International Journal of Community Medicine and Public Health
Sosmus SM et al. Int J Community Med Public Health. 2026 Jan;13(1):62-71
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20254411

Original Research Article

Genotypic characterization and antibiofilm formation of resistant *Escherichia coli* isolates causing urinary tract infections among pregnant women at Kisii, Kenya

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Received: 28 August 2025
Revised: 09 December 2025
Accepted: 10 December 2025

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ABSTRACT

Background: Urinary tract infections (UTIs) during pregnancy are among the most common infections worldwide, leading to poor perinatal and maternal outcomes. This study aimed at profiling ESBL-resistant genes and deducing the antibiofilm formation activity of *Escherichia coli* isolates obtained from pregnant women against the commonly used antibiotics.
Methods: Hospital-based cross-sectional study was conducted from March to June 2020, total of 199 pregnant women were involved. Mid-stream urine samples were collected and cultured on CLED at 37ºC overnight. Positive growths were biochemically analysed for the *E. coli* isolates identification, drug susceptibility tests were conducted by Kirby Bauer disc diffusion technique and the PCR technique was used to detect the ESBL genes. The antibiofilm formation was analyzed using the ordinary one-way ANOVA Dunnett's multiple comparison tests (GraphPad Prism, version 9.3) and data was presented in bar graphs.
Results: Out of the positive growth, 28 (23.5%) isolates, *E. coli* species demonstrate resistance to selected antibiotics. From 12 (42.9%) isolates that shows high drug resistance were investigated for ESBL gene profiling, where 8 (42.1%) of them had blaCTX-M, 6 (31.6%) had blaTEM and blaSHV 5 (26.31%) and 8 (66.7%) showed the ability to form antibiofilm against the commonly used antibiotics with 91.66% statistical significance at different levels.
Conclusions: The MDR for commonly prescribed drugs and the high prevalence of bacterial UTI were observed with a significant number of ESBL producers. In light of these findings, biofilm formation with antimicrobial resistance genes in urinary tract infection may lead to difficult-to-treat infections.

Keywords: Antibiofilm, AMR, ESβL, *E. coli*, Resistance genes

INTRODUCTION

Urinary tract infections usually affects the urinary tract and can extend from the gut to the kidney, urethra and bladder.[1] UTIs are commonly in pregnant women due to the decreased abdominal strength during micturition and a lack of the hormone oestrogen, which leads to colonization of *Escherichia coli* and UTIs recurrence.[2] As per other studies done it was revealed that most UTI infections in both community and hospital environments are due to *E. coli* infections.[3] And this high prevalence of the organism was due to its ability to coexist with other organisms as a commensal and it has been discovered to be the primary source of mobile genetic material used to

disseminate antibiotic resistance traits to other enteric pathogenic bacteria.[4]

In Europe and the United States of America, millions of people acquire antibiotic resistance every year, resulting in deaths.[5] The WHO report indicated that Africa and Southeast Asia are regions without developed antimicrobial surveillance systems.[6] A study conducted on 150 patients between June 2016 and July 2017 at Nishtar Medical University in Pakistan reveals that *E. coli* strains had high prevalence rates and overall resistance to a variety of antibiotics such as imipenem (80%), ciprofloxacin (72%) and Amoxiclav (68%).[7]

In Africa, *Escherichia coli* (43.2%) is the most common strain as was observed in a study done in Harare, Zimbabwe, where resistance rates were greatly significant to ampicillin (100%) and penicillin (70%), similarly, this organism was found to be highly resistant to cefuroxime (100%), ceftazidime (100%), Nalidixic acid (90%) and ciprofloxacin (90%) in a study at Mulago Hospital in Uganda.[8,10] In Kenya, a survey of 99 families in Nairobi revealed that *E. coli* has significant prevalence rates and a high resistance of over 80% to antibiotics such as penicillin, sulfonamides, trimethoprim and aminoglycosides.[9] In our previous publication on the antimicrobial resistance of *E. coli* among pregnant women at Kisii antenatal clinic, it is shown that Sulfamethoxazole 100% was the most resistant, followed by Amoxiclav 85%, Ceftriaxone 71.42%, Nalidixic acid 71.42%, Nitrofurantoin 57.14%, Norfloxacin 35.71%, Ofloxacin 21.42% and Gentamycin 14.28%.[10] The ESBL variants such as Cefotaximase (CTXM), Temoneira (TEM) and sulfhydryl variable (SHV), among others, are classified based on their amino acid sequence.[12] The CTX-M, TEM and SHV genes are often multidrug-resistant and are known to contribute to drug resistance in both humans and animals, creating negative clinical and epidemiological implications.[13]

According to several studies, the majority of bacterial infections are difficult to manage and control because they form biofilms that result in chronic and advanced diseases.[14] Many studies have revealed the strong influence of virulence and resistant genes in serogroups of uropathogenic *E. coli* that produce biofilms and have indicated that bacterial quorum sensing regulates the production of enzymes like β-lactamases and elastases that aid in the formation of biofilms.[15,16]

METHODS

Study design

This study was hospital-based cross-sectional study conducted between March 2020 and June 2020 among 119 pregnant women attending ANC services at Kisii Teaching and Referral Hospital in Kenya with 10 pus cells (leucocytes) /mm3 and had consented to participate in the study.

