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Phenotypic and Genotypic Characterization of Extended Spectrum Beta-Lactamases Producing Bacteria Causing Urinary Tract Infections among Expectant Women Attending Antenatal Clinic at Ruiru Sub County Hospital, Kenya

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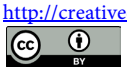

Abstract

Background: Urinary tract infection (UTI) is a bacterial infection affecting males and females but is more prevalent in expectant women. ESBLs are bacteria with enzymes that make them resistant to many antibiotics, posing a significant health challenge. This study aims to determine the characteristics of ESBL-producing bacteria causing UTIs in expectant women. **Methodology:** A self-administered survey was carried out; 300 expectant women were recruited using a random sampling method. A questionnaire was used to collect socio-demographic information. Urine samples were collected in sterile universal bottles and processed at the JKUAT Zoology laboratory. Urine samples were analyzed using urinalysis, microscopy, culture, and sensitivity testing. ESBL-producing bacteria were identified phenotypically using the double-disc synergy test (DDST) and genotyped for specific resistant genes using PCR. **Results:** UTI prevalence was 32.7% (98/300). UTI was significantly associated with the history of previous UTI (OR = 0.84, $p = 0.02$) and multigravida (OR = 0.14 $p = 0.01$). UTI was common in women aged between 28-37 years in their second trimester. Bacteria isolated were *E. coli* 57.1% (56/98), *S. aureus* 21.4% (21/98) *K. pneumonia* 11.2% (11/98) and *Proteus* spp 10.4% (10/98). Bacteria antibiotic resistance patterns were *E. coli*-tetracycline (91.1%), sulfamethoxazole (55.4%), cefotaxime (53.4%) and augmentin (53.4%). *S. aureus*-sulfamethozaxole (100%) and augmentin (71.4%), *K. pneumoniae*-sulfamethoxazole (72.2%) cefotaxime (63.6%), chloramphenicol and tetracycline (54.5%). *Proteus* spp: tetracycline (100%), nitrofurantoin (90%), cefotaxime and chlo-

ramphenicol (50%). The proportion of ESBLs bacterial producers was 37.6% (29/77) and 44.8% (13/29) possessed ESBLs resistant genes; *Bla CTX-M* 53.8% (7/13), *Bla SHV* and *Bla TEM* 23.1% (3/13) each, *Bla OXA* (0%) was not detected. **Conclusion:** The study revealed a high proportion of ESBLs producing bacteria responsible for UTI in expectant women. ESBLs screening, routine culture and sensitivity testing will guide on proper management and empirical treatment of UTI patients thus reducing multi-drug resistance.

Keywords

Urinary Tract Infections, Resistant Genes, Genotypic, Phenotypic, Extended Spectrum Beta Lactamases

1. Introduction

Urinary tract infections (UTIs) are common bacterial infections that affect part of the urinary system. UTIs are mainly caused by bacteria when they ascend from the peril urethral area to the urethra, and upper urinary tract. Women suffer from UTIs more than males due to their shorter urethral distance and proximity of the urethral tract to the anus. UTI has a spectrum of clinical entities with severity ranging from asymptomatic infection to acute pyelonephritis with sepsis and symptomatic bacterial infections resulting from upper urinary tract infection. UTI prevalence has been reported to be high in pregnant women [1]. Global records show 150 million cases occur annually where the infection accounts (40% - 50%) of women and (5%) in men [2]. A recent study in Kenya indicated a prevalence of 34% [3] at Kiambu hospital and a prevalence of 26.7% at Kenyatta National Hospital (KNH) [4]. The study done at KNH revealed that the predominant bacterial pathogen being isolated were *Escherichia coli* (40%), *Staphylococcus* species (25%) and *Klebsiella* species (10%) as well as *Enterococcus*, *Enterobacter* and *Citrobacter* species [4]. A similar study done at PCEA Kikuyu Hospital on maternal morbidity and mortality showed that urinary tract infection was the commonest maternal illness in pregnancy, accounting for (14.5%) whereas genital trauma was the commonest morbidity suffered by women during delivery estimated at (90.6%) [5]. Research performed in Hanang in Northern Tanzania found a prevalence of (16.4%) among expectant women [6].

Antimicrobial resistance is a growing threat worldwide with UTI's showing increasing resistance to antimicrobial agents of different classes [7], which may complicate the therapeutic management of urinary tract infections. The use of narrow spectrum inexpensive antimicrobial agents is becoming less feasible affecting both cost and access to good health care by the affected individuals. Inappropriate and excessive use of antibiotics has contributed significantly to antimicrobial resistance, however, beta-lactam antibiotics are the main class of drugs used for the treatment of these infections. The emergence and spread of bacterial resistance to beta-lactam drugs cause treatment failure and recurrent infections

[8]. The production of ESBLs is an important mechanism of resistance to third-generation cephalosporins which are widely used in the treatment of urinary tract infections because of lesser nephrotoxicity effects [9]. ESBLs are one of the major concerns worldwide as they cause treatment failure thus causing antibiotic resistance.

