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Occurrence of β-Lactamase Enzyme Genes and Integrons in Antibiotic Resistant *Escherichia coli* Isolates from Pregnant Women with Asymptomatic Bacteriuria in Nairobi, Kenya

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Abstract

Asymptomatic bacteriuria (ASB) is the presence of bacteria in urine without apparent symptoms of urinary tract infections. Antibiotic treatment of asymptomatic bacteriuria in pregnant women is recommended to reduce the risks associated with urinary tract infection during pregnancy. However, antibiotic resistance affects the success of treatment leading to failure with adverse outcomes. The study was to investigate the presence of mobile antibiotic resistant factors in multidrug resistance *E. coli* isolates. **Methods:** This was a cross-sectional study involving 1020 women attending antenatal clinic in Nairobi County. The urine specimens were processed using standard methods for isolation and identification of bacteria in urine. The most common uropathogen, *E. coli* was tested for antimicrobial resistance, using polymerase chain reaction (PCR) to amplify beta-lactamase genes and integrase genes. Plasmid curing using ethidium bromide was performed on plasmid positive multidrug resistant isolates. **Results:** A total 219 of women tested positive for ASB, resulting to a prevalence of 21.5 % at 95% confidence level. A total of 85 positive samples were caused by *Escherichia coli* which was at 38.8%. *E. coli* isolates from this study had resistance to most antibiotics tested except for imipenem. The resistance ranged from 11.6% for gentamycin to 90.6% for ampicillin. PCR was used for amplification of mobile antibiotic resistant determinants and results indicated 21 isolates had integrons while 26 isolates yielded Beta-lactamase enzyme genes (OXA-1 (8)) and CTX-M (18). **Conclusion:** There was a significant ASB among pregnant women included in the study from the

Nairobi county clinics. There is the presence of ESBLs genes and integrons in community-acquired *E. coli* infections.

Keywords

Asymptomatic Bacteriuria, ESBLs Genes and Integrons

1. Introduction

Asymptomatic bacteriuria is bacterial colonization of the urinary tract without symptoms, common, and potentially a serious medical complication if untreated when it occurs during pregnancy [1]. Antibiotic treatment of asymptomatic bacteriuria in pregnant women is recommended to reduce the risks associated with urinary tract infection during pregnancy. However, antibiotic resistance affects the success of treatment leading to failure with adverse outcomes. Antibiotic resistance is spread within world's bacterial populations by acquisition of resistance genes irrespective of the pattern of antimicrobial use in an area [2]. The gene mobility is achieved through elements like plasmids, integrons and transposons mobile DNA that are acquired through recombination and conjugation. Transposons and integrons transfer genes by recombination into the chromosomes and extra chromosomal DNA [3].

Acquisition of resistance genes by microorganisms is controlled by elements found on chromosomal and extra chromosomal DNA (plasmids). Plasmids often carry antibiotic resistance genes and heavy metal resistance genes [4]. Plasmids are an important factor in acquisition and dissemination of antibiotic resistance genes through conjugation within bacterial population [5]. This study investigated the presence of integrons and plasmids in multidrug resistant *E. coli* isolates from pregnant women.

2. Objective

To screen multidrug resistant *E. coli* isolates for the presence of integrons and plasmids from pregnant women with asymptomatic bacteriuria.

3. Methods

3.1. Study Design and Setting

Between May 2011 and July 2016, we conducted cross-sectional study to determine the prevalence of Asymptomatic bacteriuria in pregnant women attending antenatal clinics in selected the Nairobi county health centers. A questionnaire was used to obtain information from the study participants. The information obtained consisted of identification number, age, phone number, educational level, marital status, parity, gestational age, and human immune virus status. The inclusion criteria involved the first visits of apparently healthy pregnant women attending clinic for first the visit and those who gave their informed consent to partici-

pate in the study. However, the women excluded from the study were those who had features of urinary tract infection, fever, had taken antibiotics within 2 weeks of the study, had medical chronic conditions (HIV) retroviral disease, and those who declined to consent despite adequate counselling.

3.2. Methods for Collecting Samples

All pregnant women at the antenatal clinic, who met the inclusion criteria, were counselled on how to collect midstream urine. This involved initial instructions by the female attending trained nurses and laboratory technicians. The laboratory technicians supervised the urine-sample collection. A total of 1020 pregnant women who made the inclusion criteria on their first visit at the antenatal clinic provided mid-stream urine for investigation. The samples were collected in a clean, leak-proof container with no visible signs of contamination and were labeled properly with demographic information of patients. The specimens were transported at 4˚C temperature in a cool box from the clinic to the processing laboratory at the KEMRI centre of microbiology. The samples were microscopically examined for pus cells, bacteria and then cultured within two hours after collection. They were cultured on air-dried plates of Cysteine lactose electrolyte deficient agar (CLED) and on blood agar, using a calibrated loop delivering 0.002 ml of urine. Plates were incubated aerobically at 37˚C overnight. Colony counts yielding bacterial growth of 105 organism/ml or more of pure isolates was deemed significant.

3.3. Sample Size

The formula used for sample size calculation was $n = z^2 (p (1 - p))/e^2$ [6]. This study used an estimate of the proportion of population falling into the group of interest at 50%. The prevalence of asymptomatic bacteriuria in pregnancy in low socio-economic population and with specific inclusion criteria is unknown in Kenya. This gave the minimum sample size at 384 however due large patient numbers that are attended at the clinics of interest the sample size was increased 1020 for better representation.

3.4. Identification of *E. coli*

A colony count yielding bacterial growth of 10^5 organism/ml or more of pure isolates was deemed significant. The isolates were characterised by colonial morphology, gram stain followed by microscopic examination, motility test and biochemical tests. Colonial appearance of yellow (lactose fermenting) with deep centres were suspected to be *E. coli*. Isolates were identified to species level using standard methods according to Clinical and Laboratory Standard Institute Guideline [7].

