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Extended Spectrum Beta- Lactamase- Producing Uropathogenic *Escherichia coli* in Pregnant Women Diagnosed With Urinary Tract Infections in South-Western Nigeria

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Abstract

Extended spectrum beta-lactamase (ESBL)-producing uropathogenic *Escherichia coli* (UPEC) in symptomatic pregnant women with confirmed urinary tract infections in Southwest. Nigeria was reported. Susceptibility of UPEC isolates to β-lactam and other classes of antibiotics was determined by the Kirby-Bauer's disc diffusion method on Mueller-Hinton agar plate. Detection of plasmid DNA in multiple antibiotic resistant isolates was carried out by alkali lysis (TENS) method. Extended-spectrum-β-lactamase (ESBL) production was determined by double disk synergy test (DDST). Isolates that were positive to ESBL were mated with non-ESBL- producing *E. coli* and other enterics in a conjugation experiment. Transfer of ESBL-enzyme and other resistance phenotypes in the transconjugants was investigated by DDST. Data obtained were statistically analyzed using SPSS 17. Greater percentage of the isolates were multiple antibiotic resistant (MAR). Sixty-nine (26.1 %) of UPEC were ESBL producers. Some of the ESBL producers transferred ESBL- enzyme and other resistance determinants to the recipients. Large size plasmid DNA of molecular weight (23.13-33.04 kb) was detected in some representative MAR isolates.

Keywords: ESBL, UPEC, pregnant women, UTI, multiple antibiotic resistance

1. Introduction

Escherichia coli have been reported to be common causes of hospital acquired infections which can have severe clinical implications with corresponding multiple antibiotic resistance (Aibinu et al., 2002). Extended spectrum β–lactamases (*ESBLs*) are plasmid mediated enzymes that confer resistance to penicillin, third generation cephalosporins and aztreonam but are inhibited by clavulanic acid (Paterson and Bonomo, 2005). In Africa, *ESBLs*-producing bacteria have been reported in Egypt, Morocco, Tunisia, Senegal and South Africa (Bloomberg et al., 2005).

Extended-spectrum β-lactamase (ESBL)-producing enterobacteriaceae have become widespread in hospitals and are increasing in community settings where they cause a variety of infections. In addition to hydrolyzing most β-lactam agents, bacteria harboring these enzymes display resistance to other unrelated antimicrobial agents and thus often pose a therapeutic dilemma (Maina et al., 2013). Increase in ESBL-producing enteric Gram-negative bacteria has led to the choice of inappropriate therapy; as a result, the rate of resistance has increased. Antibiotic therapy of infections due to ESBL-producing pathogens is still a clinical challenge. In most cases, carbapenems and fluoroquinolones have been used (Ramphal & Ambrose, 2006).

Horizontal gene transfer by plasmid exchange between *E. coli* strains is a recognized source of rapid spread of antimicrobial resistance phenotypes (Fang et al., 2006). The significance of plasmids in disseminating antimicrobial resistance genes is further enhanced by the association of plasmids with mobile genetic elements, such as transposons, integrons and insertion elements (Pitout et al., 2007). Resistance to third generation cephalosporins, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin and amikacin was detected in the ESBL- producer group. Selective pressure of the antimicrobials selects those strains that are resistant to the applied antimicrobials causing the resistant strains to multiply and spread. In the last decade, CTX-M enzymes have replaced TEM and SHV mutants as the most prevalent ESBLs worldwide, with *E. coli* being the major host (Livermore et al., 2007; Paterson & Bonomo, 2005). Extended spectrum beta-lactamases production have been

reported among *E. coli* in both hospital and community settings ((Pitout et al., 2007)). They have also been detected in pets and farm animals, products of the food chain and sewage (Warren et al., 2008). The resistance rate of *E. coli* to third generation cephalosporins (3GCs) is a broad indicator of the occurrence of ESBLs. It has been discovered that, travellers to countries with high rates of ESBLPCs (e.g. Egypt, India, etc) readily acquire asymptomatic faecal carriage.

