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Phenotypic Identification and Detection of *Bla*CTX and *Tet*M in *Klebsiella* Species from Urine Samples of Patients in Ile-Ife, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author SYO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors SYO and OAO managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Klebsiella species from urine samples 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 twelve different antibiotics was determined by Kirby-Bauer's disc diffusion method on 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 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%. Most of the isolates had multiple antibiotic resistance (MAR) to at least three different classes of antibiotics. There was diversity in the MAR patterns with 21 different antibiotypes which ranged

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from two to ten different antibiotics. Plasmid DNA of molecular weight of 900 bp to 9416 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 tetracycline was not detected in any of the isolates. The study concluded that there were multiple antibiotic resistant *Klebsiella* species from urine samples of patients which harboured plasmid DNA and resistance (*bla* CTX) gene which is of great health and economic consequences.

Keywords: Klebsiella species; urine; in-patients and out-patients; multiple antibiotic resistance; resistance genes.

1. INTRODUCTION

Klebsiella species are 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 [1]. *Klebsiella* species is an important nosocomial microorganism which may cause high rate of fatality in hospital settings, affecting different age groups and sexes [2]. It 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 [3]. *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 [4]. Infections of the urinary tract are one of the most common infectious diseases [5-7].

Nosocomial infections occur worldwide, both in the developed and developing countries. More recently, hospital acquired infections (HAIs) have been shown to be a significant economic burden to patients and public health in developing countries [8,9]. Colonized patients have a higher risk of nosocomial infection than patients who are not colonized [10]. A variety of vehicles have been implicated in the spread of nosocomial pathogens [11]. In most of the patients, a variety of sites are colonized, mostly the urinary tract, with or without a serious infection [12]. In a hospital based patient survey in Saudi Arabia, 38 (33.3%) patients had hospital acquired infection [13], 6.7% was recorded among patients on admission in a study in Ghana [14].

Statistical data and evidences from researches prove that multidrug resistant bacteria are emerging worldwide which is a big challenge to healthcare. Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate using available antibiotics [15]. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and the development of multidrug resistant strains that produce extended-spectrum beta-lactamase (ESBL) [15,16]. *Klebsiella* isolates have steadily increased over the past years and they have been important sources of transferable antibiotic resistance [17]. For instance, the prevalent pathogens of UTIs have been found to be resistant to most chemotherapeutic agents [18].

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 [19]. Plasmids that carry several different resistance genes can confer resistance to multiple antibacterial agents [20]. *Klebsiella* species contain many plasmids that differ in number and molecular weight, carrying different types of genes including those encoding extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases and inhibitor resistant TEM beta-lactamases. These enzymes confer resistance to various antimicrobial agents including third and fourth generation cephalosporins, cephamycins, beta-lactamase/inhibitor combinations and carbapenems [21]. The number of resistance genes carried on the plasmids of multidrug resistant *Klebsiella pneumoniae* is usually more than one and occasionally as many as five genes are reported [22].

Indiscriminate use of third generation cephalosporins to treat Gram-negative bacterial infections is partly responsible for the emergence

of resistance to beta-lactams [17]. *Klebsiella pneumoniae* is at the forefront of antimicrobial resistance for Gram-negative pathogenic bacteria, as strains resistant to third-generation cephalosporins and carbapenems are 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 [23]. 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* species and *Enterobacter* species isolates were also detected [24].

Antibiotic resistance is a large and growing problem in the microbial world that accounts for most of Africa's disease burden [25]. Many factors play in the emergence of antibiotic resistant bacteria [26] 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.

2. MATERIALS AND METHODS

2.1 Study Population, Sampling and Bacteriological Analysis

Voided mid-stream urine samples were collected into sterile labelled universal bottles from in-patients (patients admitted into the hospital) and out-patients (patients who come to the hospital daily) in Obafemi Awolowo University Teaching Hospitals Complex and Obafemi Awolowo University Health Centre, Ile-Ife, Nigeria. The samples were collected after ethical clearance and informed consent were obtained. The subjects were educated to collect midstream urine specimen into 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 MacConkey agar plates. The identity of the isolates was further confirmed by various biochemical tests following Bergey's Manual of Determinative Bacteriology [27].