3.5. Antimicrobial Susceptibility Testing

All *E. coli* isolated from asymptomatic bacteriuria cases were screened for antimicrobial susceptibility test using the disc diffusion method [8] on Mueller-Hin-

ton (MH) agar (Oxoid). Methods recommended by the Clinical and Laboratory Standard Institute Guidelines [7] were used. Antibiotic discs from Biomerieux-France and Becton Dickinson-USA were used. Antibiotic concentrations on each disc were as follows, Kanamycin 10 mg, Gentamicin 10 mg, Nalidixic acid 30 mg, Chloramphenicol 30 mg, Ampicillin 10mg, Ceftazidime 30 mg, Tetracycline 30 mg, Cotrimoxazole (23.75/1.25 mg), Ciprofloxacin 5 mg and Cefotaxime 30 mg. Mueller-Hinton agar was used for anti-microbial susceptibility test and the inhibition zones sizes were interpreted according to WHO guidelines (CLSI, 2023). *E. coli* ATCC 25922 (Mast Group Ltd., Merseyside, UK) was used as reference strain for quality control. The class of antibiotics that were tested are: β-lactams, cephalosporins, quinolones, aminoglycosides, and macrolides. Some of these classes of antibiotics are known to have their resistant genes conferred by mobile determinants factors.

3.6. Plasmid Mediated Resistant Genes Amplification and Curing

The detection of gene sequences coding for the TEM, OXA, SHV and CTX-M-type enzymes was performed by using a commercial Plasmid Miniprep kit (GenEluteTM) (Sigma-Aldrich), following the protocol delivered by the manufacturer. PCR was applied using the ready Mix Kit (REDTaq® Ready MixTM PCR reaction Mix with MgCl2), Sigma-Aldrich, and the primers used to amplify the above-mentioned genes are listed in **Table 1.** Cycling conditions were as follows: TEM-gene initial denaturation at 96˚C for 5 min, followed by 35 cycles of denaturation at [96˚C for 1 min, 1 min at 58˚C 1 min 72˚C] and a final extension step 1 cycle at 72˚C for 10 min. SHV-gene initial denaturation1 cycle at 96˚C for 5 min, followed by 35 cycles of denaturation at [96˚C for 1 min, 1 min at 60˚C 1 min 72˚C] and a final extension step 1 cycle at 72˚C for 10 min. OXA-1 gene initial denaturation at 96˚C for 5 min, followed by 35 cycles of denaturation at [96˚C for 1 min, 1 min at 60˚C 1 min 72˚C] and a final extension step 1 cycle at 72˚C for 10 mins CTX-MU1 gene initial denaturation at 94˚C for 7 min, followed by 35 cycles of denaturation at [94˚C for 50 secs, 50˚C 40 sec 72˚C 1 min] and a final extension step 1 cycle at 72˚C for 10 min and CTX-M1 cycle of 5 min at 72˚C,1 cycle of 10 min at 94˚C, 30 cycles of [1 min at 94˚C, 1min at 48˚C, 1 min at 72˚C], 1 cycle of 7 min at 72˚C I Annealing temperatures differed according to the primer pair used. Amplified PCR products were separated on 0.8% agarose gels, stained with ethidium bromide and visualized under UV illumination. Appropriate positive and negative controls were used in all cases [9]. PCR amplification of blaCTX-M alleles was carried out with primers CTX-MU1 (5'-ATGTGCAGYACCAGTAARGT) and CTX-MU2 (5'-TGGGTRAARTARGTSACCAGA), designed on conserved regions of blaCTX-M genes. These primers target amplification of a 593-bp internal region of the blaCTX-M genes [10].

The curing was done according the method used by Letchumanan [11]. All plasmid positive isolates were subjected to a curing treatment using Ethidium Bromide (EB). The isolates were grown in fresh Tryptic Soy Broth (TSB) (HiMe-

dia, India) and TSB supplemented with 0.2 mg/mL EB, then incubated at 37˚C for 24 hours under constant agitation. After treatment with the curing agent, the profiles of resistance phenotypes were examined for the antibiotic susceptibility profile. A loopful of growth, cultured on Mueller Hinton Agar (MHA) plates, and then put the antimicrobial disk on (MHA). Absence of inhibition zones on Mueller Hinton agar was indicative of plasmids-mediated resistance (plasmid cured), while presence of zone of inhibition on Mueller Hinton agar was indicative of chromosome-mediated resistance or plasmid not cured.

Table 1. The primers used to amplify the genes.

Primer	Primer sequences	PCR cycles	Reference	Expected size (bp)
TEM-1/F TEM-1/R	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	1 cycle of 5 minutes (min) at 96˚C; 35 cycles of [1 min at 96˚C, 1 min at 58˚C, 1 min at 72˚C]; 1 cycle of 10min at 72˚C.	[53]	867
SHV-1/F SHV-1/R	GGTTATGCGTTATATTCGCC TTAGCGTTGCCAGTGCTC	1 cycle of 5min at 96˚C; 35 cycles of [1 min at 96˚C, 1 min at 60˚C, 1 min at 72˚C; 1 cycle of 10 min at 7˚C	[53]	867
OXA-1/F OXA-1/R	ACACAATACATATCAACTTCGC AGTGTGTTTAGAATGGTGATC	1 cycle 5 min at 96˚C; 35 cycles of [1 min at 96˚C, 1 min at 60˚C, 2 min at 72˚C]; 1 cycle of 10 min at 72˚C	[53]	814
CTX-MU1 CTX-MU2	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	1 cycle of 7 min at 94˚C; 35 cycles of [50 seconds (sec) at 94˚C, 40 sec at 50˚C, 1 min at 72˚C]; 1 cycle of 5 min at 72˚C	[54]	593
CTX-M1-A2 CTX-M1-B2	CTT CCA GAA TAA GGA ATC CCG TTT CCG CTA TTA CAA	1 cycle of 10 min at 94˚C, 30 cycles of [1 min at 94˚C, 1 min at 48˚C, 1 min at 72˚C], 1 cycle of 7 min at 72˚C	[38]	499

Plasmids present in strains *E. coli* PDK-9, R1 andV517 will be used as molecular weight standard.