ESBLs are enzymes that give resistance to beta-lactam antibiotics such as cephalosporins, carbapenems, penicillins and monobactams which are used for the treatment of gram-negative bacterial infections. They are derived from narrow spectrum beta lactamases namely (TEM 1, TEM 2 or SHV 1) by mutations and alter the amino acid configuration around the enzyme site [9]. ESBLs are produced by Enterobacteriaceae members especially *E. coli* and *K. pneumonia* in clinical settings [10]. Extended spectrum beta lactamases producing Enterobacteriaceae are a major player in increased antibiotic resistance.

ESBLs are classified as nine evolutionary and structural families based on amino acid sequences as CTX-M, TEM, SHV, OXA, PER, VEB, GES and BES. First ESBLs were observed in the enzyme family of *TEM* and SHV. Nowadays CTX-M family is dominant worldwide with CTX-15 being the most detected ESBL enzyme [10]. Other beta lactamases families include GES, VEB, and BES. However, the same Amber class D (OXA-2 and OXA-10) also belongs to ESBLs group of enzymes. The spread of ESBLs enzymes is due to high gene exchange within Enterobacteriaceae and a related group namely *Pseumonadales*. Many genes coding for ESBLs are found on mobile genetic elements such as plasmids which can be expressed in different species. In Kenya there is limited data on phenotypic and genotypic characterization of ESBLs bacterial producers causing UTIs among expectant women hence this study investigated the phenotypic and genotypic characteristics of bacterial uropathogens from expectant mothers Attending Antenatal Clinic at Ruiru Sub-County Hospital.

2. Materials and Methods

Study Design, Site and Ethical Approval

This study was conducted between August and October 2021. This was a cross-sectional prospective study which used systematic sampling. The study was carried out at Ruiru Sub-County Hospital which is a government level 4 facility located in Ruiru town, Kiambu County. It offers inpatient and outpatient medical services to Ruiru and its environs. It has a bed capacity of 200 with modern equipment, a theater, a maternity ward, a chest clinic and an antenatal clinic. The land area is 292 km, elevation 1565 m (5135 ft), coordinates: (01˚10'04"S, 36˚58'24"E). Ethical approval was sought from JKUAT Ethical committee REF: JKU/2/4/896B. Informed consent was sought from expectant women attending ante natal clinic at Ruiru Sub County hospital, who were 18 years and above and for the minors consent was obtained from parents or guardians.

Sampling

Participants were recruited using a systematic random sampling procedure

until the desired study sample was reached. Data on the participants' sociodemographic characteristics were gathered using a standardized questionnaire. After completing the consent form, participants received instructions on how to collect clean, catch-midstream urine. Every sample vial featured a barcode and a date of collection connected to the participant's questionnaire, which was recorded to an expectant women information file. The sample size was determined using Zepdep, 2006 formula according to Fisher 1992 using UTI postulated prevalence of 26.7% reported by [4] Nairobi, Kenya. The Calculated sample size was 300, meaning 300 expectant women were enrolled in this study.

Macroscopic, Dipstick and Microscopic Urine Analysis

All 300 participants' urine samples were subjected to macroscopy observation to test various pathological parameters. Dipstick analysis using the 10-parameter chemical reagent urine strip and microscopic examination was performed as previously documented [11] [12]. Briefly, 10 ml of the urine sample was centrifuged at 2000 - 3000 rounds per minute (rpm) for 5 minutes. The supernatant after centrifugation was poured and a drop of the deposit was placed on a glass microscope slide, covered with a cover slip and examined using a compound microscope under a 10× objective lens. This was followed by an examination under 40× objectives. This was to determine the presence of pus cells, white blood cells, bacteria or yeast, casts, crystals and red blood cells in urine. Any bacteria presence detected (1 - 4 per high power field) was considered bacteriuria. In contrast, the presence of pus cells/leucocytes ≥ 10 in a single high-power field (HPF) was treated as pyuria case [11].

Bacterial Isolation

Culture and subculture was done on CLED and MacConkey medium. They were incubated for 24 hours at 37˚C. Identification of bacteria species was done using colony characteristics, gram staining and biochemical tests following standard procedures. Gram positive bacteria were identified by catalase and coagulase test. Triple sugar iron, indole, citrate utilization and urease test were used to identify gram negative bacteria.

Antimicrobial Sensitivity Test

Kirby-Bauer disc diffusion technique was carried out. A suspension of the test organism was prepared using 0.5 McFarland standard as the reference. The entire surface of Mueller Hinton agar (MHA) plate was inoculated with a sterile swab. Antibiotics disks used were Ampicillin (AMP 10 ug), Cefotaxime (CTX 80 up), Cefuroxime (CEF), Meropenem (MEM 10 ug) and Augmentin (AUG 30 ug) were used in the first plate. This helped in the observation of synergy zones that form when a cephalosporins antimicrobial combines with Beta lactam inhibitors.