Extended spectrum β-lactamase-producing *E. coli* and other enterobacteriaceae, particularly those producing CTX-M, have spread rapidly among humans and there is evidence of spread among animal populations. Factors that influence the spread of resistance genes as well as resistant bacteria include, antimicrobial usage, co-selection for resistance genes

A number of risk factors of acquiring ESBL-producing bacteria have been identified in hospitalized patients, most of which also apply to other multi-resistant Gram-negative bacilli. These risk factors include: prolonged hospital stay; prior hospitalization; previous use of 3GCs, aminoglycosides and quinolones; presence of medical devices such as urinary catheters and mechanical ventilation (Rodriguez-Bano et al., 2006). In the case of community acquired ESBL infections, older age, female gender, recurrent UTIs/prior invasive procedures (e.g. catheterisation), known faecal carriage, contact with healthcare facilities/residents in care homes and previous antimicrobial treatment are the risk factors (Moor et al., 2008).

Travellers to areas of the world such as India where very high rates of *ESBL* are present, have been noted to become readily colonized, asymptomatically, with CTX-M-producing *ESBL* strains (Tham et al., 2010). The present study reports the prevalence of ESBL- producing UPEC in pregnant women with confirmed UTIs in Ondo and Ekiti States, south-western Nigeria.

2. Methodology

2.1 Study Design *and* Sample Collectio*n*

The study population include symptomatic UTIs pregnant women in Ekiti and Ondo States, South-western Nigeria. Ekiti State is located between longitudes 40°51′ and 50°451′ east of the Greenwich meridian and latitudes 70°151′ and 80°51′ north of the Equator while Ondo State lies between longitudes 4"30" and 6" East of the Greenwich Meridian, 5" 45" and 8" 15" North of the equators

The preliminary identification of *E. coli* recovered from 400 voided mid-stream urine samples of symptomatic pregnant women with confirmed cases of UTIs was based on colonial morphology by a characteristic green metallic sheen on EMB agar (Oxoid Ltd., Hampshire, England) plate. The identity of isolate was confirmed by various conventional biochemical tests with reference to Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.2 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of isolate was performed by Kirby-Bauer's disc diffusion method. The antibiotics tested and their concentrations (μg) include; cefadroxil (30), ampicillin (10), nalidixic acid (30), cefepime (30), augumentin (30), cefuroxime (30), ceftazidime (30), cefotaxime (30) (Oxoid, Basingstoke, Hampshire, UK); amoxicillin (30), gentamicin (10), ofloxacin (5), ciprofloxacin (30), tetracycline (25), augmentin (30), ceftriazole (30), nitrofurantoin (300), cotrimoxazole (30), and pefloxacin (Fondos, Nigeria). The standardized inoculum (0.5 McFarland Barium Sulphate turbidity standard- 10^6 CFU/ml) was seeded on Mueller-Hinton Agar (MHA) (Hi Media, Vadhani, India) plates. The antibiotic disks were firmly placed on the sterile MHA plates (Oxoid, England) and incubated at 37^{o}C for 24 h. Diameter of zones of inhibition was measured to the nearest millimeter using a transparent calibrated ruler and compared to the Clinical and Laboratory Standards Institute (2012). *Escherichia coli* ATCC 25922 was used as reference.

2.3 Detection of β-lactamase Enzymes Producing E. coli

Extended spectrum β-lactamase production among the isolates was detected following the double-disk synergy technique (DDST) (Clinical and Laboratory Standards Institute (CLSI), 2012). The standardized isolates were subjected to double-disk synergy tests (DDST) on sterile Mueller Hinton agar plates. *Escherichia coli* ATCC 25922 was used as reference. The test was performed by placing ceftazidime (30 μg) and cefotaxime (30 μg) at 20 mm (center to center) from a centre disk containing augmentin (30 μg) (amoxicillin (20 μg) plus clavulanate (10 μg). Enhancement or potentiation in the zone of inhibition of any of the antibiotic disks toward the center disk containing clavulanic acid indicates the presence of ESBLs (Clinical and Laboratory Standards Institute (CLSI), 2012).

2.4 In Vitro Conjugation Experiment

Minimum inhibitory concentrations of the antibiotics (augmentin, amoxicillin, cefadroxil, Cefotaxime, cefepime, ceftazidime, cefuroxime, alidixicAcid, ciprofloxacin, ofloxacin, pefloxacin, gentamicin, tetracycline,

cotrimoxazole) used in the conjugation experiments were determined by agar dilution method as prescribed by CLSI.[6] Conjugation experiment was performed by the mating assay using the method of Aibinu et al. (2003) in tryptic soy broth for six of the ESBL-producing *E. coli* isolates as donors and non- ESBL-producing *E. coli* ATCC 25922 and other enterobacteriaceae (*Klebsiella* sp, *Proteus* sp., *Salmonella* sp. and *Shigella* sp) as recipients. The donors were tested and confirmed to be sensitive to the antibiotic resistance markers on MHA plates. A suspension of each organism was made in the sterile double strength nutrient broth at 37°C and adjusted to 0.5 McFarland Standard. The donor and the recipient were then mixed in a ratio 1:9 (50 µl of the donor to 450 µl of the recipient) and incubated at room temperature for 3 h for conjugation to take place.