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), amoxicillin-clavulanic acid (30 µg), gentamycin (10 µg), pefloxacin (30 µg), ofloxacin (10 µg), streptomycin (30 µg), ceftriaxone (30 µg), nitrofurantoin (200 µg) and tetracycline (30 µg). 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 [28].

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 (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.

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

Primer	Sequence	Reference
*bla*CTX	(forward) 5'-ATGTGCAGYACCAGTAARGTKATGGC-3'	[29]
*bla*CTX	(reverse) 5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3'	
*tet*M	(forward) 5'-CGAACAAGAGGAAAGCATAAG-3'	[30]
*tet*M	(reverse) 5'-CAATACAATAGGAGCAAGC-3'	

2.4 Detection of *bla* CTX and *tet* M Genes in *Klebsiella* species

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 11 µg/ml of the DNA template (total DNA of bacteria), 4.0 µl of the master mix (Solis Biodyne, Estonia), 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 (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.

3. RESULTS

A total of thirty-two *Klebsiella* species were isolated from 456 urine samples of in-patients and out-patients collected. These include nineteen and thirteen *Klebsiella* isolates from in-patients and out-patients, respectively. These isolates comprised *Klebsiella pneumoniae* (59.4%), *Klebsiella ozaenae* (28.1%) and *Klebsiella oxytoca* (12.5%). Antibiotic resistance was high and varied among the isolates. Most of the isolates were resistant to amoxicillin-clavulanic acid (93.75%), tetracycline (71.88%) and ciprofloxacin (31.25%) being the least.

The multiple antibiotic resistant (MAR) isolates were described as being resistant to three or more different classes of antibiotics. There was diversity in the MAR pattern with 21 different antibiotypes which ranged from two to eleven different antibiotics. This consists of *Klebsiella pneumoniae* (59.4%), *Klebsiella ozaenae* (28.1%) and *Klebsiella oxytoca* (12.5%). The most prevalent antibiotype (C CIP AMC CN COT TE NIT) was expressed in *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Klebsiella oxytoca* isolated from urine samples of both in- and out-patients, and were resistant to all the antibiotics tested. The MAR patterns of the isolates are represented in Tables 3a and 3b.

The molecular weight of the plasmid DNA ranged from 900 to 9416 bp. However, *Klebsiella pneumoniae* from urine samples of in-patients had a multiple plasmid DNA with molecular weight of 900, 6557 and 9416 bp. *Klebsiella ozaenae* and *Klebsiella pneumoniae* from urine samples of out-patients also harboured multiple plasmid DNA of molecular weight ranging from 900 to 9416 bp. The agarose gel electrophoresis of plasmid DNA in the MAR *Klebsiella* isolates is represented in Plate 1. Tables 4a and 4b show the molecular weight of the plasmid DNA.

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

Antibiotics (µg)	Number of isolates (n)	Percentage (n%)		
		Susceptibility	Intermediate	Resistant
Chloramphenicol (30 µg)	32	37.50	18.75	43.75
Ciprofloxacin (10µg)	32	53.13	15.63	31.25
Amoxicillin (30 µg)	32	12.50	25.00	62.50
Amoxicillin-Clavulanic acid (30 µg)	32	0.00	6.25	93.75
Gentamycin (10 µg)	32	62.50	3.13	34.38
Ofloxacin (10 µg)	32	62.50	6.25	31.25
Streptomycin (30 µg)	32	46.88	12.50	40.63
Nitrofurantoin (200 µg)	32	0.00	0.00	100.00
Cotrimoxazole (25 µg)	32	34.38	18.75	46.88
Tetracycline (30 µg)	32	6.25	21.88	71.88
Ceftriaxone (30 µg)	32	3.13	28.13	68.75