3.7. PCR Assay for Integrase Genes and Gene Cassettes

Detection of class 1 and class 2 integrons conserved regions was performed by polymerase chain reaction (PCR). The primers that were used are as shown below in Table 2. A colony of each multidrug resistant isolates was suspended in 25 µl of reaction mixer containing 2.5 µl of 10x PCR reaction buffer, 1.5 µl of 50 mMMGCl2, 2 µl of 2.5 mMdNTP (dNTP, dCTP, dTTP, dGTP), 1 µl of primer (forward and reverse) together with 1 unit of TaqDNA polymerase (5 U/µl). The volume of the reaction mixture was adjusted by adding filtered deionized water. The PCR was performed in a PerkinElmer Gene Amp PCR system 9600-R (Roche Diagnostic Gmbh, Mannheim, Germany). Polymerase chain reaction was performed under the following conditions; denaturing at 94˚C for 12 minutes followed by 30 cycles of 1minutes at 94˚C, 30 seconds at Tannealing at 60˚C and Textension (2 min) at 72˚C, where Tannealing is specific annealing temperature and Textension is the specific elongation time for each reaction, with a final extension at 72˚C for 10minutes. The PCR products were separated by horizontal

mini electrophoresis on 1% agarose gel at 100 V (50 mA) and stained with 0.5 μg/ml ethidium bromide for 30 minutes and photographed. Escherichia coli ur-31 and *E. coli* ur-60 was used as positive control.

Table 2. Specific oligonucleotide primers and conditions PCR [12].

Gene	Primer sequence	Size of product (bp)	Reference	Condition
Int 1-F	GGT CAA GGA TCT GGA TTT CG	786 - 766 bp	[12]	A
Int I-R	ACA TGC GTG TAA ATC ATC GTC	303 - 324		
Int I2-F	CAC GGA TAT GCG ACA AAA AGG T	219 - 240 bp	[12]	A
Int I2-R	GTA GCA AAC GAG TGA CGA AAT G	1007 - 986		
Int I3-F	AGT GGG TGG CGA ATG AGT G	178 - 196 bp	[12]	A
Int I3-R	TGT TCT TGT ATC GGC AGG TG	777 - 758		
5'CS	GGC ATC CAA GCAGCAAG	1190 - 1206	[12]	B
3'CS	AAG CAG ACT TGA CCT GA	1342 - 1326		
Att I2-F	GAC GGC ATG CAC GAT TTG TA	1943 - 1962	[12]	B
orfX-R	**GAT GCC ATC GCA AGT ACG AG**	**4848 - 4928**		

Condition-A: 5 min at 94˚C; 32 cycles of 1 min at 94˚C, 1 min at 60˚C, 2 min at 72˚C; 10 min at 72˚C. Condition-B: 5 min at 94˚C; 35 cycles of 1 min at 94˚C, 1 min at 58˚C, 2 min at 72˚C; 10 min at 72˚C.

Gene cassettes (variable region) for class 1 was amplified using hep58 (5'-TCATGGCTTGTTATGACTGT-3') and hep59 (5'GTAGGGCTTATTATGCACGC3') as described by Machado [12]. The gene cassettes for the class 2 integrons were amplified using hep74 (5'-CGGGATCCCG-GACGGCA3') and hep51 (5'-GATGCCATCGCAAGTACGAG-3') same-sized amplicons was digested with RsaI and HinfI (Boehringer Mannheim). All the PCR amplification was conducted as for the integrase amplifications but annealing temperature at 50˚C.

3.8. Ethical Clearance

Ethical clearance for this study was obtained from the KEMRI Ethics Clearance Committee.

3.9. Data Analysis

Data analysis was descriptive and multinomial logistic regression analysis was done at 95% confidence level using SPSS version 23.0 (IBM SPSS Statistics Inc., Chicago, IL, USA). A P-value of less than or equal to 0.05 was considered statistically significant.

4. Results

The target group of pregnant women attending antenatal clinic at Health Centres were from a low socio-economic background. The County clinics were stratified into strata and those offering antenatal clinic services then were randomly selected.

One thousand and twenty (1020) women visiting the antenatal clinic for first visit who met the inclusion criteria participated in the study. A total 219 of the participants had ASB, giving a prevalence of 21.5% (95 CI range 19.1% - 23.9%).

4.1. *E. coli* Isolated from Positive Bacteria Samples

A total of 85 isolates were identified as *E. coli* from the participants who had ASB Table 3. These was the most frequent causative agent.

4.2. Antibiotic Sensitivity Profile for *E. coli* Isolates

E. coli isolates from this study had resistance to most antibiotics tested except for imipenem. The resistance ranged from 11.6% for gentamycin and to 90.6% for ampicillin see Table 3.

Table 3. Antibiotic sensitivity pattern of *E. coli isolates* in asymptomatic bacteriuria.