One milliliter of each conjugated samples was serially diluted (10-folds) and 0.1 ml from 10^{-3} and 10^{-4} dilution fractions was spread inoculated onto the surface MacConkey, eosin methylene blue agar and *Salmonella-Shigella* agar plates supplemented with appropriate minimum inhibitory concentration of antibiotics used as the recipients' markers. Transfer of resistance was read by observation of recovery of the recipient colonies on the agar plates containing the corresponding antibiotics. The transconjugants were subjected to DDST to confirm the transfer of ESBL enzymes and co-transfer of other resistance determinants present in the donor isolates.

2.5 Plasmid Profiling

Plasmid DNA extraction of selected multiple antibiotic resistant ESBL-producing isolates was performed using the alkaline lysis 'TENS' (Tris 25 mM, EDTA 10 mM, 0.1 N NaOH and 0.5% SDS- all Sigma products) method of Kraft et al. (1988) and Lech et al. (1987). The extracted plasmid DNA was separated on 0.8% agarose gel (Oxoid, Basingstoke, England) in a 20-40 µl of TE (Tris-EDTA) buffer and a 100 bp ladder (Promega, Madison, USA) was used as standard. The electrophoretic products were viewed using ultraviolet trans-illuminator and the plasmid size was compared to the reference marker.

2.6 Statistical Analysis

Significant differences and relationship between the prevalence of ESBL- producing UPEC strains in pregnant women in the study areas were compared using analysis of variance (SPSS 17 version). A value of $p < 0.05$ was set as significant

3. Results

Table 1. Prevalence of antibiotic resistance among ESBL-producing uropathogenic *E. coli* in pregnant women with UTI in Ondo and Ekiti States

Antibiotics tested		% Resistance of the (MAR) isolates (n=264)					
		ESBL (%) (n=69)			Non ESBL (%) (n=195)		
Classes of antibiotics tested	Specific antibiotics tested	Ondo	Ekiti	Total (%)	Ondo	Ekiti	Total (%)
β-Lactams	Augmentin	34	30	64 (92.8)	57	57	114 (58.5)
	Amoxicillin	33	31	64 (92.8)	57	68	125 (64.1)
	Ampicillin	35	33	68 (98.6)	75	77	152 (77.9)
	Ceftriaxone	30	32	62 (89.6)	79	87	166 (85.1)
	Cefadroxil	36	32	68 (98.6)	93	93	186 (95.4)
	Cefotaxime	31	29	60 (87.0)	54	66	120 (61.5)
	Cefepime	14	20	34 (49.3)	16	26	42 (21.5)
	Ceftazidime	36	31	67 (97.1)	92	92	184 (94.4)
	Cefuroxime	34	33	67 (97.1)	87	88	175 (89.7)
Quinolones	Nalidixic acid	28	28	56 (81.2)	57	65	122 (62.6)
	Ciprofloxacin	28	24	52 (75.4)	50	66	116 (59.5)
	Ofloxacin	28	22	50 (72.5)	50	58	108 (55.4)
	Pefloxacin	34	30	64 (92.8)	60	71	131 (67.2)
Aminoglycosides	Gentamicin	28	30	58 (84.1)	34	72	106 (54.4)
Nitrofurantoins	Nitrofurantoin	31	27	58 (84.1)	58	68	126 (64.6)
Tetracyclines	Tetracycline	36	33	69 (100)	90	91	181 (92.8)
Sulphonamides/Trimethroprim	Cotrimoxazole	32	32	64 (92.8)	68	88	156 (80.0)

Key: MAR= Multiple antibiotic resistant; ESBL= Extended spectrum β-lactamase.

Table 2. Multiple antibiotic resistance (MAR) among ESBL-producing uropathogenic *E. coli* in pregnant women with UTIs in Ondo and Ekiti States

Number of classes of antibiotics tested	ESBL producer (n=69)		Total	Percentage (%)
	Ondo State	Ekiti State		
6	15	21	36	52.2
5	10	8	18	26.1
4	7	4	11	15.9
3	4	0	4	5.8

Key: MAR = Multiple antibiotic resistant; ESBL= Extended spectrum β-lactamase.