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

Isolates	Number of antibiotic classes	MAR patterns	Frequency n	n%
Klebsiella pneumoniae	3	AMC CN NIT	1	0.0625
		AUC TE NIT	1	0.0625
		CRO TE NIT	1	0.0625
	4	AMC CN TE NIT	1	0.0625
		C AMC TE NIT	1	0.0625
	5	C AMC CN TE NIT	1	0.0625
	7	C CIP AMC CN COT TE NIT	1	0.0625
		C CIP CRO CN COT TE NIT	1	0.0625
Klebsiella ozaenae	3	AMC TE NIT	1	0.0625
	4	C AMC COT NIT	1	0.0625
	6	C AMC CN COT TE NIT	1	0.0625
		CIP AMC CN COT TE NIT	1	0.0625
	7	C CIP AMC S COT TE NIT	1	0.0625
		C CIP AMC S COT TE NIT	1	0.0625
		C CIP CRO S COT TE NIT	1	0.0625
Klebsiella oxytoca	3	AMC TE NIT	1	0.0625
Total			16	

Key: *C-Chloramphenicol, AMC-Amoxicillin-Clavulanic acid, AMX-Amoxicillin, CRO-Ceftriaxone, CIP-Ciprofloxacin, CN-Gentamycin, S-Streptomycin, TE-Tetracycline, COT-Cotrimoxazole, NIT-Nitrofurantoin.*

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

Isolates	Number of antibiotic classes	MAR patterns	Frequency n	n%
Klebsiella pneumoniae	3	AMC TE NIT	2	0.2
	5	CIP AMC COT TE NIT	1	0.1
	6	C AMC S COT TE NIT	1	0.1
	7	C CIP AMC CN COT TE NIT	1	0.1
Klebsiella ozaenae	5	C AMC CN COT NIT	1	0.1
	7	C CIP AMC S COT TE NIT	1	0.1
Klebsiella oxytoca	3	CRO TE NIT	1	0.1
	5	AMC S COT TE NIT	1	0.1
	7	C CIP AMC CN COT TE NIT	1	0.1
Total			10	

Key: *C-Chloramphenicol, AMC-Amoxicillin-Clavulanic acid, AMX-Amoxicillin, CRO-Ceftriaxone, CIP-Ciprofloxacin, CN-Gentamycin, S-Streptomycin, TE-Tetracycline, COT-Cotrimoxazole, NIT-Nitrofurantoin.*

Klebsiella pneumoniae (2) and *Klebsiella ozaenae* (1) from urine samples of in-patients harboured *bla*CTX (543 bp) gene coding for resistance to beta-lactams, respectively as depicted by Lanes 4, 11 and 15. However, none of the selected MAR *Klebsiella* isolates harboured *tet*M (974) gene. These results are shown in Plate 2.

4. DISCUSSION

The recovery of *Klebsiella* species (7.02%) from urine samples of in-patients and out-patients in the study area establishes the fact that *Klebsiella* sp. can be found in the urine of in-patients and out-patients presenting different clinical conditions, with *Klebsiella pneumoniae* (59.4%) predominating among the isolates. This is supported by other studies, though with higher prevalence such as 19.1% by [31], 10.8% by [32] and 19.72% by [33] on the investigation of *Klebsiella* species in urine samples from in-patients and out-patients in Nigeria, Ghana and India, respectively. Hospital acquired infections (HAIs) have been shown to be of serious concern in patients and public health in

developing countries. They are a major cause of death and increased morbidity in hospitalized patients. They may cause increased functional disability and emotional stress and may lead to conditions that reduce quality of life. Not only do they affect the general health of patients, but they are also a huge burden financially. The greatest contributors to these costs are the increased stays that patients with nosocomial infections require [34,35]. *Klebsiella* species commonly cause infections following intravenous and urinary catheterization, and infections complicating burns [36]. Individuals who are immunosuppressed by therapy (e.g. cancer patients or transplant recipients) or by congenital defects of the immune system may develop *Klebsiella* infections. Such infections often have lethal course [37]. The use of antibiotics can be a factor that increases the risk of nosocomial infection with *Klebsiella* sp. [38]. Sepsis and septic shock can follow entry of the bacteria into the blood, and may lead to death [39,40]. High resistance to antibiotics was observed in most of the *Klebsiella* isolates. *Klebsiella* species have been reported to show high resistance rates to various antimicrobial agents including fluoroquinolones, tetracycline and beta-lactams [15, 41-45]. *Klebsiella* isolates have been steadily increasing over the past years and they have been important sources of transferable antibiotic resistance [17]. Reports from multiple studies from different parts of Nigeria though have observed temporal trends in the prevalence of resistance among enteric organisms; they have shown increasing prevalence in the last fifteen years [45,46].The misuse of antimicrobial agents accelerates this natural phenomenon that is antibiotic resistance [45]. Poor infection control practices also encourage the spread of antimicrobial resistance (AMR) [47]. The percentage multiple antibiotic resistances are higher in isolates from urine samples of in-patients than out-patients, implying a high risk of nosocomial infection. These isolates were observed to have resistance to commonly used antibiotics in treating *Klebsiella* related infections. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and the development of multidrug resistant strains that produce extended-spectrum beta-lactamase (ESBL) [15,16].