Second plate had Gentamicin (GN 10 ug), Ciprofloxacin (CIP 5 ug), Chloramphenicol (CHL 30 ug), sulfamethoxazole (SXT 25 ug), Tetracycline (TE 30 ug). Cotrimoxazole (COT 30 ug), Nitrofurantoin (NIF 30 ug). All disks were obtained from oxoid, UK. All the plates were incubated at 37˚C for 24 hours. Incubation zones were measured and interpreted as per clinical laboratory stan-

dard institute guidelines 2020. Potency of antibiotic discs and quality media was tested with *E. coli* (ATCC 25922) standard positive control strain.

Phenotypic Detection of ESBLs

Double disk synergy method (DDST) was used to detect ESBLs phenotype where Culture of test isolates was made on Mueller Hinton agar plate, a clavulanate disc containing amoxicillin + clavulanate acid 30/10 (µg) was placed at the center of the plate and discs containing cefotaxime (30 µg) or ceftazidime (30 µg) were placed around it at a distance of 20 - 30 mm and then incubated at 37˚C for 24 hours. An increase in the zone towards the disc of amoxicillin, clavulanate was considered positive for ESBLs production. *K. pneumoniae* ATCC 700603 (ESBL producer) and *E. coli* ATCC 25922 (non-ESBL producer) served as the positive and negative controls respectively.

DNA Isolation

Colony PCR was carried out. Pure cultures were used in DNA preparation by picking two colonies from a fresh culture media plate using a sterile wire loop. It was suspended in 0.5 mL of distilled water and heated in a water bath at 100˚C and allowed to boil for 15 minutes. It was cooled down to room temperature and centrifuged at 2700 rpm for 10 minutes. The supernatant which contained the suspended DNA preparation was then harvested and stored at −4˚C. The harvested DNA preparation was Purified using, Qiaquick purification kit (Qiagen) and the concentration was checked using a Nanodrop 2000 spectrophotometer (Themo Fisher Scientific-US).

Detection of Resistant associated genes

PCR was used to detect for *Bla CTX-M, Bla TEM, Bla SHV* and *Bla OXA* resistant genes (Table 1). PCR amplification was done in 20 µl volumes containing 4 µl of 5× master mix of 0.4 µl concentrations of each primer, 12.2 µl of PCR water BSA 1 µl and 2 µl of DNA template. A programmable thermocycler was used with initial denaturation at 94˚C for 5 min; followed by 35 cycles at 94˚C for 30 s, annealing was done between 30 seconds and 1 minute depending on the primer temperature, then a short extension step at 72˚C for 1 minute and a final extension at temperature of 72˚C for 10 minutes for short fragments and 20

Table 1. Primers used for detection of resistant genes.

Gene	Primer sequence	Expected size (bp)	annealing temp (˚C)
Bla TEM	F = 5'GCGGAACCCCTATTTG3' R = 5'TCTAAAGTATATATGAGTAAACTTGGTCTGAC3'	999 bp	52
Bla SHV	F = 5'TTCGCCTGTGATTATCTCCCTG3' R = 5'TTAGCGTTTGCCAGTGYCG3'	851 bp	60
Bla CTX-M	F = 5'ATGTGCAGYACCAGTAARGTKATGGC3' R = 5'TGGGTRAARTAARTARGTSACCAGAAYCAGCGG3'	593 bp	60
Bla OXA	F = 5'ATGAAAAACACAATACATATCAACTTCGC3' R = 5'GTGTGTTTAGAATGGTGATCGCATT3'	920 bp	52

minutes for longer fragments.

Electrophoresis was done to the amplified PCR products which were visualized on 1% agarose gel in 1× TBE buffer and stained with ethidium bromide dye. This was visualized under UV trans-illumination. Positive control stains were used for the different genes and distilled water was used as a negative control.

Statistical Analysis

The data was stored in an excel sheet. The results were analyzed using SPSS 11.0 statistical software, chi square (*2), OR was used to compare associations between proportions and p-values < 0.05 was considered significant at 95% CI.

3. Results

Demographic Characteristics of Expectant Women

A total of 300 expectant women aged between 15 - 47 years were recruited for the study. Age distribution was, 15 - 17 years, 1.6% (5/300), 18-27 years 41.7% (125/300), 28 - 37 years 52.7% (158/300), 38 - 47 years 4% (12/300), their gestation period was 1 - 12 weeks 6% (18/300), 13 - 27 weeks 58.3% (175/300), 28 - 42 weeks, 35.7% (107/300), their parity; primigravida 35.7% (107/300) and multigravida 64.3% (193/300), marital status; single 20.7% (62/300) and married 79.3% (238/300), employment status; employed 35% (105/300) and unemployed 65% (195/300). The previous history of UTI was 10% (30/300) and without 90% (270/300) (Table 2).