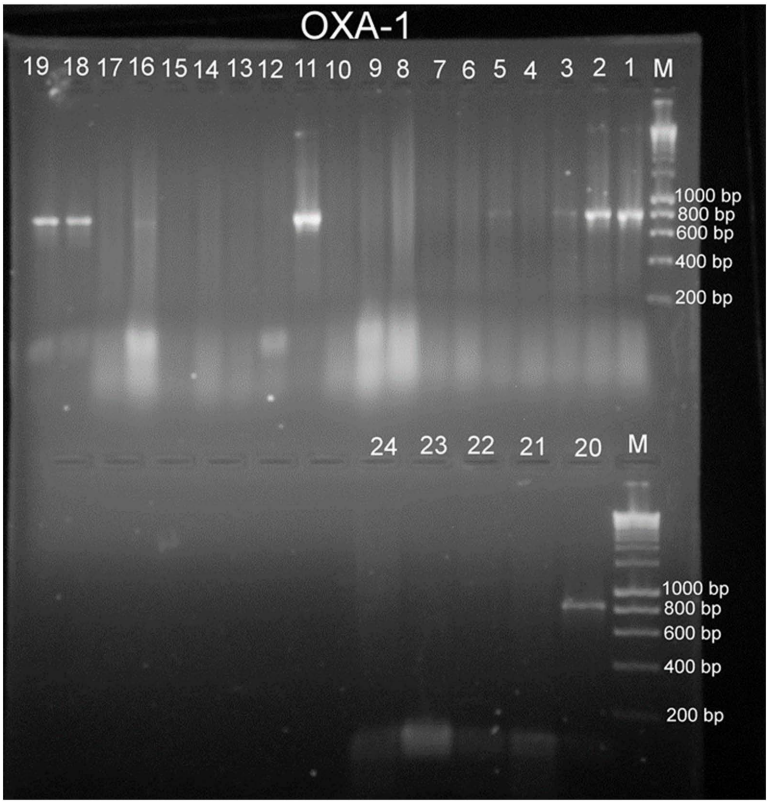
Antibiotics	Susceptibility profile	*E. coli* (n = 85)
Ampicillin	R	77 (90.6%)
	I	6 (7.0%)
	S	2 (2.4%)
Tetracycline	R	51 (60.0%)
	I	8 (9.4%)
	S	26 (30.6%)
Chloramphenicol	R	17 (17.0%)
	I	8 (9.4%)
	S	60 (70.6%)
Cotrimoxazole	R	56 (65.9%)
	I	3 (3.5%)
	S	26 (30.6%)
Ciprofloxacin	R	16 (18.8%)
	I	6 (7.1%)
	S	63 (74.1%)
Nalidixic acid	R	36 (42.3%)
	I	2 (2.4%)
	S	47 (55.3%)
Ceftazidime	R	16 (18.8%)
	I	8 (9.4%)
	S	61 (71.8%)
Gentamycin	R	10 (11.8%)
	I	4 (4.7%)
	S	71 (83.5%)
Amoxy/clavua	R	49 (57.6%)
	I	17 (20.0%)
	S	19 (22.4%)

Continued

Cefotaxime	R	63 (74.1%)
	I	20 (23.5%)
	S	2 (2.4%)
Imipenem	R	0 (0%)
	I	0 (0%)
	S	85 (100%)
Kanamycin	R	66 (77.6%)
	I	5 (5.9%)
	S	14 (16.5%)

4.3. PCR Detection of the BlaOXA-1 and Bla CTX-M Gene in *E. coli* Isolates

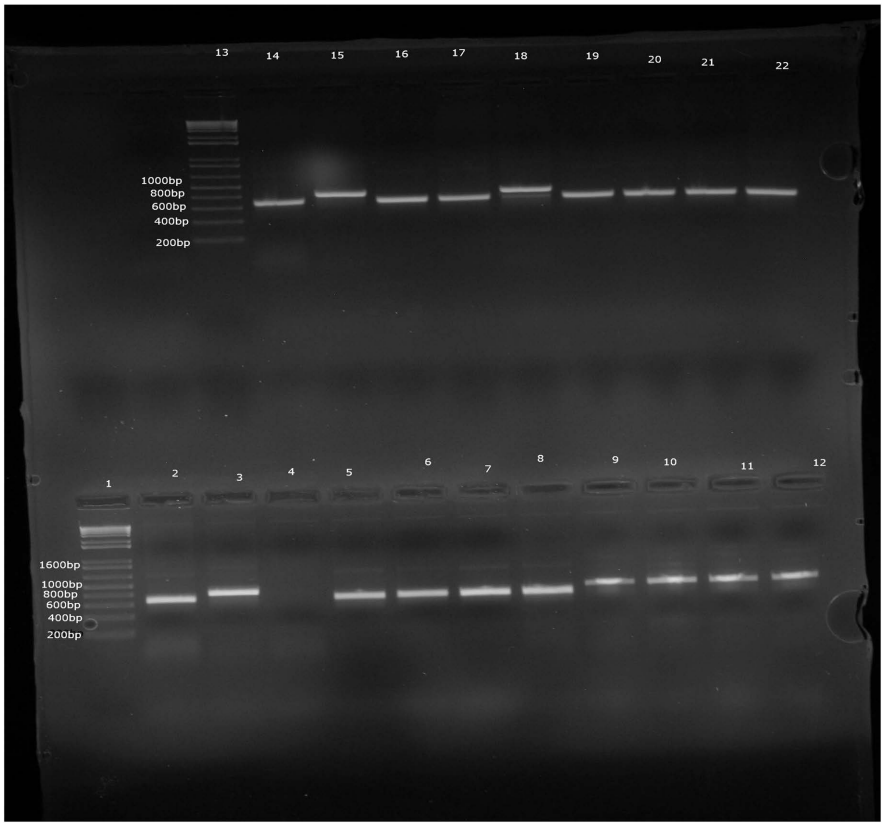
Figure 1 demonstrated the presence of blaOXA-1 and reported in eight isolates. The gene was reported at 813 bp band on PCR products on 1% agarose gel. Eight of the multidrug resistance isolates reported presence blaOXA-1 genes.



Electrophoresis of PCR products on a 1% agarose gel. Lane M. Ladder 100 bp. Lane 11. Positive control. Lanes 10 negative control Lane 1, 2, 3, 5, 16, 18, 19, and 20 Positive samples that indicated 813 bp PCR product.

Figure 1. PCR detection of the $bla_{OXA\text{-}1}$ gene in *E. coli* isolates.

PCR detection of the blaCTX-M gene was reported in 17 *E. coli* isolates and indicated in Figure 2. The amplicons ranged from 600 to 700 pb.