Isolation and identification of bacteria

From our previous study 12 isolates were found to be MDR to commonly used antibiotics within our study site (unique codes as *E. coli* K001, *E. coli* K006, *E. coli* K008, *E. coli* K019, *E. coli* K030, *E. coli* K043, *E. coli*K048, *E. coli* K059, *E. coli* K064, *E. coli* K080, *E. coli* K110 and *E. coli* K117) were further considered in this study.[10]

Phenotypic characterization, culture and biochemical tests were performed as per the CLSI recommendation.[17] the samples were inoculated on CLED media agar (Oxoid Ltd, UK), using wire loop (0.001 ml) then incubated aerobically at 37˚C for 24 hours. Colony count growth of ≥104-105CFU/ml (colony-forming units per milliliter) was considered significant. The negative growth plates were further re-incubated overnight. Colony characteristics, Gram reactions and a series of biochemical reactions, including catalase, coagulase, oxidase, urease, indole, citrate utilization, lysine decarboxylase, glucose, lactose fermentation, gas and H2S production and motility tests were used for the isolations of bacteria.[18]

DNA extraction

The MDR *E. coli* isolates were subjected to DNA extraction using the QIAmp DNA extraction Minikit (QIAGEN) as per the manufacturer's instructions, where the isolates suspension was enzymatically lyzed using proteinase K and purified by the use of ethanol-containing washes, followed by the elution of the DNA with distilled water. Spectrophotometry (NanoDrop ND1000, Thermo Fisher Scientific Inc, Delaware, USA) was used for quantity and quality confirmation of the DNA extracted.

Gel electrophoresis

The agarose in a gel tray with the well comb was placed at room temperature to solidify. A loading buffer was added to each 50 µl of extracted DNA samples from *E. coli*isolates suspected to contain beta-lactamase (blaSHV, blaCTX-M and blaTEM) genes to increase the density. The agarose gel was placed into the electrophoresis unit, filled with 1xTAE (or TBE) until the gel was covered and then the molecular weight ladder was loaded into the first lane of the gel.

The gel was run at 80-150 V until the dye line was approximately 75-80% of the way down the gel. The power was turned off, the electrodes disconnected from the power source and the gel removed from the gel box. The gel was placed into a container filled with 100 mL of TAE running buffer and 5 µl of EtBr put on a rocker for 20-30 minutes. The EtBr solution was replaced with water and de-stained for 5 minutes. The DNA fragments (bands) were visualized as they appeared on the gel in a UV light device.[20]

Molecular characterization and bioassays using PCR

Polymerase Chain Reaction (PCR) for beta-lactamase genes, blaSHV, blaCTX-M and blaTEM, were performed using generic primers sourced from Bioneer Corporation, similar to the ones obtained from an 18 years study in Kenya (Supplementary 1).[21] Amplification reactions were performed in a volume of 50 µl containing 1.25 units of Taq DNA polymerase, 1.5 mM $MgCl_2$, 0.2 µM of each forward and reverse primer, 200 µM of each dNTP, 12.5 µl of PCR water and 2.5 µl of DNA template. The thermocycling conditions were as blaSHV at 94°C for 5 minutes followed by 30 cycles at 94°C for 30 seconds, 68°C for 60 seconds and 72°C for 60 seconds, with a final extension of 72°C for 10 minutes; blaTEM at 94°C for 2 minutes followed by 30 cycles of 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute with a final extension at 72°C to 7 minutes; blaCTX-M at 94°C for 2 minutes followed by 30 cycles of 95°C for 20 seconds, 51°C for 30 seconds, 72°C for 30 seconds with a final extension at 72°C to 3 minutes.

PCR products were resolved by electrophoresis on 1% agarose gels at 100 V run for 1 hour and visualized using a UV Trans illuminator (BioDoc-It Imaging System, UVP-Upland, USA). All procedures were performed and compared with the National Committee for Clinical Laboratory Standard (NCCLS 2003) guidelines for the detection of ESBL-producing *E. coli* spp. The CF2 strain of Enterobacter cloacae, a blaCTX-M-producing bacteria, was used as a positive control for *E. coli* while S1b and T1b strains of *E. coli* were used as controls on blaSHV and blaTEM producing *E. coli*, respectively.[22]

Antibiofilm formation

The *E. coli* isolates with high resistance to tested drugs were subjected to antibiofilm formation activity against the drugs (Amoxiclav, Sulfamethoxazole, Nalidixic acid and Ceftriaxone). An aliquot of 190 µl of M9 broth with antimicrobials (Ceftriaxone (30 µg), Nalidixic Acid (5 µg), Sulfamethoxazole (25 µg) and Amoxyclave (20/10 µg) were inoculated with a bacterial suspension per well at 37°C for 48 hr without shaking. Pseudomonas aeruginosa (a biofilm producer) was used as a positive control and M9 broth was used as a negative control. The flat-bottomed polystyrene tissue culture microplate was sealed with Parafilm to prevent medium evaporation before incubation.

The wells were then rinsed gently with double-distilled water to remove the loosely attached cells. These microplates were air-dried, then 200 µl of 0.4% crystal violet solution were added per well to the adhered cells and left at room temperature for 15 min.

The excess stain was removed by gently rinsing with distilled water. The microtiter plates were again air-dried for 1 hour, after which 200 µl of absolute ethanol were added per well to solubilize the dye. The intensity was measured at OD590 nm by using a Safire Tecan-F129013 Microplate Reader (Tecan, Crailsheim, Germany). The tests were done in triplicate and an average of OD590nm was evaluated. Abf A (%) = (1- (OD Test sample – OD Blank)/ (OD Untreated sample – OD Blank) ×100.

Data management and analysis

The antibiofilm activity data entry and cleaning were performed using Microsoft Excel and analysis was done at a 95% confidence level using ordinary two-way ANOVA GraphPad Prism (Windows, version 9.3). The data on isolated pathogens were presented using bar graphs and ANOVA Dennett's multiple comparison tests.