Prevalence of UTI in Expectant Women Attending Antenatal Clinic at Ruiru Sub County Hospital

The prevalence of UTI in expectant women attending antenatal clinic at Ruiru Sub County was 32.7% (98/300) (Figure 1).

SOCIO-Demographic Characteristics of Expectant Women Associated with UTI

The history of previous UTI (OR = 0.84; 95% CI: 1.40, 40. 23, $p = 0.02$) and multigravida (OR = 0.14; 95% CI: 0.01, 0.51, $p = 0.01$) were significantly associated with the occurrence of bacterial UTI. Further, no significant association was observed between age, marital status, employment status and gestation period of pregnancy (Table 3).

Distribution of Bacterial Pathogens Causing UTI among Expectant Women Attending Antenatal Clinic at Ruiru Sub County Hospital

The predominant bacteria isolates causing UTI were *E. coli* (57.1%), *S. aureus* (21.4%) *K. pneumoniae* (11.2%) and *Proteus spp.* (10.2%) (Figure 2).

Antibiotic Susceptibility Pattern of Bacterial Pathogens Causing UTI in Expectant Women

E. coli was highly resistant to tetracycline (91.1%), sulphamethoxazole (55.4%), cefotaxime (53.4%) and Augumentin (53.4%). *S. aureus* was highly resistant to sulphamethoxazole (100%) and Augumentin (71.4%). *K. pneumoniae* was resistant to sulphamethoxazole (72.2%) cefotaxime (63.6%), chloramphenicol and tetracycline (54.5%) each and *Proteus spp* were resistant to tetracycline

(100%), nitrofurantoin (90%), cefotaxime and chloramphenicol (50%) (Table 4).

Proportion of ESBLs Bacterial Producers Causing UTI among Expectant Women Attending Antenatal Clinic at Ruiru Sub County Hospital

A total of 77 isolates were tested for ESBL producers. Out of the isolates tested

Table 2. Demographic characteristics of pregnant women.

	Frequency N = 300
Age category	
15 - 17 years	5 (1.6%)
18 - 27 years	125 (41.7%)
28 - 37 years	158 (52.7%)
38 - 47 years	12 (4%)
Gestation period	
1 - 12 weeks	18 (6%)
13 - 27 weeks	175 (58.3%)
28 - 42 weeks	107 (35.7%)
Parity	
Primegravida	107 (35.7%)
Multigravida	193 (64.3%)
Marital status	
Single	62 (20.7%)
Married	238 (79.3%)
Employment status	
Unemployed	195 (65%)
Employed	105 (35%)
Previous UTI	
Yes	30(10%)
No	270(90%)

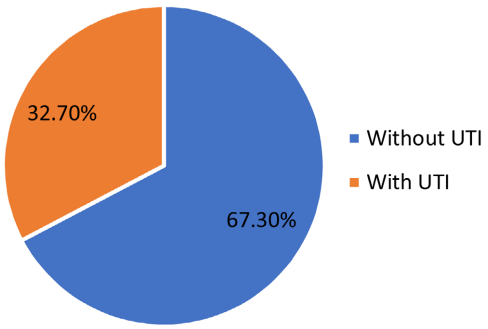

Figure 1. Prevalence of UTI in expectant women attending antenatal clinic at Ruiru Sub County Hospital.

Table 3. Socio demographic associations of UTI in expectant women.

	n = 98	Odds ratio	95% CI	P value
Age category				
15 - 17	0 (0%)	0	0 - 0	0
18 - 27	45 (45.9%)	0.79	0.07 - 8.17	0.84
28 - 37	47 (48%)	4.91	0.47 - 50.90	0.18
38 - 47	6 (6.1%)	3.29	0.29 - 36.93	0.33
Gestation period				
1 - 12 weeks	7 (7.1%)	1.01	0.31 - 3.70	0.82
13 - 17 weeks	36 (36.7%)	1.12	0.33 - 3.73	0.85
28 - 42 weeks	55 (56.2%)	0.47	0.06 - 3.65	0.47
Parity				
Primegravida	31 (31.6%)	0.02	0.01 - 0.51	0.21
Multigravida	67 (68.4%)	0.14	0.03 - 0.57	0.01
Marital status				
Single	26 (26.5%)	0.01	0.01 - 0.85	0.12
Married	72 (73.5%)	0.01	0.02 - 0.89	0.14
Employment status				
Employed	37 (37.8%)	0.52	0.16 - 2.19	0.64
Unemployed	61 (62.2%)	0.71	0.18 - 3.18	0.76
Previous UTI				
Yes	30 (30.6%)	0.84	1.40 - 40.23	0.02
No	68 (69.4%)	7.93	1.50 - 41.76	0.12

