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Original Research Article

Social demographic factors, antimicrobial profiles and genotypic characterization of extended beta- lactamase producing uropathogens among adult diabetic patients

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Abstract

Introduction: Urinary tract infections among diabetic patients have been on the rise in Kenya and globally. They have been worsened by the rise of multi-drug-resistant strains against the commonly used Beta -lactam drugs. Relapse and re-infection have been observed among these patients.
Objectives: The study aims to investigate the antimicrobial resistance patterns, and socio-demographic factors associated with urinary tract infections (UTIs) among adult diabetic patients attending P.C.E.A Kikuyu and Lussiggetti Sub-County Hospitals in Kenya. Specifically, it seeks to determine how common UTIs are in the patient groups and to identify any socio-demographic characteristics that may influence their occurrence. The research will also focus on isolating and identifying the bacterial pathogens responsible for these infections and evaluating their antimicrobial susceptibility profiles. Additionally, the study will examine the presence of selected Extended Spectrum Beta-Lactamase (ESBL) resistance genes in the bacterial isolates, providing insight into the genetic mechanisms underlying antibiotic resistance in this population.
Materials and Methods: The study was carried out among diabetic patients attending Presbyterian Church of East Africa Kikuyu and Lussiggetti Sub- County hospitals in Kenya. A cross-sectional study where 95 diabetic patients were randomly selected. Data was collected in excel sheets and analyzed using SPSS.version 26: 2018.The Chi square was used to determine the relationship of UTI to several demographic factors with a P value of < 0.05 considered to be significant. Presence of UTI from the 95 samples was detected using dipstick analysis and urine culture on CLED agar. Isolate identification and Antimicrobial Susceptibility was done using the Minimum Inhibition Concentration technique on Vitek 2 compact machine. DNA extraction was done using QiaAmp kit and Multiplex PCR for the detection of ESBL genes from the ESBL producers identified using Double disk synergy testing.
Results: All the factors age, sex, weight, education, occupation, smoking none had significant relationship .10 /95 samples had a growth of 10^5CFU/ul with *E. coli* as the predominant, the rest were *Enterococcus faecalis, Klebsciella pneumonia and Staphylococcus epidermidis*. The organisms showed resistance to the Beta lactam drugs used, Multi drug resistance for the study was at 70%. *E. coli* susceptibility was found at 28.6% for Ampicillin, Ampicillin Sulbactam, Sulphamethazole. The drugs susceptible for the gram-negative organisms were Meropenem and Ciprofloxacin. The gram positive were resistant to Gentamicin, Sulphamethazole and clindamycin .8/10 samples were tested by molecular method and 5 were confirmed to harbor at least one ESBL gene. Bla TEM at 62.5% 5/8 followed by CTX-M at 25%, no SHV was isolated.
Conclusion: Multidrug resistance at 70% was observed thus still a burden among diabetic patients with UTI and intervention should be done to offer the right treatment. Studies on more genes of resistance that will aid on antimicrobial resistance reduction.

Keywords: Antibiotic susceptibility, Extended Spectrum β-lactamases, UTI infections

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1. Introduction

Urinary tract infections have been found to be one of the most common microbial diseases in medical practice people worldwide irrespective of their age. Although there is a wide variety of etiological agents linked to UTIs, bacteria are the main pathogenic organisms, accounting for more than 95% of UTI cases.[1] A large population of the world is generally affected by diabetes; 463 million adults worldwide had diabetes in 2019 which leading to 4.2 million fatalities.[2]

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Association (ADA) reports that type 2 diabetic patients are more likely to suffer from a urinary tract infection (UTI) and even repeat UTI compared to patients without diabetes. Diabetic patients are at increased risk for urinary tract infections (UTIs) due to factors such as impaired immune function, poor glucose control, and autonomic neuropathy, which can lead to incomplete bladder emptying.[3] Female, obese, and overweight diabetic individuals are particularly vulnerable, as are those with hypertension, diabetic nephropathy, and those on insulin therapy. Diabetic patients are also more prone to resistant pathogens, such as fluoroquinolone- and carbapenem-resistant bacteria, leading to more severe UTI outcomes like longer hospital stays and higher mortality rates. Men with diabetes, particularly after prostate manipulations, are at risk for acute bacterial prostatitis, which can progress to chronic prostatitis.[4] Complications of UTIs in diabetic patients include emphysematous pyelonephritis (a severe infection with gas formation in the kidneys) and bacteremia, which can lead to kidney damage, pyelonephritis, and septic shock. Emphysematous pyelonephritis has a high mortality rate, and other issues such as relapse, re-infection, and prolonged hospitalizations are common. High blood glucose levels can also damage nerves, impairing bladder function .The bladder fails to recognize the presence of urine, which overstays without release causing infection. Early diagnosis and treatment are crucial for preventing serious complications in diabetic patients.[5]