RESULTS

Bacterial identification, isolation and susceptibility patterns of E. coli

From the previous study, 10 it was revealed that out of 119 samples tested, 28 isolates were identified to be *E. coli* with resistance pattern of 28 (100%) for the sulfamethoxazole, 26 (85%) to Amoxiclav, 20 (71.42%) to both Ceftriaxone and Nalidixic acid, 16 (57.14%) to Nitrofurantoin, 10 (35.71%) to Norfloxacin, 6 (21.42%) to Ofloxacin and lastly 4 (14.28%) to Gentamycin. A total of 12 MDR *E. coli* isolates (K001, K006, K008, K110, K019, K059, K064, K030, K043, K048, K080 and K117) were selected for molecular and antibiofilm formation assaying based, resistant genes of *E. coli* isolates.

Out of the 12 MDR *E. coli* isolates. 8(66.7%) (K001, K006, K008, K110, K019, K059, K064 and K030) were positive for extended-spectrum genes, blaCTX-M, blaTEM and blaSHV of which 5 (41.7%) contained the 3 genes combination (i.e., blaCTX-M, blaTEM and blaSHV), 2 (16.7%) had 2 genes combination (i.e., blaCTX-M, blaTEM) and lastly 1 (8.33%) had 1 gene (blaCTX-M), while 4 (33.33%) of the isolates (K043, K048, K80 and K117) did not show any of these ESBL genes as shown in table 1. On the gel electrophoresis plate, blaCTX-M was observed at 593bp, blaTEM at 865bp and lastly blaSHV at 747bp as shown in Figure 1.

Antibiofilm formation activity

The selected high resistant *E. coli* isolates (K001, K006, K008, K110, K019, K059, K064, K030, K043 K048, K080 and K117), were run against the four drugs which shows MDR (sulphamethoxazole, amoxiclav, ceftriaxone and nalidixic acid) for the antibiofilm formation. Out of these isolates, 8 (66.6%) were positive for the antibiofilm activity toward one or more drugs as shown in Figure 2-5.

Sulphamethoxazole

The produced antibiofilm activity varied as in some biofilm formation was partially inhibited by this antibiotic while in other isolates there was complete inhibition on biofilm formation as shown in Figure 2.

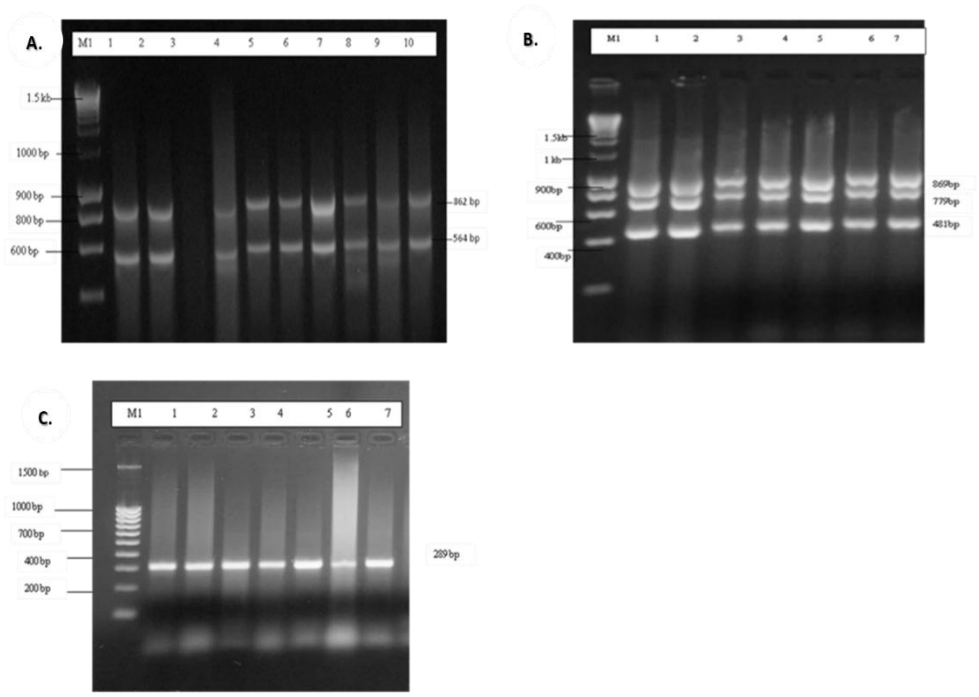

Figure 1 (A-C): Gel electrophoresis results. Lane M1 for plate A represents 2kb DNA size marker-hyper ladder I SHV band 747 bp; lane M1 for plate B represents 2 kb DNA size marker- hyper ladder I TEM band 865 bp and lane M1 for plate C represents 2 kb DNA size marker- hyper ladder I, CTX-M band 593 bp.