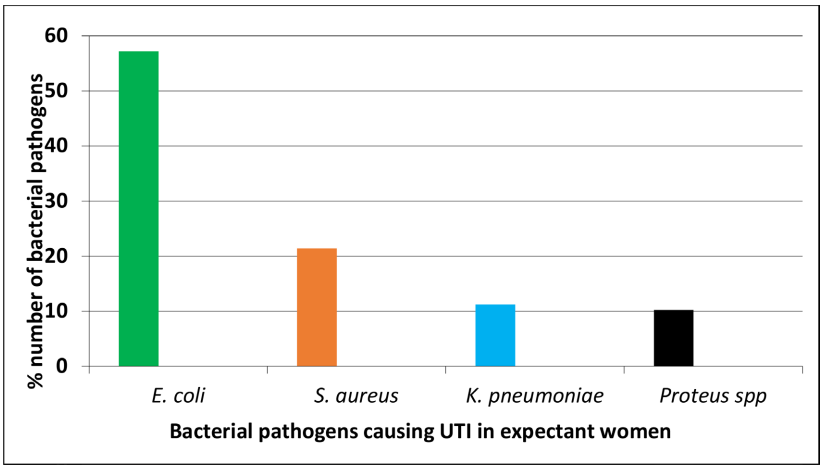

Figure 2. Bacterial pathogens causing UTI in expectant women attending antenatal clinic at Ruiru Sub County Hospital.

37.6% (29/77) were found to be ESBL producers (Table 5).

Distribution of ESBLs Genes in Bacterial Pathogens Causing UTI in Expectant Women

A total of 44.8% (13/29) isolates tested positive for ESBLs genes. Their genotypes

Table 4. Antibiotic resistance frequency in bacterial pathogens to various antibiotics.

Class of antibiotics	*E. coli* n = 56		*S. aureus* n = 21		*K. pneumoniae* n = 11		*Proteus spp* n = 10	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Cefotaxime	26 (46.4)	30 (53.4)	16 (76.2)	5 (23.8)	4 (36.4)	7 (63.6)	5 (50)	5 (50)
Cefuroxime	53 (94.6)	3 (5.4)	15 (71.4)	6 (23.6)	10 (90.9)	1 (9.1)	10 (100	0 (0%)
Meropenem	34 (60.7)	22 (39.3)	17 (80.9)	4 (19.1)	9 (1.8)	2 (18.2)	7 (70)	3 (30%
Nitrofurantoin	53 (94.6)	3 (5.4)	16 (76.2)	5 (23.8)	10 (90.9)	1 (9.1)	1 (10)	9 (90)
Sulphamethoxazole	25 (44.6)	31 (55.4)	0 (0.)	21 (100)	3 (27.3)	8 (72.7)	8 (80)	2 (20)
Ampicilin	30 (46.4)	26 (46.4)	18 (85.7)	3 (14.8)	6 (54.5)	5 (45.5)	7 (70)	3 (30)
Augmentin	30 (53.6)	30 (53.4)	6 (28.6)	15 (71.4)	7 (63.6)	4 (36.4)	5 (50)	5 (50)
Ciprofloxacin	56 (100)	0 (0)	16 (76.2)	5 (23.8)	9 (81.8)	2 (18.2)	9 (90)	1 (10)
Chloramphenicol	29 (51.8)	27 (48.2)	19 (90.5)	2 (9.5)	5 (45.5)	6 (54.5)	6 (60)	4 (40)
Tetracycline	5 (8.9)	51 (91.1)	14 (66.7)	7 (33.3)	5 (45.5)	6 (54.5)	0 (0)	10 (100)
Gentamicin	34 (60.7)	22 (39.3)	20 (95.2)	1 (4.8)	10 (90.1)	1 (9.1)	10 (100)	0 (0)

Table 5. Proportion of ESBLs producers causing UTI in expectant women.

Bacterial pathogen	Frequency n = 29
E. coli	20(69.0%)
K. pneumoniae	7(24.1%)
Proteus spp	2(6.9%)

Table 6. Frequency of ESBLs genes detected in bacterial pathogens causing UTI in expectant women.

Gene	*Bacteria Isolates*		
	E. coli	*K. pneumoniae*	*Proteus spp*
Bla CTX-M	3 (23.1%)	3 (23.1%)	1 (7.7%)
Bla SHV	1 (7.7%)	1 (7.7%)	1 (7.7%)
Bla TEM	3 (23.3%)	0	0
Bla OXA	0	0	0
Total	7	4	2

were *E. coli Bla CTX-M* (23.1%), *SHV* (7.7%), *TEM* (23.1%), followed by *K. pneumoniae* with *CTX-M* (23.1%), *SHV* (7.7%) and lastly *Proteus spp* with *CTX-M* (7.7%), *SHV* (7.7%). *TEM* was not detected in *K. pneumoniae* and *Proteus spp*. *Bla OXA* gene was not detected in any of the isolates (Table 6).

Figures 3-5 below represents electrophoresis gel results for *Bla CTX-M*, *Bla TEM* and *Bla SHV* genes detected in expectant women.