The Urinary tract infections have become a severe public health problem caused by a wide range of pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus.* The Extended-Spectrum β-Lactamase (ESBL) producing *Enterobacteriaceae*, which produce a range of illnesses, have spread widely in hospitals and are rising in community settings. Bacteria with these enzymes not only hydrolyze the majority of beta-lactam drugs but also show resistance to other unrelated antimicrobial drugs, which frequently creates a therapeutic conundrum.[6] The most common drugs used for the treatment of bacterial infections are Beta-lactam antibiotics.They have been used widely since 1980 for the treatment of gram-negative bacteria infections; however resistance against these antibiotic groups occurred quickly worldwide. They have been found to develop resistance against this class of antibiotics by production of beta-lactamase enzymes.[7]

The overall prevalence of urinary tract infections in a study carried out in Kisii Referral Hospital in Kenya was 20.6% with 37 participants testing positive for urinary tract infection. Gender and level of education showed no significant association with urinary tract infections among diabetic patients while age was the biggest association factor.[8] There is limited knowledge of UTI infections occurrence among Diabetes patients in relation to social demographic factors in Kenya.

2. Materials and Methods

2.1. Study site, period and socio-demographic data

Sampling method used was random sampling, Sample size 95 Samples (Fisher`s et., al 1999) previous prevalence of 9.6% (Forson *et al*., 2021)

Z = A standard normal variate at 5% type 1 error P < 0.05 it is at 1.645
P = Prevalence from previous studies = 9.2% A similar study in Ghana (Forson*et.al*, 2013).
d= Absolute error of precision 0.05-90% Confidence interval, an error of 10%

$$Sample\ size = \frac{Z^2\ P\ (1-P)}{d^2}$$

$$\frac{1.645^2 * 0.902(1-0.902)}{0.05^2}$$

–95

The study was a cross-sectional study carried out at P.C.E. A Kikuyu and Lussiggetti Sub- County hospitals from April 2023 to August 2023.Patients who were 18 years and above, with symptoms of UTI and consented to have the study done were included. The socio-demographic factors were filled by the patients following their consent. The patients who could not collect midstream urine and on antibiotics for the last one week were not included.

2.2. Lab procedure

Midstream urine was collected from patients with UTI symptoms from the study sites with sterile urine containers. The standard urinalysis method was used to process the collected samples. The samples with pus cells, nitrite, and presence of protein using dipstick method qualified for culture. The urine was centrifuged for microscopy to check for epithelial, red and pus cells. Centrifugal urine sediment was cultured in CLED agar by streaking method using the standard quantitative wire loop followed by incubation at 37°C for 18-24 hours. They were observed for growth. The cultures with a characteristic growth of $>10^5$ colony forming units per ml were considered significant of a single bacterial species. The colonies on the plate were counted and multiplied by the calibration factor from the standard wire loop of 10ul, where each colony equals to 100cfu/ul according to culture guidelines.[9] The culture plates with no growth or non-significant growth were discarded according to biological waste segregation procedure of the laboratory. The positive cultures were processed for identification by the colonial morphology; size, shape. Gram stain was performed before inoculation to AST GN-ISI-1240705071 and GP.-ISI-2422879103

Quality control ATCC 25922 and pure cultures of bacterial isolates were suspended in 3ml of sterile saline in a clear polystyrene tube to achieve a turbidity of 0.50-0.63. This was measured by the densicheck turbidimeter. The suspensions were inoculated into the different identification cards and also with the AST cards where dilution was done as per the Standard Operating Procedure for Vitek VK2C6482. Minimum Inhibition Concentration was the method used to determine the susceptibility patterns.