Electrophoresis of PCR products on a 1% agarose gel. Lane1. Ladder 100 bp. Lane 2. Positive control. Lane 3. Positive control. Lanes 4, negative control 5, 6, 7, 8, and 9, 10, 11, 12 to 22 Positive samples that indicated 600 bp to 700 bp PCR product.

Figure 2. PCR detection of the *blaCTX-M* gene in *E. coli* isolates.

4.4. Plasmid Curing

In this study it was observed that fifteen isolates became susceptible to antibiotics they had previously shown resistance to as indicated **Table 4**. It was noted that those isolates that demonstrated the presence of the BLACTX-M genes only were fully cured. Five isolates that demonstrated plasmids with a combination of BLAOXA-1 and BLACTX-M genes remained resistant to some antibiotics after curing.

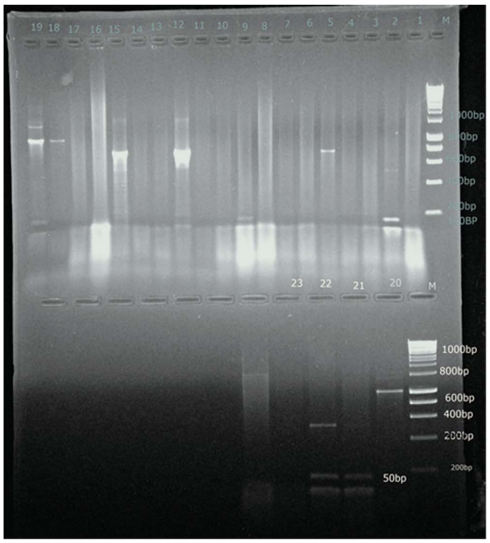
Table 4. Comparison of the presence of Variable regions positive, plasmids and antibiotic resistance patterns after curing with Ethidium bromide.

	Bla-OXA	*blaCTX-M*	*Integron*			*Antibiotic resistant pattern*	*Phylogroups*	*Cured isolate antibiotic resistant profile*
			Int1	*Int2*	*int3*			
***R*193**	+	+	430,800 bp		100 bp	AMP-TET-COT-NAD-AM-CFX-	B2	COT-NAD
***R*243**	+	+	766 bp	-	-	AMP-CHLO-AM	B2	CHLO
***R*66**	+	+	800 bp	1000 bp	-	AMP-TET-COT-NAD-AM-CFX-	B2	AMP-TET-COT-NAD-AM-CFX
***R*387**	+	-	-	-	100 bp	AMP-CFX	B1	-

Continued

***R*375**	+	+	-	280 bp	-	AMP-TET-COT-CFX-	D	COT
***R*351**	-	+	-	-	100pb	AMP-TET-COT	D	COT
***R*123**	-	+	430 bp		-	AMP-CAZ-AM-CFX	A	-
***R*88**	-	+	766 bp	-	-	AMP-TET-COT-NAD-CAZ-AM-CFX-	B2	AMP-TET-COT-NAD-CAZ-AM-CFX-
***R*129**	+	+	-	-	700 bp	AMP-TET-COT-NAD-CFX	B2	COT-NAD
***R*348**	-	+	800 bp	-	600 bp	AMP-TET-CHLO-COT-CIP-NAD-CAZ-AM-CFX	B2	CHLO-COT-CIP-NAD
***R*94**	-	+	430 bp	-	-	AMP-AM	B1	-
***R*202**	+	+	800 bp	-	600 bp	AMP-TET-COT-CIP-NAD-CAZ-GEN-AM-CFX-	B2	
***R*597**	+	+	766 bp	-	-	AMP-TET-COT-CIP-NAD-CFX-	D	COT-CIP-NAD
***R*656**	-	+	788 bp	-	-	AMP-TET-CHLO-COT-NAD-CAZ-CFX-	B1	-
***R*538**	-	+	800 bp	-	600 bp	AMP-TET-CHLO-CIP-NAD-CAZ-GEN-AM-CFX	D	-
***R*476**	-	+	800 bp	-	600 bp	AMP-TET-COT-CIP-NAD-CAZ-GEN-AM-CFX	B2	-
***R*403**	-	+	-	-	100 bp	AMP-TET-CFX	A	-
***R*317**	-	+	800 bp	-	-	AMP-TET-CHLO-COT-NAD-CAZ-AM	A	-
B455	-	+	788 bp	-	-	AMP-TET-COT-CIP-NAD-AM-CFX	B1	-
***R*335**	-	+	766 bp	-	-	AMP-TET-CHLO-COT-AM-CFX	B2	-
***R*481**	-	+	800 bp	-	600 bp	AMP-TET-COT-CIP-NAD-CAZ-GEN-AM-CFX	B2	-
***R*519**			800 bp	1000 bp	-	AMP-TET-CHLO-CIP-NAD-CAZ-GEN-AM-CFX	B2	-

