may pose serious health threat; these highly resistant strains may raise difficult therapeutic options and clinical problems.

This study showed the presence of multiple antibiotic resistant *Klebsiella* sp. harbouring plasmid DNA and resistance genes in the study area that could be transferred from one patient to the other or to health care providers and vice versa especially when basic infection control measures are not taken. This is very important in the pathogenesis of community and hospital acquired infections. Promoting rational use of antibiotics and proper patient care, enhancing infection prevention and control in health care settings and fostering innovation, research and development are ways to overcome multiple antibiotic resistance problems.

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