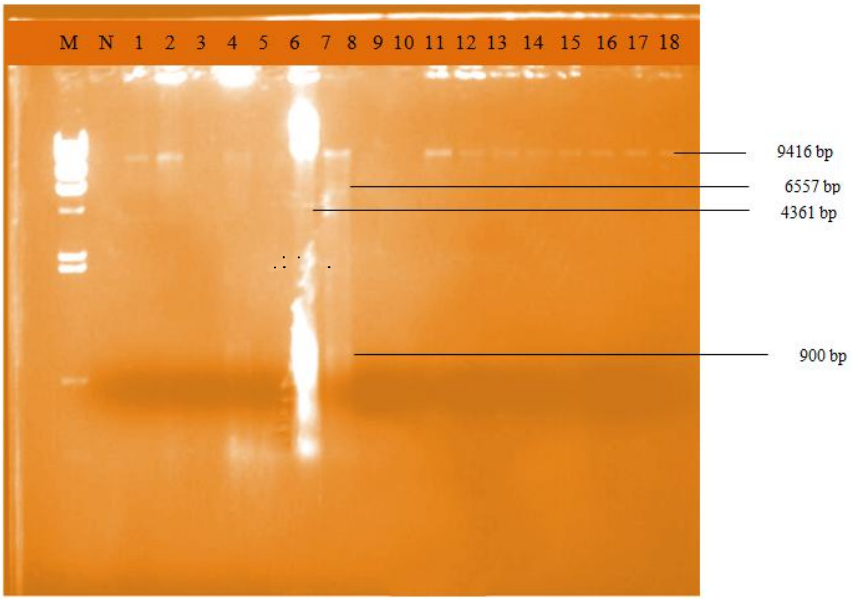

Plate 1. Plasmid profile of MAR *Klebsiella* sp. from urine samples of in-patients and out-patients

Lane M = Lambda DNA/HindIII Marker, 2, Lane N = Negative control, Lane 1-5 (K402, K391-UTI, K95-Fibroid in pregnancy, K84, K108-?UTI) = Klebsiella pneumoniae, Lane 6 (K85) = Klebsiella oxytoca, Lane 7 (K151) = Klebsiella ozaenae, Lane 8, 9 (K326-?UTI, K37) = Klebsiella pneumoniae, Lane 10, 11 (K14, K339-Bladder outlet obstruction) = Klebsiella ozaenae, Lane 12, 13 (K152, K88-Hypertension) = Klebsiella pneumoniae, Lane 14 (K272-Hypertension) = Klebsiella ozaenae, Lane 15 (K301-Benign prostate enlargement) = Klebsiella pneumoniae, Lane 16 (K105-UTI) = Klebsiella ozaenae, Lane 17 (K158) = Klebsiella pneumoniae, Lane 18 (K255-Urethral stricture) = Klebsiella oxytoca

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

Lanes/Isolates	Number of Plasmids	Molecular Weight (bp)	MAR Patterns
L1=402	1	9416	AMC TE NIT
L2=391	1	9416	AMC CN NIT
L3=95	-	-	CRO TE NIT
L4=84	1	9416	C CIP AMC CN COT TE NIT
L8=326	3	9416, 6557, 900	AMC CN TE NIT
L11=339	1	9416	CIP AMC CN COT TE NIT
L13=88	1	9416	C AMC TE NIT
L14=272	1	9416	C CIP AMC S COT TE NIT
L15=301	1	9416	C AMC CN TE NIT
L16=105	1	9416	CH CIP CRO S COT TE NIT
L18=255	1	9416	AMC TE NIT

Key: LI-L4, L8, L12, L13, L15 = Klebsiella pneumoniae, L11, L14, L16 = Klebsiella ozaenae, L18 = Klebsiella oxytoca, C-Chloramphenicol, AMC-Amoxicillin-Clavulanic acid, CRO-Ceftriaxone, CIP-Ciprofloxacin, CN-Gentamycin, S-Streptomycin, TE-Tetracycline, COT-Cotrimoxazole, NIT-Nitrofurantoin.

