Higher prevalence of ESBL producing uropathogenic Escherichia coli among Diabetic patients from a Tertiary Care Hospital of Kathmandu

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Research article

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Abstract

Background: The increased rate of urinary tract infection (UTI) in immunocompromised patients especially diabetic patients is a major public health problem in adults. Moreover, the infection with multidrug resistant strains producing extended spectrum beta-lactamase (ESBL) is a key obstacle in disease management among such vulnerable population. An immediate proper treatment depends on rapid diagnosis of UTI and screening of antimicrobial resistant pattern with highly sensitive methods which also reduces the possible urinary complications among the diabetic patients. Hence, this study was aimed to determine the occurrence of antibiotic resistant genes for β-lactamases; blTEM and blaCTX-M in uropathogenic Escherichia coli isolates from UTI suspected diabetic and non-diabetic patients.

Methods: A hospital-based cross-sectional study was conducted in Kathmandu Model Hospital in association with Central Department of Microbiology, TU from June to December 2018. A total of 1267 non-duplicate mid-stream urine specimens from diabetic and non-diabetic patients were obtained and processed immediately for isolation of uropathogens. The isolates were subjected for antibiotic susceptibility testing and ESBL confirmation. Finally, blaTEM and blaCTX-M ESBL genes were screened by using specific primers.

Results: The overall prevalence of the urinary tract infection UTI was found to be 17.20%(218/1267), out of which diabetic patients were significantly more infected with UTI accounting for 32.29%(31/96) as compared to non-diabetic persons, 15.97%(187/1171). A total of 221 bacterial were from 218 culture positive specimens in which E. coli was a most predominate one; 67.9%(150/221.). Forty-four percent (66/150) of the total E. coli was MDR and 37.33%(56/150) were ESBL producers. Among 56 isolates, 92.3%(12/13) from diabetic patents and 83.0% (44/53) were from non-diabetic patients. Furthermore, 84.85% of the screened ESBL producers were confirmed to possess either single or both of the blaTEM and blaCTX-M genes. The blaTEM and blaCTX-M genes were detected in 53.57% and 87.5% of the phenotypically ESBL confirmed E. coli.

Conclusions: The UTI infection is an increasing problem in diabetic patients and infection with multidrug resistant strains specially ESBL producing uropathogens are causing a huge problem in disease management leading to high rate of mortality and morbidity of diabetic patients.

Background

Diabetes mellitus is an emerging chronic non-infectious disease characterized by hyperglycemia occurs due to insufficient production of insulin from pancreas. Out of top ten diseases with higher mortality, diabetes is the one causing around four million deaths worldwide in 2017 in adults [1]. The patients with diabetes are highly susceptible to infectious diseases most frequently by urinary tract infection (UTI). The major predisposing factors for UTI in diabetic patients are impairment in immune system, poor metabolic control and incomplete bladder emptying due to autonomic neuropathy [2–4]. Older age is another crucial influencing factor to enhance the risk of UTI in diabetic patients [5].

UTI involves the invasion of microbes, their multiplication, and colonization in genito-urinary organs [6]. The degree of UTI in the diabetic patients ranges from asymptomatic infection to various severe lower urinary tract infections including cystitis, pyelonephritis and urosepsis. UTI also remains widespread nosocomial infection, is commonly diagnosed in outpatients and inpatients [7, 8]. Escherichia coli is the most dominant bacteria for
causing UTI followed by other members of Enterobacteriaceae; *Klebsiella pneumoniae*, *Citrobacter* spp., *Proteus* spp., *Enterobacter* spp. etc[7, 9, 10]. Recently, the rapid widespread of Multi-Drug Resistance (MDR) bacteria has been the major public health problem especially caused by β-lactamases producing MDR strains. The production of Extended-Spectrum β-lactamases (ESBLs) has helped bacteria to expand their activity even against the newly developed β-lactam antibiotics [11]. ESBL producing microorganisms pose tremendous therapeutic consequences and significant clinical challenges if they remain undetected. They confer resistance or decreased susceptibility to narrow and expanded-spectrum cephalosporins and monobactams but do not affect cephemycin and carbapenem compounds [12]. They are usually resistant to fluoroquinolones, aminoglycosides, and co-trimoxazole [13]. There are more than 300 different ESBL variants, TEM and SHV being the major type with increasing occurrence of CTX-M enzymes [14, 15]. Almost all Enterobacteriaceae harbor ESBL with higher prevalence among *E. coli* isolates in community-acquired infections.