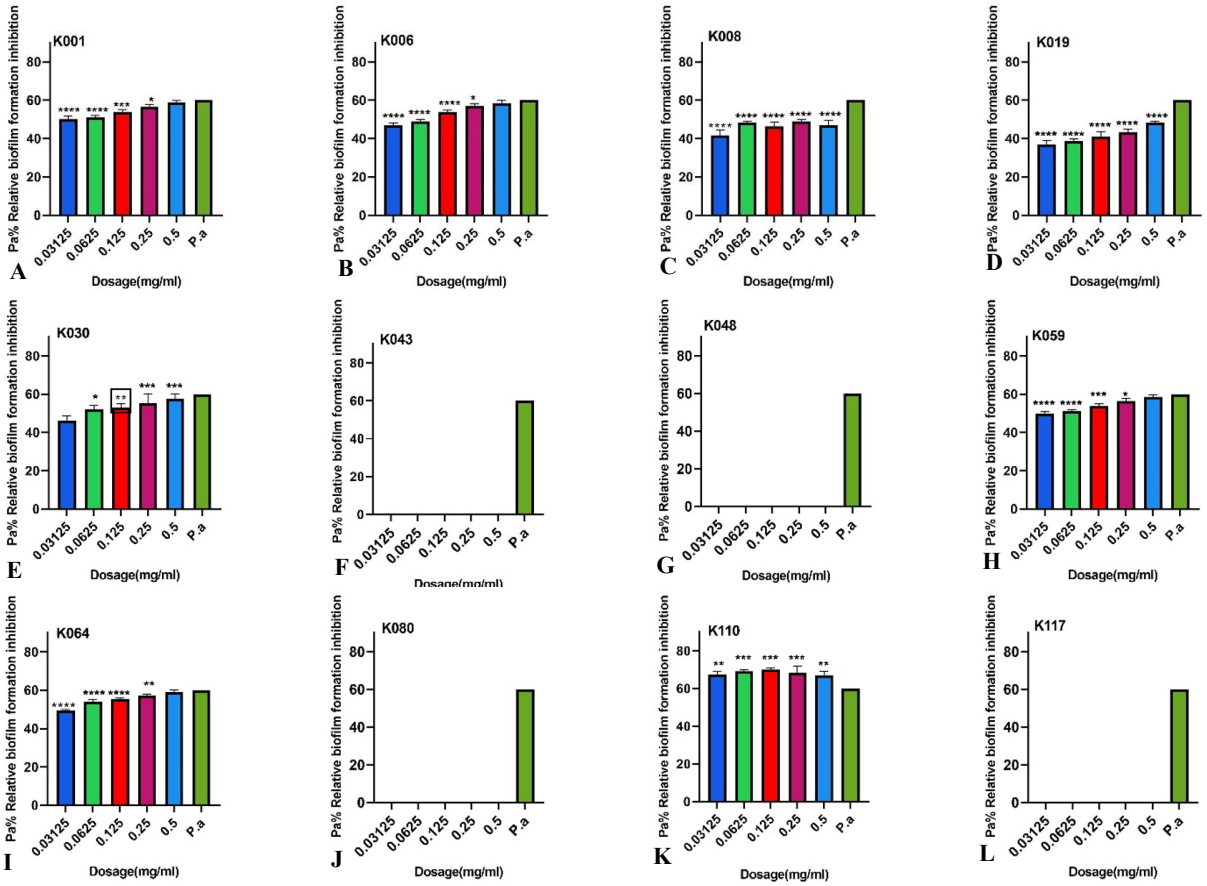

Figure 2 (A-L): Sulphamethoxazole antibiofilm formation activity against, *E. coli* K001, *E. coli* K006, *E. coli* K008, *E.coli* K019, *E. coli* K030, *E. coli* K043, *E. coli* K048, *E. coli* K059, *E. coli* K064, *E. coli* K080, *E. coli* K0110 and *E. coli* K0117, PC=P. aeruginosa – positive control.

(n=3, Dunnett's multiple comparisons ANOVA test; *p=0.05; **p=0.01; ***p=0.001; ****p=0.0001).