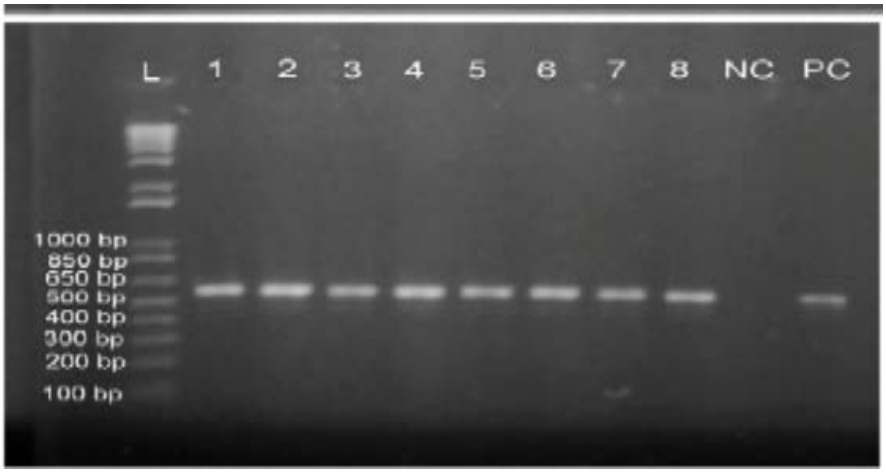

Figure 3. Electrophoresis gel results for *Bla CTX-M* gene (593 bp): From left 100 bp molecular ladder, lane 1 - 8 target gene NC: Negative control (distilled water), PC: Positive control (*K. pneumoniae* ATCC 700603).

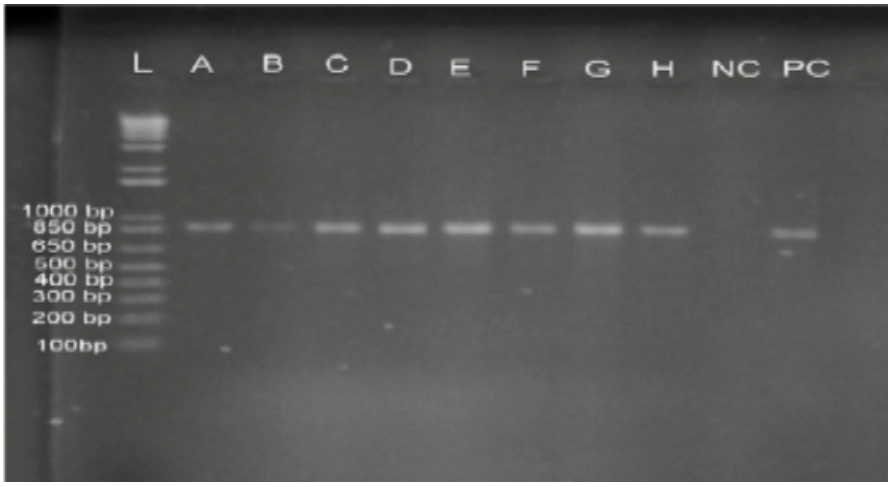

Figure 4. Electrophoresis gel results for *Bla TEM* genes (999bp): From left 100bp Molecular ladder, lane A-G NC: Negative control (distilled water), PC: Positive control (*K. pneumoniae* ATCC 700603).

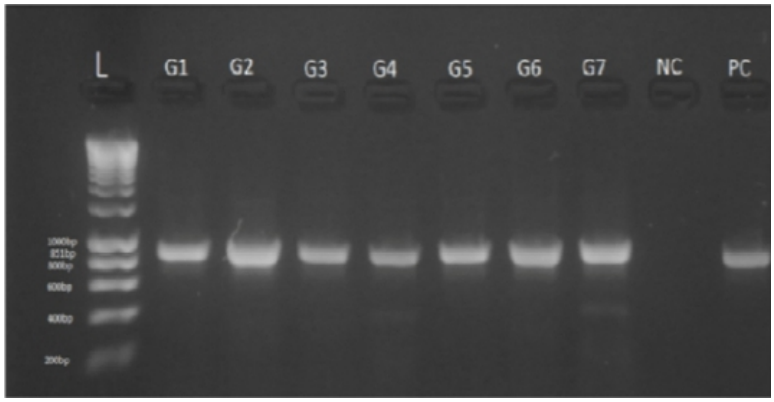

Figure 5. Electrophoresis gel results for *Bla SHV* gene (851 bp): From left 100 bp molecular ladder, Lane G1 - G7 target gene NC: Negative control (distilled water); PC: Positive control (ATCC *K. pneumoniae* 700603).