This was done by filling the cards with the suspension using a vacuum device. The filled cassette/cards were manually loaded into the Vitek 2 compact machine which detects bacterial growth and metabolic changes in the cards using a fluorogenic methodology. The results were read after 12-18hours.The antibiotics used with a concentration ranging from 0.25ug/Ml/32ug/Ml. The drugs used for Gram Negative bacteria were: Ampicillin, Amoxicillin/Clavulanic Acid, Ampicillin Sulbactam, Piperacillin/Tazobactam, Cefazolin, Cefuroxime, Cefuroxime-Axetil, Cefoxitin, Cefotaxime, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Nitrofurantoin, and Sulphamethazole. Gram positive bacteria Benzylpenicillin, Oxacillin, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Linezolid, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Fusidic acid, Rifampicin, Trimethoprim/Sulphamethazole for Gram positive bacteria. The AST cards used consists of 60 biochemical method and substrates for identification where reporting procedure was CLSI M100 Guidelines/2023.

The positive samples were also tested for ESBL production using done disc synergy. This was done by measuring the zone of inhibition between clavulanate and cefotaxime/ceftriaxone/ceftazidime. The test was performed on Mueller Hinton agar with a 30mcg disk of ceftriaxone and a disk of amoxicillin-clavulanate containing 10 mcg of clavulanate positioned at a distance of 30 mm (center to center). The test was considered as positive when a decreased susceptibility to ceftriaxone combined with a clear-cut enhancement of the inhibition zone of ceftriaxone in front of the clavulanate-containing disk, resulting in a characteristic shape zone referred to as ‘champagne-cork’ or ‘keyhole’.[10] The ESBL producers were transported to KEMRI for PCR and genotypic characterization. The DNA was extracted from the colonies using QiaAmp DNA mini kit which was yielded into 1.5ml micro-centrifuge tube. PCR amplification was done using multiplex PCR machine with primers for *blaSHV, blaTEM, blaCTX-M* as described by Monstein[11] (expected amp size *TEM* -445bp, *CTX* -M593bp, *SHV-747bp*). The PCR products were loaded into gel wells where electrophoresis was done. The PCR products were run alongside a known *E. coli* isolate as a positive control and distilled water as Negative control. Gel staining with Gel red and image viewing and captured under UV Transilluminator.

2.3. Ethical consideration

Permission to carry out the study was sought from JKUAT Ethical commission, NACOSTE and also from the two Hospitals PCEA Kikuyu and Lussiggetti Sub- County. Consent forms were issued to patients before collection of samples which were written in languages understood by the community. The illiterate signed the consent after it was read to them in the presence of a relative/guardian.

3. Results

A total of 95 patients were recruited in the study. Results were recorded as follows; 10 samples were positive for UTI with a growth of ≥10^5/ul CFU which accounted for 10.5% of the total samples analyzed. Sixty-one samples were collected from female patients (64.2%) and 34 (35.8%) from male patients as shown in **Table 1**. A Chi-square evaluation for relationship between the social demographic factors was done where a p value of 0.05 was considered significant. None of the factors showed any association with urinary tract infection.

The distribution of bacterial pathogens from the 10 positive cases were 80% (8/10) for gram negative while gram positive 20% (2/10). The predominant organism was *E. coli* making a total of 70% (7/10), the rest of the 30% were Klebsciella pneumonia, Staphylococcus epidermidis and Enterococcus faecalis. Out of 7 E coli isolates, 5 were possible Multidrug Resistant (MDR) and one was a possible extensive Drug resistant (XDR) Isolate, the other two were neither MDR nor XDR. The Klebsciella pneumonia and Staphylococcus epidermidis were MDR and possibly Pan drug resistant strains while enterococcus faecalis was neither an MDR nor PDR. Multidrug Resistant (MDR) drug- defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (That is, resistant to at least 33%) of the drugs tested. Extensive drug Resistance (XDR) drug- defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (bacterial isolates remain susceptible to only one or two categories), (That is, resistant to at least 50% of the drugs tested). Pan Drug Resistant (PDR) drug- Pan Drug-Resistant (PDR) refers to a type of bacterial or microbial infection that is resistant to all or nearly all available antimicrobial drug classes available according WHO guidelines.

Two Gram positive species isolated: Enterococcus faecalis were neither multidrug resistant neither pan drug resistant while Staphylococcus epidermidis was a multidrug resistant organism where 50% of the drugs used were resistant as illustrated in **Table 2**.

The gram positives were resistant against Gentamicin, Erythromycin and Sulphamethazole.

The overall resistance patterns for the *E. coli* isolates to the 18 antibiotics used is as summarized in **Table 3**.