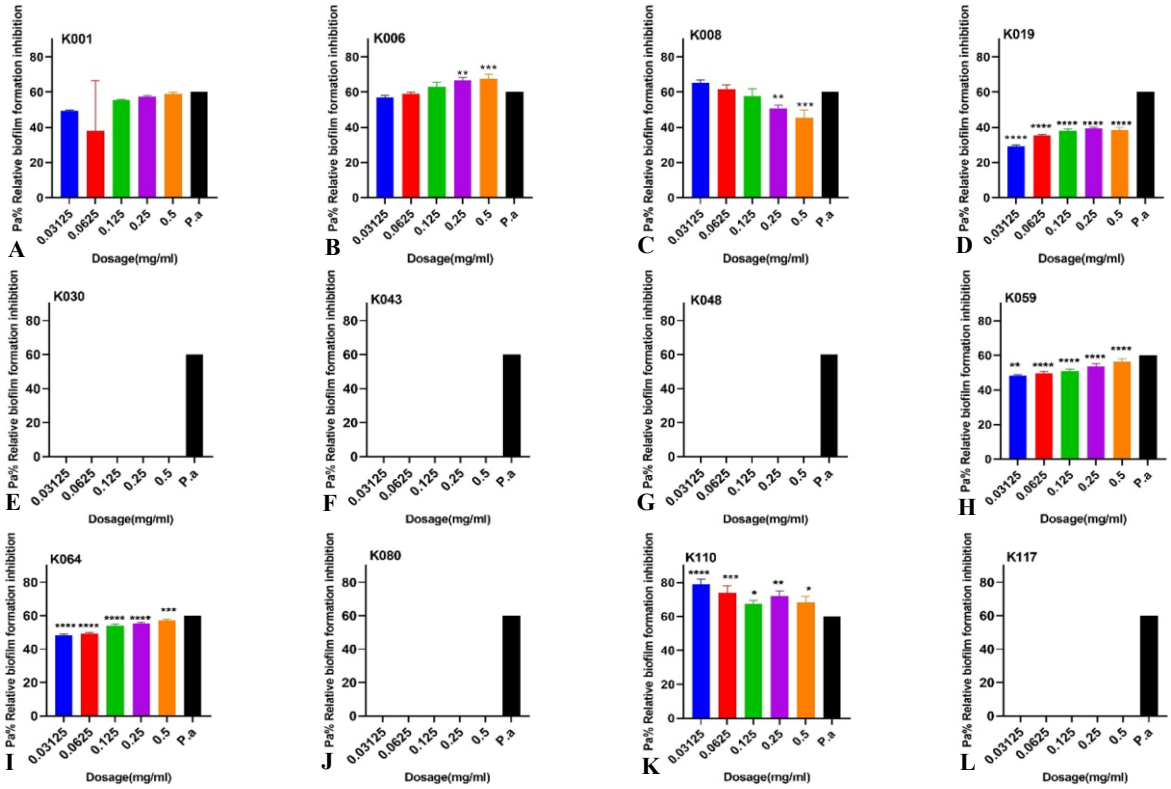

Figure 3 (A-L): Amoxyclave antibiofilm formation activity against, *E. coli* K001, *E. coli* K006, *E. coli* K008, *E. coli* K019, *E. coli* K030, *E. coli* K043, *E. coli* K048 *E. coli* K059, *E. coli* K064, *E. coli* K080, *E. coli* K0110 and *E. coli* K0117, PC= P. aeruginosa – positive control.

(n=3, Dunnett's multiple comparisons ANOVA test; *p=0.05; **p=0.01; ***p=0.001; ****p=0.0001).

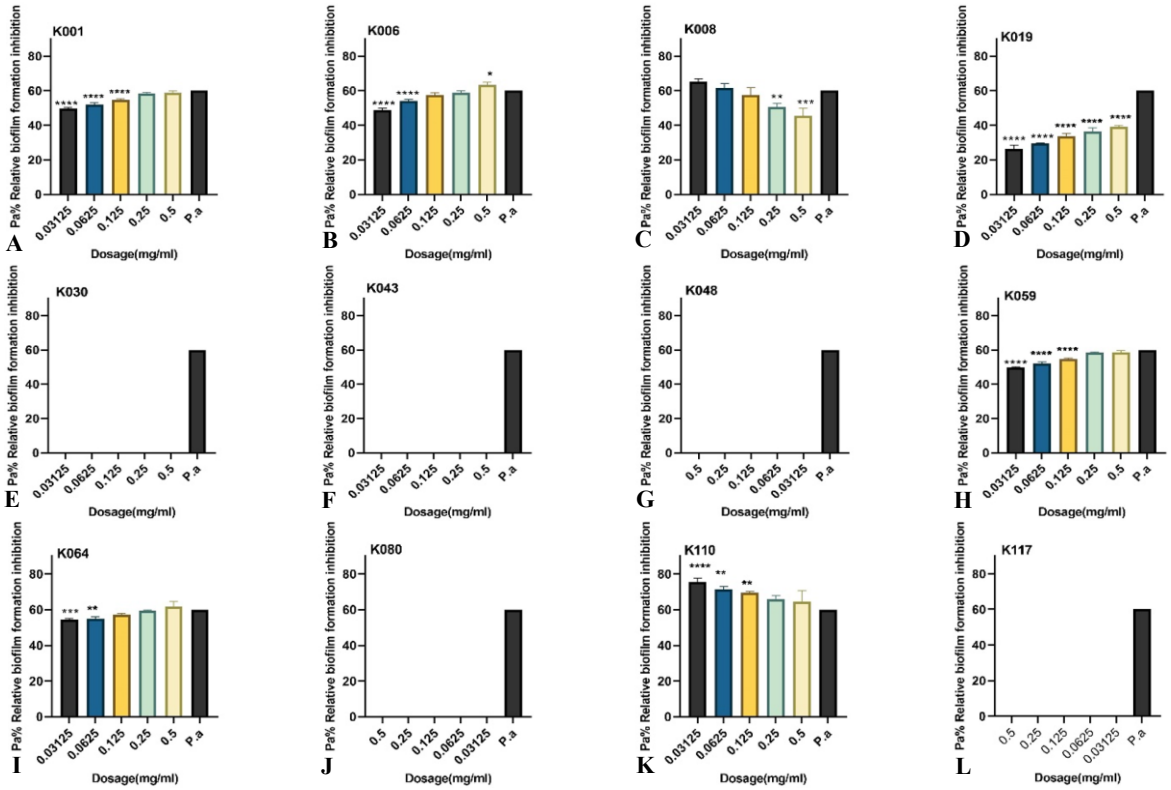

Figure 4 (A-L): Ceftriaxone antibiofilm formation activity against, *E. coli*K001, *E. coli*K006, *E. coli* K008, *E.coli* K019, *E. coli*K030, *E. coli* K043, *E. coli* K048, *E. coli*K059, *E. coli* K064, *E. coli* K080, *E. coli* K0110 and *E. coli* K0117, PC=P. aeruginosa – positive control.

(n=3, Dunnett's multiple comparisons ANOVA test; *p=0.05; **p=0.01; ***p=0.001; ****p=0.0001).