4. Discussion

Bacterial UTI is one of the most common causes of seeking treatment in expectant women. Effective treatment of UTI patients depends on the organism causing the infection and the selection of antimicrobial agents efficient for the treatment of the uropathogens. ESBLs producing uropathogens have become a great problem worldwide [13]. This study reports on urinary tract bacterial pathogens and ESBLs production among urine isolates obtained from expectant women attending the antenatal clinic at Ruiru Sub County Hospital. We found an overall prevalence of UTI at 32.7%. This prevalence is within the global prevalence range of 13% - 33% [14]. High UTI prevalence leads to negative birth outcomes due to the infection causing a challenge in treatment leading to antibiotic resistance [15]. The overall prevalence of UTI has been reported to range between 11.6% - 49.4% in various studies in sub-Saharan Africa and India. The variation in UTI prevalence within different geographical locations could be attributed to the modes of screening and risk factors such as age, parity and previous UTI history [3] [13] [14] [16] [17].

This study showed that the highest UTI prevalence was in expectant women between the ages of 28 - 37 years and in their second trimester. This may be due to the fact that this age group is the most sexually active and reproductive stage. The finding is similar to a study done by [14] in Nairobi, Kenya, which reported that most UTIs occur during the second trimester of pregnancy. The high prevalence of UTI in the second trimester can be attributed to physiological changes during pregnancy and poor personal hygiene among expectant women. Physiological changes in pregnancy affect the natural history of UTI during gestation, these changes are likely to occur in expectant women who have pregnancies in rapid succession [15]. Our study showed a significant association between parity and previous history of UTI, which means that multigravida or women who have had several pregnancies and those with a history of previous UTI infection are more likely to get UTIs. This study is comparable to [18] in Mwanza, Tanzania, [17] Ibadan, southwest Nigeria and [19] from northwest Ethiopia which reported a strong association between multi-party and previous history of UTI. In [20] study from Menoufia, Egypt revealed an association between low-income level and UTI. This could be due to a lack of funds to buy essentials like toilet paper and soap leading to poor personal hygiene coupled with a history of previous UTI infection. Our study disagrees with a study done by [14] from Pumwani, Kenya who found no association between UTI age, parity, income, previous history of UTI and marital status. A study done by [21] Khartoum, Sudan found no association between parity and UTI in pregnancy.

In this study the most predominant uropathogens isolated were, *E. coli* (57.1%), followed by *S, aureus* (21.4%) *K. pneumoniae* (11.2%) and *Proteus spp* (10.2%). The uropathogens were resistant to commonly prescribed antibiotics. *E. coli* was highly resistant to tetracycline (91.1%), sulphamethozaxole (55.4%), cefotaxime and augmentin (53.4%) each. Resistance can be brought about by in-

appropriate antibiotic prescription, extensive use of over-the-counter antibiotics, failure to complete the antibiotic dose as well as spouse not getting treated [22]. Multi-drug resistance limits the therapeutic options leading to increased morbidity health care costs [23]. A similar study by [24] in Kisii, Kenya revealed *E. coli* as the most common organism causing UTI in pregnancy and the isolates demonstrated high resistance to sulfamethoxazole (100%), augumentin (85.8%) and ceftriaxone (71.4%). Our study disagrees with [7] in Burkina Faso where among *E. coli* isolates resistance rates to ampicillin and cotrimoxazole were (65.8%) and (64.4%) respectively. A study done in Tanzania by [18] revealed *E. coli* was resistant to sulfamethoxazole (64.4%), tetracycline (58.5%) and ampicillin (53%). In [25] from North West Ethiopia recorded *E. coli* at (49.2%) and *S. aureus* at (18%). A higher number of *E. coli* isolates were resistant to ampicillin (90%) cefotaxime (83.3%), augumentin (56.7%). However, a study done by [26] in Edna Aden, Somaliland revealed majority of gram-negative isolates were resistant to ampicillin. The predominance of *E. coli* bacteria isolated in different studies is an indication of infection by fecal contamination due to poor hygiene, anatomy of genitourinary area in females and urinary stasis in pregnancy [27].

Our study revealed *K. pneumoniae was* resistant to sulphamethozaxole (72%), cefotaxime (63.6%), chloramphenicol and tetracycline (54.5%) resistance may occur due to altered binding to the target site and increased discharge drug inactivation. Many strains of *K. pneumonia*e produce ESBLs or form biofilms hence aggravating resistance. A study done by [28] Karnataka, India showed that *K. pneumoniae* was resistant to ampicillin (75.6%), nitrofurantoin and cefuroxime (73.1%) each and all the isolates were susceptible to imepenem. The uropathogen *K. pneumoniae* poses a new problem to health care professionals worldwide thus complicating and limiting therapeutic options [29].