Table 1: Social demographic factors associated with UTI among diabetic patients

Variables	Frequency	%	Positive UTI cases	%
Gender				
Male	34	(35.8)	4	(11.8)
Female	61	(64.2)	6	(9.4)
Age in years				
20-29	5	(5.3)	0	(0)
30-39	6	(6.3)	0	(0)
40-49	21	(22.1)	1	(4.76)
50-59	25	(26.3)	3	(12)
60-69	26	(27.4)	5	(19.2)
70-79	10	(10.5)	0	(0)
80-89	2	(2.1)	1	(50)
Education				
Tertiary	24	(25.3)	1	(4.2)
High school	14	(14.7)	2	(14.3)
Primary school	57	(60)	7	(12.3)
Alcohol intake				
Non-alcoholics	85	(89.5)	9	(10.5)
Alcoholics	10	(10.5)	1	(10)
Smoking				
Non-smokers	90	(94.7)	10	(11.1)
Smokers	5	(5.3)	0	(0)
Weight				
55-59	1	(1.1)	0	(0)
60-64	5	(5.3)	0	(0)
65-69	11	(11.6)	1	(9.1)
70-74	22	(23.2)	2	(9.1)
75-79	32	(33.7)	3	(9.4)
80-84	15	(15.8)	2	(13.3)
85-89	6	(6.3)	2	(33.3)
90-94	2	(2.1)	0	(0)
95-99	1	(1.1)	0	(0)

Note: The social demo graphic factors according to the population N=95

Table 2: Sensitivity and resistance patterns of Gram-positive species isolated N=2 Pattern = S/R Sensitive/Resistant

Antimicrobial drugs	*Enterococcus faecalis*	*Staphylococcus epidermidis*
Benzylpenicillin	S	R
Oxacillin	S	R
Gentamycin	R	R
Tobramycin	R	S
Levofloxacin	S	R
Moxifloxacin	S	R
Erythromycin	R	R
Clindamycin	R	S
Linezolid	S	S
Teicoplanin	S	S
Vancomycin	S	S
Tetracycline	S	S
Tigecycline	S	S
Fusidic	R	S
Rifampicin	S	R
Trimethoprim/ Sulphamethazole	R	R

Note: Interpretation of Susceptibility patterns by CLSI M100 Guidelines

Table 3: The overall resistance patterns for the *E. coli* isolates to the 18 antibiotics

Antibiotics	**Pattern**	***E. coli* (non-ESBL producers) % N=3**	***E. coli* (ESBL producers) % N=4**
Ampicillin	S R	66.7 33.7	0 100
Ampicillin Sulbactam	S R	66.7 33.7	0 100
Piperacillin/Tazobactam	S/I R	66.7 33.3	50 /50
Cefazolin	I R/I	66.7 33.3	 50/50
Cefuroxime	S R	100 0	100 0
Cefuroxime Axetil	S R	100 0	100 0
Cefoxitin	S R	100 0	100 0
Cefotaxime	S R	100 0	75 25
Ceftazidime	S R	100 0	75 25
Ceftriaxone	S R	100 0	75 25
Cefepime	S/I I	66.7 33.7	50/25 25
Aztreonam	S R	100 0	75 25
Meropenem	S R	100 0	100 0
Amikacin	S R	100 0	100 0
Gentamicin	S R	100 0	100 0
Ciprofloxacin	S R	100 0	75 25
Nitrofurantoin	S R	100 0	100 0
Sulphamethazole/Trimethoprim	S R	66.7 33.3	0 100

Note: Interpretation of Susceptibility patterns by CLSI M100 Guidelines/2023

Table 4: Sensitivity and resistance patterns of Gram-positive species isolated N=2

Antimicrobial drugs	**S (%)**	**R (%)**
Benzylpenicillin	1(50)	1(50)
Oxacillin	1(50)	1(50)
Gentamycin	2 (100)	0
Tobramycin	1(50)	1(50)
Levofloxacin	1(50)	1(50)
Moxifloxacin	1(50)	1(50)
Erythromycin	0	2(100)
Clindamycin	1(50)	1(50)
Linezolid	2(100)	0
Teicoplanin	2(100)	0
Vancomycin	2(100)	0
Tetracycline	2(100)	0
Tigecycline	2(100)	0
Nitrofurantoin	2(100)	0
Fusidic	1(50)	1(50)
Rifampicin	1(50)	1(50)
Trimethoprim/Sulphamethazole	0	2(100)