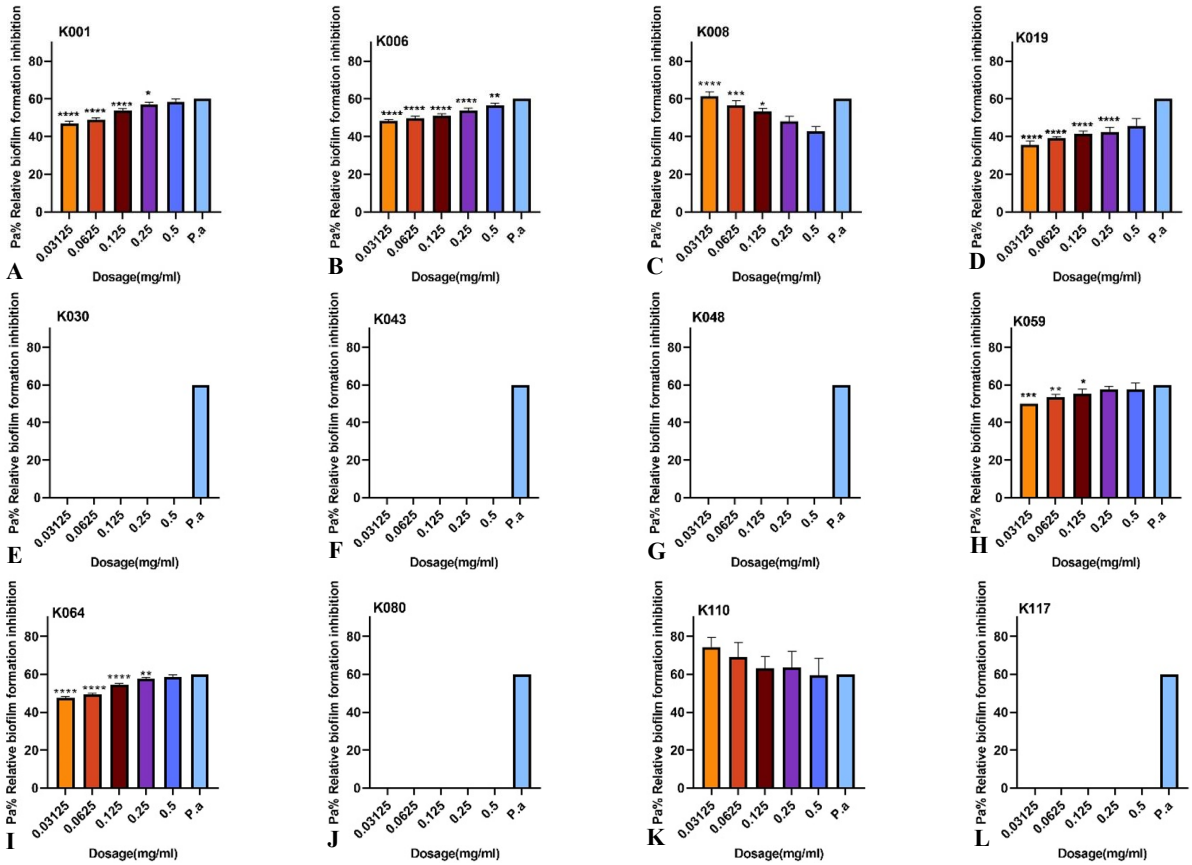

Figure 5 (A-L): Nalidixic acid antibiofilm formation activity against, *E. coli* K001, *E. coli* K006, *E. coli* K008, *E.coli* K019, *E. coli* K030, *E. coli* K043, *E. coli* K048, *E. coli* K059, *E. coli* K064, *E. coli* K080, *E. coli* K0110 and *E. coli* K0117, PC=P. aeruginosa – positive control.

(n=3, Dunnett's multiple comparisons ANOVA test; *p=0.05; **p=0.01; ***p=0.001; ****p=0.0001).

Table 1: Resistant genes occurrence.

E. coli isolates	blaCTX-M	blaTEM	blaSHV
K001	+	+	+
K006	+	+	+
K008	+	+	+
K019	+	+	+
K030	+	-	-
K043	-	-	-
K048	-	-	-
K059	+	+	+
K064	+	-	-
K080	-	-	-
K110	+	+	-
K117	-	-	-
Gene presence frequency n/N (pa %)	8/12 (66.7%)	6/12 (50%)	5/12 (417%)

+, Positive; -, Negative.

The isolates showed significance at different levels and it is also clear from our findings that at higher dosages (0.5 mg/ml), more biofilm formation inhibition was observed in these isolates as compared to the lower dosages.

Amoxyclave

Six isolates except isolate *E. coli* K001 show significance differences at different levels as shown in Figure 3. The higher dosages (0.5 mg/ml) again show more inhibitory activities as compared to lower dosages in the majority of the isolates except for isolates *E. coli* K008 and *E. coli* K110.

However, with isolate *E. coli* K008 the reverse activities were observed about dosage with more biofilm formation inhibitory activity being seen at lower dosages as compared to higher dosages.

Ceftriaxone

Total of 7(58.33%) (*E. coli* K001, *E. coli* K006, *E. coli* K008, *E. coli* K019, *E. coli* K059, *E. coli* K064 and *E. coli* K110) isolates had substantial levels of antibiofilm formation activities against the drug ceftriaxone with a significance at different levels as indicated in Figure 4.

More inhibition was observed at a higher dosage (0.5 mg/ml) as compared to lower dosages except for isolate *E. coli* K008 and *E. coli* K110.

Nalidixic acid

Only 7 (58.33%) (*E. coli* K001, *E. coli* K006, *E. coli* K008, *E. coli* K019, *E. coli* K059, *E. coli* K064, *E. coli* K110) had antibiofilm formation activities against the drug Nalidixic acid in which six of them (except for isolate *E. coli* K110) had significance at different levels as shown in Figure 5.