Table 1 shows the prevalence of antibiotic resistance among ESBL- producing UPEC isolates in Ondo and Ekiti States. Sixty-nine (26.1%) of the UPEC were ESBL- producing strains with concomitant multiple antibiotic resistance profiles. Isolates that produced ESBL-enzymes showed high rate of resistance to the extended spectrum β-lactam and other classes of antibiotics as well. There was a significant statistical difference in the incidence of resistance between ESBL- and non- ESBL- producing *E. coli* isolates in Ondo and Ekiti States ($P < 0.05$) (Table 1).

Each of the ESBL producers was resistant to more than one class of antibiotic. The ESBL-producing UPEC (52.2%) were resistant to all the six classes of antibiotics tested, 26.1% to five, 15.9% to four, and 5.8% to three classes (Table2).

Figure 1 shows the percentage rate of transfer of resistance phenotypes to the transconjugants. Extended spectrum β-lactamase enzyme and other antibiotic resistance phenotypes were transferred to non-ESBL- producing *E. coli, Salmonella* sp., *Shigella* sp., *Proteus vulgaris* and *Klebsiella* sp. recipients. Consequently, resistance to other antibiotics was also transferred in the same trend, augmentin, cefotaxime and ceftazidime were 100% transferred to the transconjugants, followed by amoxicillin (98.0%), tetracycline (94.0%) and cotrimoxazole (90.0%). However, pefloxacin, ciprofloxacin, ofloxacin and nalidixic acid resistance traits were not transferred (Figure 1).

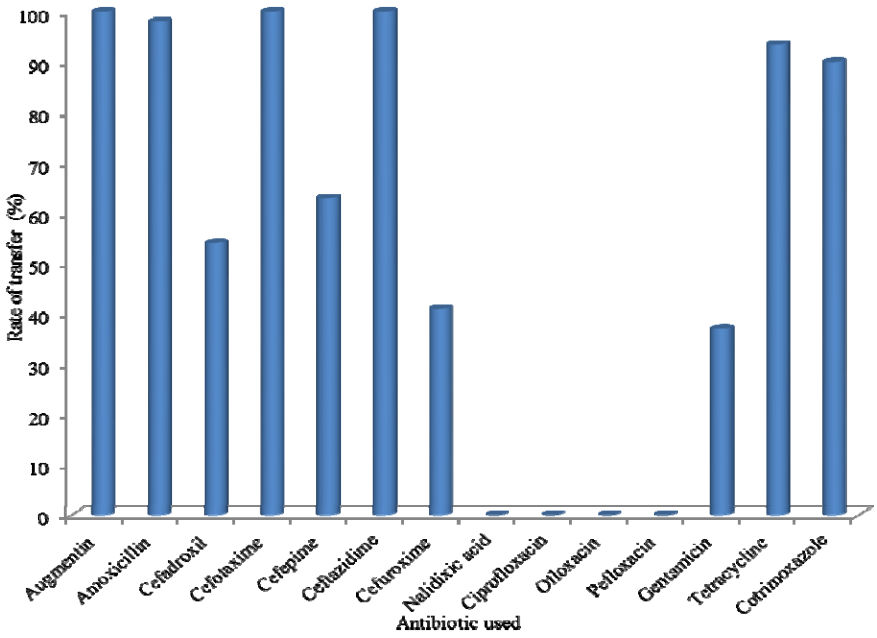

Figure 1. Rate of transfer of resistance phenotypes to the transconjugants (%)

AUG=Augmentin, AMX=Amoxicillin, CFR=Cefadroxil, CTX=Cefotaxime, FEP=Cefepime, CAZ=Ceftazidime, CXM=Cefuroxime, NAL=NalidixicAcid, CPX=Ciprofloxacin, OFL=Ofloxacin, PFX=Pefloxacin, GEN=Gentamicin, TET=Tetracycline, COT=Cotrimoxazole.

Molecular weights of plasmid DNA in the representative MAR -UPEC isolates are presented in table 4 and the gel electrophoresis of the amplified plasmid DNA is shown in Figure 2. Large plasmid size of molecular weights ranging from 23.13– 33.04 kb were harboured by the selected MAR isolates, and 62.5% of the isolates were ESBL- producing strains. Some of the non-β-lactam antibiotic resistant isolates had same plasmid DNA size as those of ESBL-producing UPEC (Table 3).