In this study, *Proteus* spp showed high resistance to nitrofurantoin (90%) and cefotaxime (50%) but was susceptible to cefuroxime and gentamicin at (100%) hence should be the recommended drug of choice for the *Proteus spp* treatment. *Proteus mirabilis* is capable of causing symptomatic infections of urinary tract including cystitis and pyelonephritis and is present in cases of asymptomatic bacteriuria in the elderly. In addition, this species can cause infection in the respiratory tract eye, ear, nose, skin, throat, burns and wounds. *Proteus* possesses virulence factors like pilli or fimbriae for adherence to uroepithelium. *Proteus* isolates possess flagella for motility and are naturally resistant to antibiotics such as oxacillin, tetracycline and macrolide. A study done by [1] in Dezful, Iran reported that *Proteus spp* showed high resistance to ampicillin, amoxicillin, imipenem, amikacin and gentamicin. *Proteus spp* can acquire resistance to ampicillin through plasmid mediated Beta lactamase and chromosomal lactamases expression. Resistance to carbapenems is also observed in *Proteus spp*. Moreover, data from United States and European Union collected between 2009 and 2011 reported less than 10% of Proteus isolates were resistant to amikacin, aztreonam, ceftazidime, ceftriazone, meropenem and piperacillin (SENTRY) 2009.

In the present study gram positive *S. aureus* was resistant to sulfamethoxazole (100%) and augmentin (71.4%). Different studies reported by [14] in Nairobi, Kenya reported *S. aureus* was resistant to sulphamethoxazole, oxacillin and cefoxin. *S. aureus* is a common human pathogen which can colonize the skin, nose pharynx causing major diseases due to its ability to escape the innate immune response such as phagocytic complement mediated killing which assists survival in blood and other tissues during persistent infections. In recent years methicillin resistant *S. aureus* strains have developed resistance to all Beta-lactam antibiotics including penicillins, cephalosporins and carbapenem [30].

One of the leading antimicrobial resistant mechanisms for UTI bacteria is the production of ESBL that hydrolyse Beta-lactam ring thus conferring bacterial resistance to commonly prescribed antibiotics including penicilins, aztreonams and third generation cephalosporins [13]. This study revealed an overall of 37.7% (29/77) of bacteria being ESBLs producers. The rise in the prevalence of ESBLs producing uropathogens might be attributed to the habit of empirical treatment practice without antibiotic susceptibility testing. Lower prevalence was reported in North West Ethiopia who recorded a prevalence of ESBLs producers of (15.9%). Lower findings could be due to differences in study population and health care trends [25].

In our study the ESBLs genes observed in uropathogens among expectant women were *Bla CTX-M* (53.8%), *Bla SHV* (23.1%) and *Bla TEM* (23.1%). The variation in ESBLs gene detection could be due to differences in screening methods, study settings and study population [15]. The main mechanism of resistance to B lactam antibiotics may be chromosomal and/or plasmid mediated. The beta lactamases genes often coexist on the same plasmid [9]. Plasmid mediated production is acquired by vertical genetic information transfer from one organism to another and also horizontal transfer to other microbial agents [31]. Our study results agree with [32] who reported *Bla CTX-M* as the most predominant gene isolated in UTIs. [33] also reported *Bla CTXM* (95.6%), *Bla TEM* (95.6%) and *Bla SHV* (21.7%) from Kenya. Our study [32] is also comparable to [34] Beirut Lebanon who reported *Bla CTX-M* (90.7%) as the most predominant gene, followed by *Bla TEM* (88.4%) and *Bla SHV* (44.2%). A similar study done by [26] Hargesia Somalia reported *Bla TEM* (71.4%), *Bla CTX-M* (66.7) and *Bla SHV* (3.2%). The high ESBL production by bacterial isolates might be due to broad spectrum antibiotics in hospitals and the lack of laboratory screening of ESBLs production in clinical isolates. Our study differs with [26] from Hargesia, Somalia who reported *Bla TEM* (60%) as the most predominant gene, followed by *Bla CTX-M* (24%) and *Bla SHV* (16%). This is because the ESBLs genotype is spreading around the community and is affected by degree of antibiotic usage. The ESBLs are produced in hospitals where the use of antibiotics is very high and ESBLs plate to the communities and lack of screening of ESBLs in hospitals. Also, a study done by [1] in Dezfull city, Iran reported the most common genes as *Bla TEM* (66.7%) and *Bla CTX-M* (33.3%). This could be due to the growing

level of self-medication without prior laboratory investigation and difficulty in detecting and reporting ESBLs within community settings. The study had a few limitations; the sample size was small which may affect the estimation of ESBLs producing bacteria strains among expectant women. Secondly, the study was done in a hospital setup hence the results are not representative of other expectant women attending other health centres in the same region.

5. Conclusion

In conclusion the prevalence of UTI was high and significantly associated with previous UTI history and multigravida. Multidrug resistance was high among uropathogens, therefore treatment of UTIs during pregnancy should be based on antimicrobial susceptibility testing. The isolation of ESBLs producing uropathogens in the study calls for continuous follow-up on antibiotic usage in pregnant women. Moreover, conducting molecular studies will help characterize various drug-resistant strains.

6. Recommendations

The presence of multidrug resistance bacteria during pregnancy and isolation of ESBLs producing pathogens in this study requires periodic and continuous follow-up of antibiotic usage during pregnancy.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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