DISCUSSION

The World Health Organization (WHO) has listed the majority of microorganisms, including E. coli, as priority bacteria for disease research, discovery and development of new medicines since the evolution of resistant bacteria, which poses a serious global public health concern.[23]

According to the current study findings, it is clear that *E. coli* isolates possess extended spectrum beta-lactamase (ESBL) genes where the majority of these genes appeared in double and triple gene combinations. The triple ESBL gene combination with a prevalence of 5 (41.7%) and that of double gene combinations being 2 (16.66%) existed as CTXM/TEM/SHV and CTXM/TEM, respectively. Only 1 (8.33%) blaCTX-M gene stayed singly while the remaining 4 isolates (33.33%) lacked any of the three ESBL genotypes that were found in this investigation. The most common gene was blaCTX-M (42.1%), followed by blaTEM (31.57%) and blaSHV (26.31%). There is a possibility that one of the causes of bacterial drug resistance is the incorrect use of antibiotics by individuals, consequently resulting in the creation of these resistant genes in the population under study.

Some drugs like sulfamethoxazole are inexpensive and easy to obtain over-the-counter without a prescription in many developing nations, making them more vulnerable to abuse and thus contributing to resistance. Another drug that has fallen victim because of its easy access from drug stores is the Amoxiclav whose resistance may be associated with the hyper production of the chromosomal class C -lactamase of *E. coli* and the plasmid-mediated TEM enzymes.[24] The majority of *E. coli* possess beta-lactamase genes, as noted in the current study and this has easily contributed to treatment failure by many drugs. Early studies have also highlighted the increasing ESBL resistance targeting the penicillin, cephalosporin, cephamycins and monobactams class of drugs, which happen to form the majority of the antibiotics used in this study.[12]

Research conducted at the Agha Khan University Hospital in Kenya shows that *E. coli* and Klebsiella are some of the bacteria that cause antibiotic resistance in humans where blaTEM, blaSHV and blaCTX-M genes were isolated.[25] Similarly, a study in Tunisia has reported the isolation of triple gene combinations of TEM/SHV/CTX-M, whereas in Tanzania, another study isolated a double gene combination of TEM/CTX-M.[26,27] This has shown a worrying trend, bearing in mind that the production of β-lactamases encoded by blaCTX-M, blaSHV and blaTEM genes has consequently limited the drug choices commonly used in the developing world, leading to increased mortality, morbidity and inflated hospital costs.[28] The witnessed high antimicrobial resistance from *E. coli* can be linked to a possible lack of advanced laboratory quality control and assurance practice. The study finds these resistant genes silent killers, especially in the communities that are economically challenged, who ignorantly and unknowingly misuse drugs. These results again agree with those in a study at Agha Khan University, Kenya, which identified blaCTX-M as the predominant genotype and another in Turkey where the same gene at 92% dominated in occurrence, followed by TEM 39% and SHV 5%.[25,29] This purely reflects this enzyme's ability to hydrolyze the drug cefotaxime, a third-generation cephalosporin drug, killing its potency.[30] Our findings, however, do not concur with those obtained from a study at a Machakos hospital in Kenya, which established SHV 22 (43.14%) as the most isolated ESBL gene, followed by blaTEM 18 (35.29%) and blaCTXM 16 (31.37%).[31]

The differences between this study's results and those of other authors could be attributed to variations in the prevalence and type of ESBL genes isolated from different geographical regions.[12] There is no doubt that the presence of CTX-M, TEM and SHV -lactamases in clinical isolates indicates the presence of multidrug resistance cases with clinical implications in both community and hospital settings.[32] This can easily be attributed to inadequate surveillance and monitoring of the commonly used antibiotics, which has rendered the empirical therapy ineffective.[33] Notably, over-the-counter drug acquisition and the excessive administration of oral antimicrobial agents can create pressure on the targeted bacteria to make them gain resistance towards these drugs.[34] The most efficient, secure and often prescribed antibiotics have been the beta-lactam drugs such as Amoxiclav and ceftriaxone; however, due to abuse and overuse, they have become an easy target by bacterial ESBL enzymes, particularly Klebsiella pneumoniae and Escherichia coli, as reflected in the current study.[12]

From the previous findings, this study also aimed at determining the E. coli's antibiofilm formation activity after exposing them to selected antibiotics such as sulfamethoxazole, Amoxiclav, Ceftriaxone and Nalidixic acid.[10] It was observed that of the 12 isolates (n=12), 4 (33.3%) were not inhibited from forming biofilms by the four drugs used, while 8 (66.6%) of the isolates were partially inhibited from forming biofilms by one or more drugs. Sulphamethoxazole drug is a sulfanomide which acts as an antimetabolite and interferes with bacterial folic acid synthesis. The antibiofilm formation of the *E. coli* isolates indicated unequivocally that the majority of the isolates (8 out of 12 isolates) had biofilm formation inhibited and the level of inhibition by the drugs used depended on the specific isolate and dosage. These isolates were partially inhibited by the sulphamethoxazole

drug from forming biofilms, showing significance at different levels. These findings indicated that at higher dosages (0.5 mg/ml), more inhibition was observed in the majority of the isolates as compared to the lower dosages. The isolates *E. coli* K043, *E. coli* K048, *E. coli* K080 and *E. coli* K117 had their biofilm formation not inhibited by this antibiotic; hence, it cannot be recommended for the biofilm management of the *E. coli* isolates.