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

Lanes/Isolates	Number of plasmids	Molecular Weight (bp)	MAR Patterns
L5=108	-	-	AMC TE NIT
L6=85	2	9416, 4361	AMC S COT TE NIT
L7=151	3	9416, 4361,900	C CIP AMC S COT TE NIT
L9=37	-	-	C AMC S COT TE NIT
L10=14	-	-	C AMC CN COT NIT
L12=152	1	9416	CIP AMC COT TE NIT
L17=158		9416	AMC TE NIT

Key: L5, L9, L12, L17 = Klebsiella pneumoniae, L6= Klebsiella oxytoca, L7, L10 = Klebsiella ozaenae, C-Chloramphenicol, AMC-Amoxicillin-Clavulanic acid, CRO-Ceftriaxone, CIP-Ciprofloxacin, CN-Gentamycin, S-Streptomycin, TE-Tetracycline, COT-Cotrimoxazole, NIT-Nitrofurantoin

The presence of plasmid DNA among the multiple antibiotic resistant *Klebsiella* isolates might have conferred antibiotic resistance in them. In hospital-acquired infections, for example, the 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 [20,48,49]. Plasmids have been found to confer drug resistance to their host bacteria by various mating processes such as conjugation [50].

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 [51]. The beta-lactamases produced by bacteria are known to protect them 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 [52]. Although *Tet*M (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 (*tet*K, *tet*A, *tet*O etc.) 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

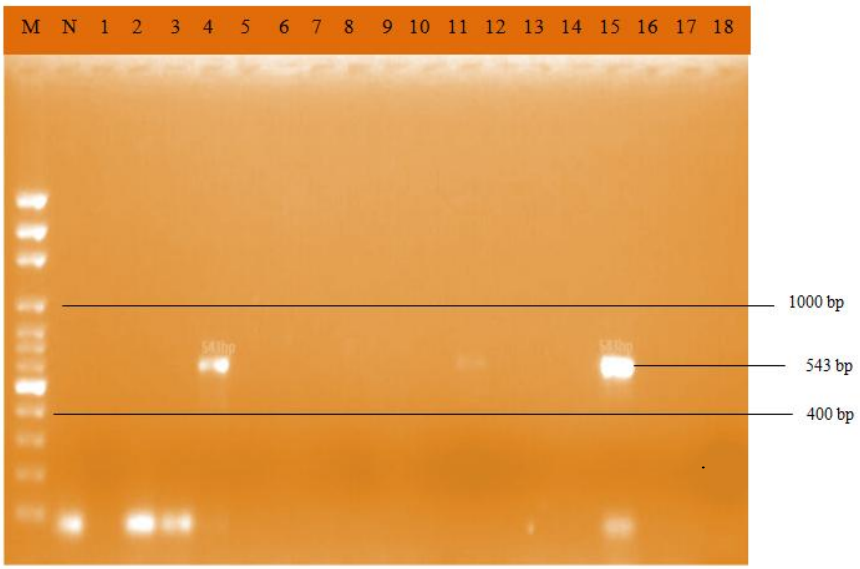

Plate 2. Agarose gel electrophoresis of the amplification products coding *bla*CTX (543 bp) gene in MAR *Klebsiella* sp. from urine samples of in-patients and out-patients

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

serious health threat; these highly resistant strains may raise difficult therapeutic options and clinical problems.

5. CONCLUSION

This study showed the presence of multiple antibiotic resistant *Klebsiella* species harbouring plasmid DNA and resistance *bla*CTX (543 bp) gene 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.

6. LIMITATION

During this study, there was restriction in sample collection as patients who did not give their consent were exempted.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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