Table 3. The molecular weight of plasmid DNA in the representative MAR uropathogenic *E. coli* in pregnant women with UTIs in Ondo and Ekiti States

Isolate code	Antibiotics to which isolates were resistant	Plasmid (Estimated) Mol. Wt. [kb]
EKNG028	AUG, AMX, AMP, CRO, CFR, CTX, CAZ, CXM, GEN, NIT, TET, COT	25.70
EKNG114	AUG, AMX, AMP, CRO, CFR, CTX, FEP, CMX, GEN, NIT, TET, COT	25.70
ODNG051	AUG, AMX, CRO, CFR, CAZ, CXM, GEN, NIT, TET, COT	25.70
ODNG172	AUG, AMX, AMP, CFR, FEP, CAZ, CXM, GEN, NIT, TET, COT	25.70
EKNG099	AUG, AMP, CRO, CFR, FEP, CAZ, CXM, NIT, TET, COT	25.70
EKNG004	AUG, AMX, AMP, CRO, CRO, CFR, CTX, FEP, CAZ, CXM, GEN, TET, COT	25.70
EKNG060	AUG, AMX, AMP, CRO, CFR, CTX, FEP, CAZ, CXM, GEN, NIT, TET, COT	25.70
ODNG124	AUG, AMX, AMP, CRO, CFR, CTX, CAZ, CXM, GEN, NIT, COT	25.70
ODNG059	AUG, AMX, AMP, CRO, CFR, CTX, FEP, CAZ, CXM, GEN, NIT, TET, COT	33.04
0DNG024	AUG, AMX, AMP, CRO, CFR, CTX, CAZ, CXM, GEN, NIT, TET, COT	33.04
EKNG007	AUG, AMX, AMP, CRO, CFR, CTX, FEP, CAZ, CXM, GEN, NIT, TET, COT	33.04
EKNG080	AUG, AMX, AMP, CRO, CFR, CTX, CAZ, CXM, GEN, NIT, TET, COT	33.04
ODNG021	AUG, AMX, AMP, CRO, CFR, CTX, CAZ, CXM, GEN, NIT, TET, COT	23.13
EKNG022	AUG, AMP, CRO, CFR, CTX, CAZ, CXM, GEN, NIT, TET, COT	23.13

EKNG= Isolates from Ekiti State, Nigeria; ODNG= Isolates from Ondo State, Nigeria.

AUG= Augmentin AMX=Amoxicillin AMP=Ampicillin CRO=Ceftriaxone CFR=Cefadroxil
CTX=Cefotaxime FEP=Cefepime CAZ=Ceftazidime CXM=Cefuroxime GEN=Gentamicin
NIT=Nitrofurantoin TET=Tetracycline COT=Cotrimoxazole AMC=Amoxicillin/Clavulanic Acid

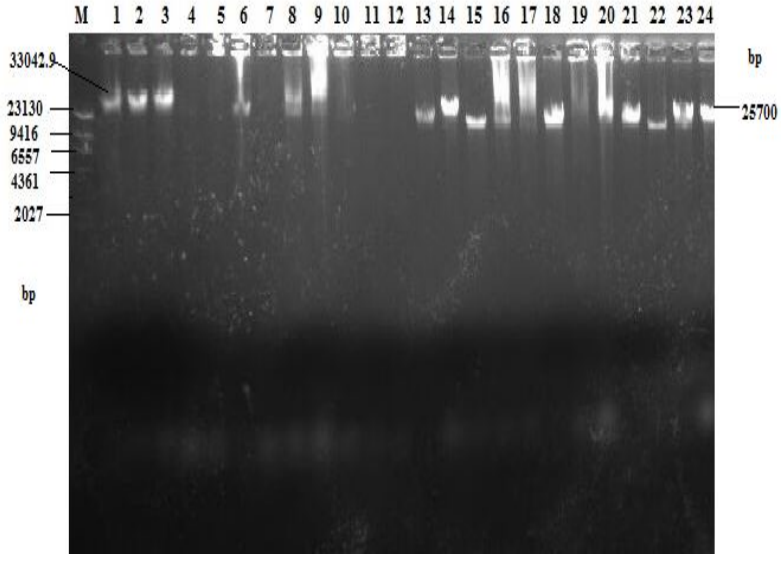

Figure 2. Gel electrophoresis of the amplified plasmid DNA of selected multiple antibiotic resistant uropathogenic *E. coli* in Ondo and Ekiti Sates

Lane M: DNA marker, Lanes 1-24: the test isolates.