Amoxiclav is a bactericidal antibiotic inhibiting cell wall synthesis. These findings indicate that 7 (58.33%) of the 12 *E. coli* isolates had antibiofilm formation against this drug and six of these isolates (except for *E. coli* K001) showed significance at different levels. The higher dosages (0.5 mg/ml) showed more inhibition as compared to lower dosages in the majority of the isolates, except for isolates *E. coli* K008 and *E. coli* K110. Finding concurs with antibiofilm formation activity, resistant genes profiling and detection of virulence factors of toxigenic vibrio cholerae isolates from Kisumu County, Kenya, whereby we expected more activity at higher dosages as compared to lower dosages because they have more inhibition activity against the isolates.[35]

The isolate *E. coli* K008 and *E. coli* K110 showed higher inhibition of biofilm formation at lower dosages as compared to higher dosages. This could be attributed to the aggregation of the treatment drug as dosage increases and, therefore, it does not penetrate the relevant poly-matrices to act at the point of need and, hence, encourages biofilm formation at higher dosages.[17] The isolates *E. coli* K030, *E. coli* K043, *E. coli* K048, *E. coli* K080 and *E. coli* K117 had their antibiofilm formation activity not inhibited by this drug; therefore, it is not the drug of choice for biofilm management.

The results from this study again prove that 7 (58.33%) of the 12 *E. coli* isolates had antibiofilm formation against the drug ceftriaxone and there was significance shown at different levels. Similarly, the results showed that 7 (58.33%) of the 12 *E. coli* isolates had antibiofilm formation against the drug Nalidixic acid and six of them (except for isolate 110) showed significance at different levels. These two drugs again indicated that the higher dosages (0.5 mg/ml) showed more inhibition as compared to lower dosages except for isolates K008 and K110. The isolates K030, K043, K048, K080 and K117 had the antibiofilm formation not inhibited by this drug.

The continued use for a long time and over subscription of Nalidixic acid would be the reason for the observed treatment failure, although its resistance is primarily associated with the presence and development of bacterial conjugative plasmids. Ceftriaxone, a third-generation cephalosporin, gains its resistance when the bacteria alters the penicillin-binding protein sites on its cell wall, drug efflux from bacterial cells and the acquisition of ESBL genes.[36] Just like sulphamethoxazole and Amoxiclav, ceftriaxone and Nalidixic acid show some incomplete and partial inhibition towards the various *E. coli* isolates, a clear indicator of multidrug resistance. The problems associated with antibiofilm formation have a global picture whereby even developed countries have had their share. In Bulgaria, a study indicated that 87.5% of the 16 *E. coli* isolates showed resistance to several drugs and 31.25% showed strong antibiofilm formation.[37] Similar observations were made in Iran where of the 130 *E. coli* isolates, 80 (61.53 %) could make antibiofilm.[16] A study on extra intestinal pathogenic *E. coli* strains from four hospitals in Catalonia Spain reported 73 (19.4%) of the *E. coli* isolates as ESBL-producing strains such as blaCTX-M-15 (n=43.58.9%), blaTEM-1 (n=33. 45.2%), blaCTX-M-14 (n=8.12.3%) and blaSHV-1 (n=7. 9.6%) and the majority of them produced antibiofilms.[38] In Hungary, another study indicated that 57(22.8%) of *E. coli* isolates were multidrug resistant (MDR) and those that produced biofilm were less common among the ones resistant to third-generation cephalosporin and trimethoprim-sulfamethoxazole.[39]

Kenya and Uganda are sub-Saharan nations that struggle with similar social and economic challenges, including the lack of basic health facilities. According to one study done in Uganda, 62.5% (125/200) of the *E. coli* isolates developed biofilms and 78% (156/200) of them were multi-drug resistant (MDR) specifically to amoxicillin (93%), trimethoprim sulfamethoxazole (78%), gentamycin (87%) and imipenem (0.5%). This would easily be attributed to misuse and over-the-counter acquisition of drugs without proper prescriptions.[40] In western Kenya, a study on wastewater samples contaminated with *E. coli* revealed that 58.8% of its isolates contained multi-drug resistant (MDR) genes, of which 85.3% showed resistance to ampicillin. The genes blaTEM at 65% and blaSHV 8.8% were encoded in those isolates where 108 (43.2%) of the total isolates were positive for biofilm formation, making *E. coli* more dangerous even at the environmental level.[39]

The biofilms, as earlier described, are multi-cellular communities (matrices) formed by bacteria to offer protection against antimicrobial intervention and host immune response.[15] The bacteria growing in the biofilm are intrinsically resistant to many antibiotics and demand a high drug dose to suppress them.[36] The higher ability of the ESBL-producing organisms to form biofilms worsens the treatment efforts, increasing the mortality and production of severe infections.[36] As such, their resistance to these antibiotics could be attributed to biofilm formation, which has been found to protect these bacteria.[25] From the findings, four isolates were not inhibited by the drugs bio-assayed from forming biofilms even though they had shown drug resistance during susceptibility in our previous published study.[10] This creates a gap for further research to involve all other ESBL genes.[40]

CONCLUSION

Resistance was found against the routinely used antibiotics of Ampicillin, Amoxicillin and clavulanic acid to the majority of the biofilm-forming *E. coli* isolates in

the current investigation. Therefore, AMR surveillance is needed to monitor the effect of biofilm throughout the UTIs' causative agents. In treating UTI cases in pregnant women, the screening of antimicrobial susceptibility patterns before the prescription of antibiotics is highly recommended. Furthermore, studies should be conducted especially in all UTIs causative agents to detect biofilm and its association with antibiotic susceptibility. This will, in turn, improve understanding particularly in UTIs diagnosis, which has a bigger impact on treatment management. Finally, the MPA methods should be introduced in the AMR surveillance program which is affordable and quantitative in examining the biofilm formation of every microorganism undergoing resistance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Sosmus SM, Omwenga EO, Musyoki SK, Awuor SO. Genotypic characterization and antibiofilm formation of resistant *Escherichia coli* isolates causing urinary tract infections among pregnant women at Kisii, Kenya. Int J Community Med Public Health 2026;13:62-71.