The increased risk of UTI among diabetic patients and enhanced urinary complication related with MDR strains may pose high morbidity and mortality. Therefore, screening for UTI and its causative agents in diabetic patients is very crucial to enable infections to be properly treated. Moreover, identifying exact drug resistant pattern is equally important to manage the disease and further prevent the development of urinary related complications in diabetes patients [16]. Hence, the study was carried out to evaluate microbiological agents and their resistant pattern by both phenotypic and genotypic methods of UTI in diabetics and non-diabetics. The aim of this study was to investigate if there are any differences between the resistance genotyping of *E. coli* isolated from Diabetic and non-diabetic patients with reference to *bla*TEM and *bla*CTX–M genes.

**Methods**

**Study design, duration and site**

A hospital-based cross-sectional study was carried out from 20th June to December 2018, among the clinically suspected Urinary Tract Infected (UTI) patients visiting the hospital. The clinical sample processing followed by identification of uropathogens were carried out in Microbiology Laboratory of Kathmandu Model Hospital, Kathmandu, while the nucleic acid extraction and PCR amplification were done in Department of Microbiology, GoldenGate International College, Kathmandu.

**Inclusion and exclusion criteria**

This study included the patients of both sexes and all age group attending the hospital with suspicion of UTI infection. The samples which were adequately collected and properly labeled were included in the study. Those samples which were not collected with standard collection procedure, inadequately collected, improperly labeled and samples with visible contamination were excluded in the study. A repeated sample was requested in such cases. The urine samples from the patients whose health status was not mentioned as the diabetic and non-diabetics and those who were taking antibiotic less than 24 hours before visiting hospital were also excluded.

**Study variables**
The study variables included were age, sex, out-patients, in-patients department, health status including diabetic or non-diabetic and history of antibiotic taking within 24 hours of visiting hospital using standard data form assigned by the hospital.

Sample size

Consent for participation was collected from all participants during enrolment and before data and sample collection. UTI patients or suspected patients were instructed to collect mid-stream urine sample in sterile, clean and leakproof vials. A total of 1267 non-duplicate mid-stream Urine (MSU) specimens were obtained and processed immediately in the Microbiology laboratory of Kathmandu Model Hospital. The adequately collected samples from both sexes and all age groups were included while the improperly collected and poorly labelled samples with visible contamination were excluded for the study. They were requested to repeat the sample collection. Besides demographic data, medical status of patients (diabetic or non-diabetic and inpatients or outpatients) had also been recorded.

Sample processing

The urine samples were cultured onto the Cysteine Lactose Electrolyte Deficient (CLED) agar by the semi-quantitative culture technique using a standard calibrated loop [17]. The agar plates were incubated at 37°C overnight. The bacteria developed in the pure culture with a load greater than $10^5$ CFU/ml were considered as significant growth and included in this study. The bacteria were identified by standard microbiological procedures including microscopy, colony microbiology and biochemical tests as described by American Society of Microbiology (ASM). Among different bacterial isolates, only *E. coli* isolates were further processed for molecular studies [18].

Antimicrobial susceptibility testing

Susceptibility tests of the bacterial isolates towards various antibiotics were performed by the modified Kirby-Bauer disc diffusion method using Mueller Hinton Agar. The *E. coli* (ATCC 25922) was used as the control strains. Antibiotics Amoxicillin (10 µg), Cefexime (5 µg), Cotrimoxazole (25 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Levofloxacin (5 µg), Ofloxacin (5 µg), Norfloxacin (5 µg) and Nitrofurantoin (300 µg) were used in the primary testing while for MDR isolates Amikacin (30 µg), Amoxicillin-clavulanic acid (20/10 µg), Cefoperazone/Sulbactam (75/30 µg), Ceftazidime (30 µg) and Piperaclillin/Tazobactam (80/10 µg) were used for further testing. Further Polymyxin B (300 µg), Colistin (10 µg) and Tigecycline (15 µg) was used for 14 bacterial isolates [19]. Subsequently, the rate MDR *E. coli* was determined [20].