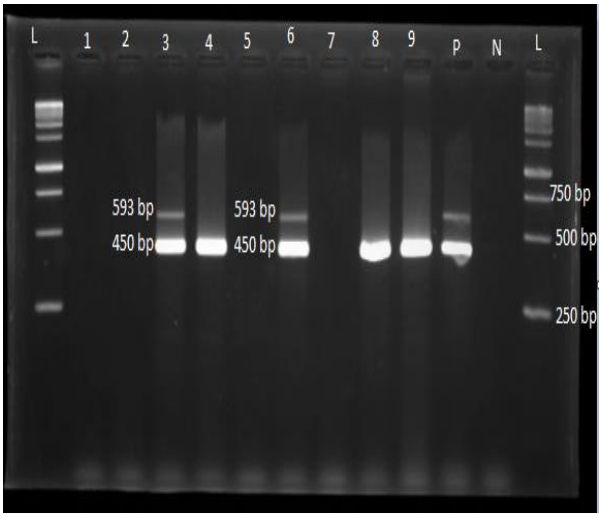

Figure 1: The genes results upon translumination with gel red. Key - L-ladder-1 kb; 1-*E.coli* isolate negative for the three genes; 2-9 Samples; P-*E.coli* positive control; N-Negative control.

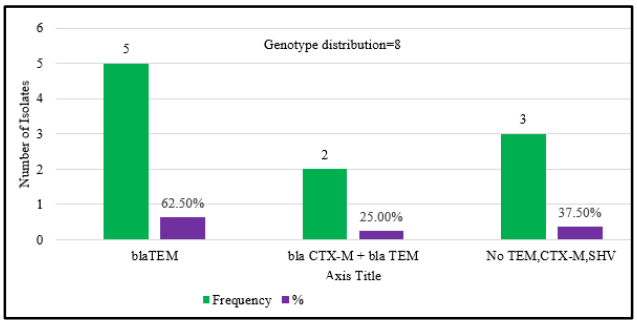

Figure 2: Genotypic distribution of the Beta lactamase producing isolates

The highest resistance was found on Ampicillin, Ampicillin Sulbactam and Sulphamethazole on Esbl producers and non-Esbl producers at 100% and 33.7%respectively No resistance was noted from Nitrofurantoin, Gentamicin, Amikacin and meropenem. Out of 7 E coli isolates, 5 were possible Multidrug Resistant (MDR) and one was a possible extensive Drug resistant (XDR) Isolate, the other two were neither MDR nor XDR. The Klebsciella pneumonia and Staphylococcus epidermidis were MDR and possibly Pan drug resistant strains while enterococcus faecalis was neither an MDR nor PDR.

Two Gram positive species isolated: Enterococcus faecalis was neither multidrug resistant neither pan drug resistant while Staphylococcus epidermidis was a multidrug resistant organism where 50% of the drugs used were resistant as illustrated in Table 4.

The gram positives were resistant against and Erythromycin and Sulphamethazole.

A double disk synergy test was performed on the 8 Gram negative bacteria to detect ESBL producers' .It was done by measuring the zone of inhibition between clavulanate and cefotaxime/ceftriaxone/ceftazidime.The ESBL producers were 5/8.The same isolates were tested for genotypic expression of selected genes *bla SHV, bla TEM, bla CTX-M.*

The PCR products from the gram-negative bacteria were loaded into gel wells where electrophoresis was done. The PCR products were run alongside a known *E. coli* isolate as a positive control and distilled water as Negative control. Gel staining with Gel red and image viewing and captured under UV Transilluminator. The results are displayed in **Figure 1**.

Genotypic characterization among the uropathogens isolated was as follows: 5/8(62.5%) Esbl bacterial isolates were confirmed by molecular analysis to harbor at least one ESBL gene. All the isolates had *blaTEM* and two isolates had both *blaCTX + blaTEM*, none of the isolates was found to harbour *SHV.BlaTEM* =5/8 (62.5%), *blaCTX+ blaTEM* =2/8 (25%) 3/8-(37.5%) did not harbor any of the three genes 5/8 -(62.5%) harbored *blaTEM*2/9- (25%) both *blaTEM +blaCTX*-. The distribution is as shown in **Figure 2**. The *E.coli* isolates harbouring the *blaTEM* genes were four except one that had both genes. The *Klebsciella pneumonia* isolate was found to have both *blaTEM + blaCTX.*