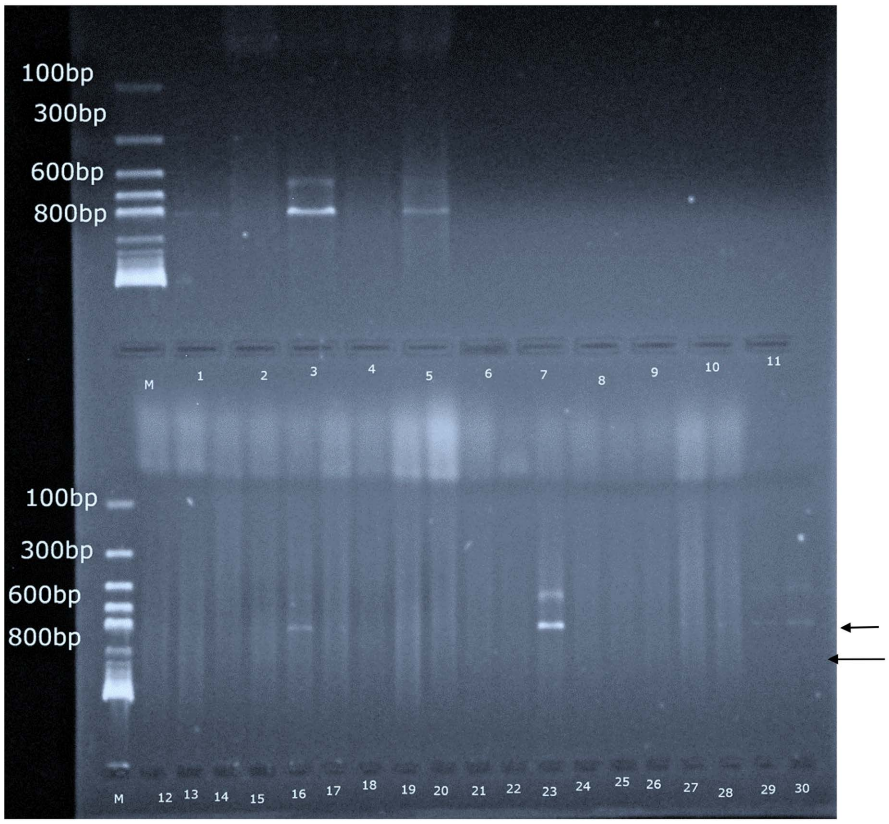
4.5. Detection of Integron Variable Region (VR)



Electrophoresis of PCR products on a 1% agarose gel. Lane M. Ladder 100 bp. Lane M ladder. Lane 12 is Positive control. Lanes 11 negative control, Lane 2 and 15 Positive samples that indicated 766bp PCR product. Lane 18 and 19 indicated 788 bp PCR product. Lane 15 and 19 indicated 1000 bp PCR product. Lane 2, 9 and 19 indicated 100 bp PCR product. Lane 12 indicated 700 bp PCR product. Lane 22 indicated 280 bp PCR product. Lane 21 and 23 indicated two bands below 100bp mark this was also observed in lane 2, 3, 6, 8, 21 and 22 positive control lane and lane 16. Lane 2 and 7 demonstrated 491 bp.

Figure 3. Detection of integron variable region (VR).

Amplification of integron variable regions (VR) indicated in Figure 3 and Figure 4 showed that 21 of the isolates produced variable region. One (1) isolate yielded three VR bands (430 bp, 100 bp, & 100 bp), Seven (7) of the isolates demonstrated two VR bands (2 - 1000 bp & 800 bp and 5 - 800 bp & 600 bp) and 13 yielded a single VR band. A total twenty-one isolates yielded integron VR and yielded twenty-three bands. A total of eight 800 pb, five 600 bp, four 100 bp, three 430 bp, two 1000 bp and one 280 bp.



Electrophoresis of PCR products on a 1% agarose gel. Lane M. Ladder 100 bp. Lane M ladder. Lane 3 is Positive control. Lanes 2 negative control, Lane 1, 5, 16, 23 29 and 30 Positive samples that indicated 788bp PCR product. While Lane 5, 16, 23 29 and 30 Positive samples that indicated 600bp PCR product.

Figure 4. Class 1 and class 3 integron profile.

5. Discussion

In this study the Gram-negative bacteria were more prevalent with *E. coli* as the most frequent at 38.8%. This was in agreement with a study done by Agarwal in India which reported Escherichia coli at 39.2% [13]. *E. coli* was a major pathogen isolated from the urine cultures and accounted for one-third of the positive cultures with significant bacteriuria. *E. coli* is considered major uropathogen due to a number of virulence factors specific for colonization and invasion of the urinary epithelium [14]. In overall gram-negative bacteria have a unique structure, which assists in attachment to the uro-epithelium and prevent pathogens from being

washed away by urine allowing growth and tissue invasion resulting colonization and infection during pregnancy [15]. While the range of etiological agents causing asymptomatic and symptomatic UTI in pregnant women is relatively constant, the susceptibility profile is different in different geographical locations.

5.1. The Antibiotic Susceptibility Profile for the Bacterial Isolates

E. coli isolates from this study had resistance to most antibiotics tested except for Imipenem. The resistance ranged from 11.6% for gentamycin to 90.6% for ampicillin. The current study showed high level of resistance to first line antimicrobial drugs such as cotrimoxazole. These findings agreed with a previous study done in Tanzania [16]. The high resistance to cotrimoxazole observed in the current study call for the need to strengthen surveillance to identify changes in sensitivity pattern among urinary tract isolates.

The current study reported high level of cefotaxime resistance with the highest resistance reported for *E. coli* at 74.1%. Cefotaxime is a second line antimicrobial agent and is a third generation of cephalosporins. A possible explanation for the resistance found might be the presence of mobile resistant factors in these strains. There were 17 (14.0%) of the gram-negative isolates which were resistant to Cefotaxime, Ceftazidime and amoxicillin-clavulanic acid. An earlier study done in Kenya reported a similar resistance pattern in uropathogen *E. coli* in Kenya [17]. This could be an indication of co-selection of the genes conferring resistance to these antibiotics. The emergence of ESBLs in community-acquired infections has been reported in earlier studies [18]. *E. coli* recorded 18.8% resistance to ceftazidime, which is a third-generation cephalosporin like Cefotaxime. The difference could be attributed to the type of antibiotic resistance mobile determinants in the isolates. The high resistance level recorded for cefotaxime in this study is associated with the presence of plasmid mediated blaCTX-M gene demonstrated in multidrug resistant *E. coli*. The presence of blaCTX-M enzyme in bacteria is known to hydrolyse cefotazime with limited effect on ceftazidime [19] [20]. Cephalosporin are safe in pregnancy therefore ceftazidime as per this study could be the antibiotic of choice yet *E. coli* isolates reported high resistant to cephalosporins. This therefore poses a risk to pregnant women and the pregnancy.