4. Discussion

Present study reveals 26.1% prevalence of ESBL-enzyme production among UPEC in symptomatic UTIs pregnant women in Ondo and Ekiti States, Nigeria. This contradicts the earlier reports of 75% by Padmavathy et al. (2014), 52.4% by Yusuf et al. (2005), 52.4% by Iroha et al. (2008), and 37.3% by Aibinu et al. (2003) on prevalence of ESBL enzymes detected in *E. coli* from UTI patients in the East, South and Northern Nigeria, respectively. Prevalence of ESBL-producing UPEC in this study is higher than reports from other countries and other parts of Nigeria. For instance, in Cameroun, Gangoue-Pieboji (2005) reported 12.0% prevalence of *E. coli* with ESBL, El-Khizzi and Bakheshwain (2006) obtained 15.8% prevalence in Riyadh, Saudi Arabia and Yushau et al. (2007) reported 9.3% prevalence in Kano, Nigeria. In general, prevalence of ESBLs in *E. coli* in UTIs cases varies from country to country and from health institution to another (Iroha et al., 2010). The Pan European Antimicrobial Resistance Using Local Surveillance (PEARIS) study between (2001- 2002) showed that, the percentage of ESBL producer among *E. coli* in UTIs cases was 5.8% for all the study sites (Bouchillon et al., 2004). In Egypt, a high rate of 38.5% was observed, 27.4% were reported from Greece, 2.0% from the Netherlands and 2.6% in Germany. In Japan, Korea and Hong Kong, the percentage of ESBL production among *E. coli* was also low (Ho et al., 2000).

In South and Eastern Nigeria, prevalence of ESBL among *E. coli* isolated from pregnant patients from 2003-2007 was very high (52.4%) (Aibinu et al., 2003; Iroha et al., 2008) and is at variance with the findings of the present study. This may likely due to geographical location and antimicrobial usage. The implication of ESBL-producing UPEC in pregnant women may include prolonged stay in the hospital, cost, treatment failure and relapsed cases. A number of risk factors of acquiring ESBL-producing bacteria have been identified in hospitalized patients, most of which also apply to other multi-resistant Gram-negative bacilli. These risk factors include: prolonged hospital stay; prior hospitalization; previous use of 3GCs, aminoglycosides and quinolones; presence of medical devices such as urinary catheters and mechanical ventilation (Rooney et al., 2009). In the case of community acquired ESBL infections, older age, female gender, recurrent UTIs/prior invasive procedures (e.g. catheterization), known faecal carriage, contact with healthcare facilities/residents in care homes and previous antimicrobial treatment are the risk factors (Moor et al., 2008). Transfer of ESBL enzyme and other antibiotic resistance phenotypes in some of the isolates is plasmid-linked. This may be due to the fact that the ESBL genes were located on transposable element or integron thus resulting in transfer function (Akortha et al., 2011). Inter-generic transfer rate in this study is lower than intra species transfer rate. This has been linked to fertility inhibition, incompatibility, inability to synthesize adhesion or narrow host range (Akortha, 2009). Transmission in the community is probably quite complicated. Individuals in long-term care homes where high carriage rates of CTX-M producing enterobacteriaceae have been observed may spread strains (Rooney et al., 2009) to other noncare-home residents. The evidence of a significant spread amongst household contacts has been presented in a Spanish study which showed that 70% of index cases of patients with ESBL-producing strains causing UTI in the community had positive contacts with 16.7% of their household members. The finding of this study explains co-selection of resistance as resistance to other non-β-lactam antibiotics is located on the same plasmid as ESBL factor.

The presence of plasmid-mediated ESBL resistance among the isolates in the present study is an evidence of its transfer capability of ESBL and other resistance phenotypes between its species and other genera. This implies that under favourable condition, horizontal gene transfer of resistance plasmid by conjugation could occur *in vivo* (Yah et al., 2008). This indicates that plasmid carrying ESBL gene in one bacterium can spread rapidly to members of the same or organisms of different species in the same or different individual. Greater percentage of UPEC in the study area MAR capable of transferring ESBL enzymes and other resistance phenotypes.

5. Conclusion

In conclusion, antibiotic resistance remains one of the nature's never ending process whereby organisms develop tolerance to new environmental condition. The development and spread of ESBL- producing UPEC and horizontal transfer of resistance in the study is of great concern especially in therapeutic management of UPEC-induced urinary tract infections.

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