4. Discussion

Urinary tract infection in diabetic patients was not influenced by any of the factors studied age, sex, smoking, drinking alcohol, marital status, and occupation. A similar study in Kisii, Kenya age showed a significant relationship[8] P =0.002. Ugandan study[12] showed an association of UTI with gender (p=0.017) in Sudan[13] none of the characteristics studied showed any association. The risk factors in Ghana were age, diabetes duration and prior history of UTI[14] and symptomatic UTI, in Addis Ababa Ethiopia.[15] The following factors, comorbidity, gender, duration of diagnosis were risk factors for diabetic patients with UTI in Northeast Ethiopia,[16] different populations thus with different risk factors.

UTI in diabetes was caused by both Gram negative and Gram-positive bacteria at 80% and 20% respectively with *E. coli* as the most isolated organism at 70% (7/10) in Kisii, Kenya at (60%) with 21 isolates out of 35 positive isolates.[5] A study in Ethiopia[15] at 63.6% as compared to other organisms isolated at 70.4%[17] in Europe.

All the 8 Enterobacteriaceae isolates were susceptible to Cefoxitin, Meropenem and Amikacin 100%. The drugs can be recommended for treatment according to this study and another in Northwest Ethiopia[18] where resistance was at 5.5%. Resistance to other drugs ranging from 100%-25% for Esbl producers and 33.7% -0% for non-ESBLs Sulphamethazole, Ampicillin and Ampicillin Sulbactam with the highest resistance. They were intermediate to Cefazolin (50%), Cefuroxime (50%) and Cefepime (37.5%). MDR resistance at 70%, 5 *E. coli* isolates, Klebsciella pneumoniae and Staphylococcus epidermidis. Gram negative MDR at 6/8 (75%) at 100%[19] in a Pakistan study from Uropathogens isolated from diabetic patients thus resistance still high

among the mentioned bacteria and Gram positive at 50% (1/2). Ampicillin showed the highest resistance at 75% for the Enterobacteriaceae thus least sensitivity. The gram negatives were sensitive to Meropenem similar to a study in India[20] where *E. coli* was found susceptible to the Carbapenems.

Gram positives were 100%susceptible to Nitrofurantoin, Gentamicin, and Vancomycin similar to a study in Karachi, Pakistan[21] Teicoplanin, Linezolid, Vancomycin, Tetracycline, Tigecycline and Penicillin (50%). They were resistant at 100% to Erythromycin, in Kiambu Kenya[22] and Trimethoprim at 100% similar still to an Ethiopian study.[1]

The 5 Esbl producers, showing Multidrug resistance and also genes of resistance blaTEM, *blaCTX* which possibly be causing resistance against Beta-lactam drugs which are commonly used for UTI treatment. Genetic analysis showed that almost all of the positive samples produced blaTEM5/8(62.5%) gene similar to a study done in Kenya[22] and in Nepal South Asia[23] at a frequency of 83.8% followed by *bla-CTX* at (59.1%), followed by *blaSHV* (27.3%) and no *CTX-M* was detected in any of tested isolates in yet another study in Egypt.[24] Coexistence of *blaTEM* and blaCTX-M 2/8 (25%), 43 isolates (50.58%) in India.[25] None of the diabetic Uropathogenic *E. coli* harbored CTX and SHV alone in diabetic isolates. Three isolates (37.5%) did not harbour any of the genes according to the study. Bla TEM gene being the dorminant gene differs from a study[26] in Bangladesh, Asia stating that CTX is more prevalent

5. Conclusion

Observation that all Diabetic patients irrespective of age, sex, weight, occupation, education are at risk of UTI, monitoring should be done through routine urinalysis and culture for the right treatment for the positive cases. The study shows 70% of the organisms were MDR where 5 of the 7 were gram negative positive for Esbl genes thus causing resistance to Beta lactam drugs that are commonly used for UTI infections. Thus, multidrug resistance is still a burden and more studies should be done on reduction of AMR burden among diabetic patients with UTI. The isolates showing Genes of resistance at 62.5% *bla TEM* followed by its co-existence with *bla CTX* which could be the cause of reduced utility of Beta Lactam drugs.

6. Study Limitations

The low positive cases constrained the elaborate comparison of the distribution of the cases among the sub-groups in the population**.**

7. Source of Funding

None.

8. Conflict of Interest

None.

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