Resistance to ciprofloxacin was at 18.8% for the *E. coli* isolates although relatively low, this trend needs to be watched closely. Since Fluoroquinolones are thought be still the most effective antibiotic agents against *E. coli* infection [21]. However, this is only true to different localities otherwise resistance to Fluoroquinolones has been increasing. There is increased resistance to Fluoroquinolones such as ciprofloxacin and levofloxacin. Kenya has reported development of resistance to Fluoroquinolones and extended-spectrum beta-lactams in uropathogenic *E. coli* [17]. This study found 18.8% resistance to ciprofloxacin by the *E. coli* isolates. Quinolones have been associated with teratogenicity in the first trimester and risk of auditory and vestibular toxicity in the foetus in last trimesters,

and are therefore contraindicated in pregnancy [21]. However, this study reported low resistance to quinolones; therefore, they could be used with caution in late pregnancy, especially if they are found to be the only sensitive drug in any specific case.

The multiple antibiotic resistance index (MAR index) value ranged between 0.18 and 0.75. The predominant MAR index for gram negative was 0.33 and 83.5% of the isolates had MAR index equals or more than 0.2. MARI is a tool that reveals the spread of bacteria resistance in a given population [20]. In this study MARI ≥ 0.2 was expressed by 83.2% of gram-negative isolates. This implies that a very large proportion of the bacterial isolates had been exposed to several antibiotics. A similar report was demonstrated by Prakash and Saxena [22]. A study done in 2016 demonstrated that urinary tract infection is caused by *E. coli* strains that have a high MARI [23]. This agreed with current study, which had high MARI for gram-negative isolates.

5.2. Plasmid Mediated Resistant Genes Amplification

Fifteen isolates demonstrated blaCTX-M gene followed by eight isolates for blaOXA-1 while TEM and SHV genes were not reported from the isolates. The findings agreed with a study done in Palestinian hospital that demonstrated blaOXA-1 gene from seven *E. coli* isolates [24]. This study reported CTX-M in 15 *E. coli* isolates which was the predominant ESBL gene. In recent years the *E. coli* strains expressing CTX-M have increased and replaced previous predominant types of ESBLs TEM and SHV; Moreover CTX-M type ESBLs have emerged within the community, particularly among *E. coli* and K. pneumonia isolated from urinary tract infections [25]. This study was done on women attending antenatal clinic who were not hospitalized. Therefore, the isolates were from a community setup. The dissemination of this gene may be associated with mobile factors that control resistance genes movement among microorganisms. This occurrence was demonstrated by Barlow in 2008 when high mobilization of the encoding genes reported an increase of tenfold in movement of blaCTX-M genes via plasmid [26].

Co-occurrence of extended spectrum β lactamase genes in isolates was reported in six *E. coli* isolates. Six isolates reported the occurrence of both blaCTX-M and blaOXA-1 in combination. This finding agrees with earlier study done in Kenya that demonstrated majority of blaOXA-1 genes were detected in strains bearing other ESBL genes such as blaCTX-Ms or blaTEM-52 [27]. In another study done in Kenyan hospital set up Sixteen isolates demonstrated blaCTX-M/TEM, whereas five had blaTEM/CTX-M/SHV [28]. A study done in USA demonstrated that blaOXA-1 gene was found in combination of blaCTX-M-15 gene in 38 *E. coli* isolates [29]. Several studies have shown that blaCTX-M genes are found in large plasmids which often carry other genes particularly blaOXA-1, blaTEM-1, tetA and aac conferring resistance to a range of antimicrobial agents. This may explain the high rate of transmission of the blaCTX-M gene among the *E. coli* strains by

acquiring R-plasmid. The occurrence of blaCTX-M resistance gene combined with other resistance genes in *E. coli* is therefore a common occurrence [30]. The demonstration of more than one ESBL gene in the isolates is an indication of presence of resistant plasmids in the isolates in this study.

The OXA-type enzymes are a growing family of ESBL, which differ from the TEM and SHV enzymes as they belong to molecular class D and functional group 2d [31]. The OXA-type lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin. blaOXA-1 has frequently been isolated from *E. coli* [32]. *E. coli* isolates were reported to have blaOXA-1 genes from Lebanon [33]. They predominantly occur in Pseudomonas aeruginosa but have been detected in many other gram-negative bacteria [30]). This agrees with this study which demonstrated the OXA-1gene from the *E. coli* isolates. However, all isolates in this study that demonstrated OXA-type ESBLs genes also demonstrated CTX-M genes. This result agreed with an earlier study that reported a similar occurrence where all OXA genes were produced alongside with CTX-M-1 [34].

Most of the *E. coli* isolates that demonstrated the presence of OXA-1 and CTX-M genes were resistant to other antibiotics like quinolones, co-trimoxazole, and tetracycline. However, these antibiotics do not possess the beta lactam ring in their structures. Therefore, the ability of the isolates to develop resistance to these antibiotics is an indication of presence of other mobile factors in the isolates. Other studies have reported similar results where ESBL producing organisms have been found to be resistant to ciprofloxacin, co-trimoxazole, and gentamicin [35]. Studies have found out that genes encoding for β-lactamases enzyme are often located on large plasmids that also encode genes for resistance to other antibiotics, including aminoglycosides, tetracycline, sulfonamides, trimethoprim, quinolones, and chloramphenicol [36]. All the *E. coli* isolates that demonstrated production of ESBL enzymes were sensitive to imipenem. This was similar to earlier studies that had reported high susceptibility of isolates to imipenem [37].

Our study involved community-acquired E coli infections. The prevalence of CTX-M-type ESBLs in these isolates followed by OXA genes is a subject of concern for the future of antibiotics. The recognition of CTX-M β-lactamases as the predominant type of ESBL among community strains is increasing concern in many countries [38]. Previously ESBL-producing Enterobacteriaceae have been reported in the hospital setup where there is heavy antimicrobial selection pressure on microorganism [38]. However, there is now evidence of dissemination of CTX-M ESBL-producing urinary pathogens into the community and the current emergence and spread of these bacteria is disturbing [39]. The association of CTX-M β-lactamase-encoding genes with mobile elements such as ISEcp1, could explain the ease with which these enzymes are spreading among bacteria in the community setting [38].

In this study isolates that demonstrated presence of both OXA-1 gene and CTX-M and did not agree with a similar study in a Kenyan hospital environment that

demonstrated presence plasmid genes TEM and SHV genes [40]. In another study carried out in a Kenyan hospital reported presence of Plasmid-mediated CTX-M-15 beta-lactamases and CMY-2 AmpC enzymes [17]. This study agreed partly with current study in the demonstration of CTX-M genes in multidrug resistance *E. coli* isolated from urine. The difference in the distribution of the genes in the isolates in this study could be due to the fact that the isolates were community based and not hospital based. Eight of the multidrug resistance isolates reported presence of both blaOXA-1 and blaCTM genes. These β-lactamases genes can either be chromosomal or plasmids mediated and invalidate β-lactams activity by hydrolyzing the β-lactam ring. Development of multi-drug resistant uropathogenic *E. coli* strains is a growing concern to practical treatment of urinary tract infections in many countries including Kenya [41]. This is made worse when it occurs in pregnant women whose range of antibiotic treatment is limited.

5.3. Plasmid Curing

In this study it was observed that fifteen isolates became susceptible to antibiotics they had previously shown resistance. This agreed with a study bacteria lost ability to produce bactericin after curing [42] [43]. It was noted that those isolates that demonstrated the presence of the BLACTX-M genes only were fully cured. Five (5) isolates that demonstrated plasmids with a combination of BLAOXA-1 and BLACTX-M genes remained resistant to some antibiotics after curing. The failure of ethidium bromide to cure some antibiotic genes may be related to mechanism of action. Ethidium Bromide inhibits plasmid replication that carry antibiotic resistance by introducing an abnormal insertion or deletion in the plasmid gene which results into frameshift mutation that alters the sequence [44].

5.4. Detection of Integron Variable Region (VR)

Variable regions of integrons were screened in 40 selected multidrug resistant *E. coli* isolates that were resistant to more than three classes of antibiotics. They yielded 27.5% of class 1 integron, 7.5% class 2 integron, and 22.5% class 3 integrons. This result agreed with a study done in Niger that demonstrated prevalence of all three classes of integron, class 1, 2 and 3 integron from *E. coli* isolates [45] This study reported 2 isolates yielded PCR product of 1000-bp fragment which were classified to be class 2 integron as per the amplification primers sequences as reported by Machado [12]. A single isolate was found to harbor both 1000bp and 800 bp fragment, and five reported 800 bp and 600 bp which meant the isolate had more than one variable region. Nine (9) of the isolates harbored class 3 integron at 600-bp and 100 bp, while eleven harbored class 1 intergron Eight (8) isolates demonstrated base pairs between 766 bp to 800 bp and three (3) isolates demonstrated 430 bp. Nine of the isolates harbored class 3 integron at 600-bp and 100 bp. However, though class 3 integrons are rarely demonstrated in *E. coli* isolates Kargar reported class 3 integron at 26.09% MDR *E. coli* isolates [46].

The prevalence of class 1 integrons, class 2 integrons has also been detected in

Kenya while no isolate contained a class 3 integron [27]. The common types of genes among class 1 and class 2 integron gene cassettes amplified are dfrA1 and aadA1 genes in *E. coli* isolated from urine cultures [47]. The integrons may contain various numbers, kinds and combinations of gene cassettes within their variable regions. The types, combinations and frequency of the gene cassettes in integrons may reflect the specific selective pressures to which the isolates were exposed [48]. Integrons have an alarming capacity for the enrolment, spread, and expression of resistance genes, and studies show that they are widespread among gram-negative bacteria [49]. Studies are increasingly reporting presence of class 3 integron in *E. coli* isolates [50]. The detection of class 3 integron is increasing and has been reported in strains from patients with urinary tract infections [50]. Class 3 integrons could be involved in the dissemination of antibiotic resistance in both clinical and the environmental setting [51]. The presence of class 3 integron in this study is a significant finding that indicates the evolution of bacteria for survival.

6. Limitations

The limitation of this study was the plasmids genes and integrons demonstrated in the multidrug resistant isolates were not sequenced for specific identification.

7. Conclusion

There was significant ASB among pregnant women involved in the study from the Nairobi county clinics. The presence of ESBLs genes and intergron was demonstrated in the multidrug resistant *E. coli* isolates from pregnant women with ASB. The MAR index reported in this study indicated the women involved in the study were from antibiotic use high risk environment.

8. Recommendation

It is recommended that more studies be done to explore the incorporation of plasmid curing agents into antibiotics formulation during the drug development process.

What Is Already Know on This Topic

ESBL producers with blaCTX-M blaTEM and blaSHV genes have been demonstrated in Kenyan hospital setup [28].

Class 1 and class 2 integrons were detected in 3 isolates but no isolate contained a class 3 integron was reported in a study in a hospital setup [27].

Studies have reported decrease in number of antibiotic resistance profile of the isolates after plasmid curing [52].

What This Study Adds

ESBL producers blaCTX-M and blaOXA-1 genes demonstrated in community setup.

Class 1 and class 2 integrons including class 3 integrons detected In *E. coli* isolates from this study in a community setup.

Antibiotic resistance ability of multidrug resistant *E. coli* lost or reduced to some antibiotics after treatment with plasmid curing agent.

Authors' Contributions

Adelaide Ayoyi conceived the study, drafted the proposal, carried out data collection, laboratory examination, data analysis, interpretation of the results and ultimately finalized write up of the manuscript. Gideon Kikuvi, Christine Bii and Samuel Kariuki gave technical advice in proposal development, in-process consultation and review of the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors report no conflicts of interest in this work.

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