Phenotypic characterization of the ESBL producers

The *E. coli* isolates were screened for possible ESBL production using Ceftazidime (30 µg) and Cefotaxime (30 µg). The suspected ESBL producing *E. coli* were subjected to Combined Disk (CD) assay using Ceftazidime (30 µg) with Ceftazidime plus Clavulanic acid (30/10 µg) and Cefotaxime (30 µg) with Cefotaxime plus Clavulanic acid (30/10 µg) for phenotypic confirmation [19].
Molecular characterization of bla TEM and bla CTX−M genes: The plasmid DNA was extracted from phenotypically confirmed ESBL producing E. coli by the alkaline lysis method followed by the phenol: chloroform purification method [21, 22]. A conventional linear PCR was used to amplify the bla TEM and bla CTX−M genes in the extracted plasmid DNA. The bla TEM gene was amplified by using a primer with forward nucleotide sequence 5'-GAGACAATAAGGGTGTAAT-3' and reverse nucleotide sequence 5'-AGAAGTAAGTGGCAGCAGTG-3' [23]. Similarly, the bla CTX−M gene was amplified by using a primer with forward nucleotide sequence 5'-TTTGCAGTGTGCAGTACCAGTAA-3' and reverse nucleotide sequence 5'-CTCCGCTGCGGTTTTAT-3'. The master mix containing 200 µM of dNTPs, 0.5 U/µl of Taq polymerase in 1X PCR buffer and 25 mM MgCl 2 from Qiagen was used [24]. The PCR was carried out in 25 µl volume which was prepared by mixing the 13 µl of the master mix, 8 µl of the double-distilled water, 0.5 µl each of the forward and reverse primer and 3 µl of the template DNA. Amplification reactions were carried out using the reaction conditions: initial denaturation at 95°C for 15 minutes; followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for bla TEM genes and 56°C for the bla CTX−M gene for 30 seconds, extension at 72°C for 3 minutes and a final extension at 72°C for 10 minutes. The PCR products were analyzed on 1.5% agarose gel electrophoresis with 0.2 µg/ml concentration of ethidium bromide and then visualized by the UV-trans illuminator. The protocol from Sharma et al., and Edelstein et al., were followed with slight modification [23, 24].

Quality control:

E. coli ATCC 35218 and E. coli ATCC 25922, obtained from Central Department of Microbiology, TU were used as control strains for screening of bla TEM and bla CTX−M genes.

Data analysis

All the data were analyzed by the Statistical Package for Social Science (SPSS) software (Version 22.0) and MS Excel (Version 10).

Results

Bacterial growth

Among the 1267 mid-stream urine specimens, 17.20% (218/1267) specimens showed significant growth (Fig. 1).

Bacterial infection among different age and sex groups

Out of the 218 significant positive, 22.02% (48/218) were from the male while 77.98% (170/218) were from female. The prevalence of UTI was the highest in the patients of age group 21–40 and least in age group 1–20. The culture positivity was significantly (p = 0.001) associated between gender and the age group (Table 1).

Bacterial growth in the samples from diabetic, non-diabetic patients, outpatients and inpatients

Significantly higher percent of diabetic patients were found to be having UTI, accounting for 32.29% (31/96) as compared to non-diabetic persons, 15.97% (187/1171). Likewise, the percentage of inpatients with UTI was
23.15 (25/108), which was higher than that of outpatients with UTI, 16.65% (193/1159) (p = 0.087) (Table 2).

**Frequency of uropathogenic bacteria**

A total of 221 bacteria were isolated from 218 culture-positive specimens. Out of which, a single bacterium was isolated from each of 215 (98.62%) specimens whereas 3 (1.38%) specimens showed poly-microbial infection. Gram-negative and Gram-positive bacterial isolates were 89.14% (197/221) and 10.86% (24/221) respectively. *E. coli* was the most predominant Gram-negative bacteria accounting for 67.9% of total isolates followed by *K. pneumoniae* (14.94%). The coagulase-negative staphylococci were the pre-dominant Gram-positive bacteria. Gram-positive bacteria included 3.17% *Enterococcus faecalis*, 7.24% *Staphylococcus saprophyticus* and 0.45% *S. epidermidis*. There was no statistically significant association between the distribution of the bacterial isolates among the diabetic and non-diabetic UTI patients. *E. coli* was found to be the most predominant bacteria among both diabetic 67.2% and non-diabetic 71.86% patients followed by *K. pneumoniae* with 14.81% in non-diabetic and 15.63% in diabetic patients respectively.

**Antibiotic susceptibility pattern of E. coli**

The highest number of the *E. coli* isolates was resistant to amoxicillin followed by the norfloxacin and third generation cephalosporins whereas the highest number of *E. coli* was sensitive to nitrofurantoin. The amikacin was the most effective drug against the MDR isolates. Among 127 *E. coli* from the non-diabetic patients, the highest number of the bacteria (65.35%) was resistant towards amoxicillin. The least number of the bacteria (4.72%) were resistant towards nitrofurantoin. Similarly, out of 23 *E. coli* from diabetic patients, the highest number of bacteria (65.22%) and the least number of bacteria (8.7%) were observed to resist towards amoxicillin and nitrofurantoin respectively. Among 44 MDR *E. coli* from non-diabetic patients, the highest number (86.36%) of bacteria was resistant to amoxycillin clavulanic acid and the least number of the bacteria (13.64%) to amikacin and carbapenems. Similarly, out of 12 MDR *E. coli* from diabetic patients, the highest number (66.67%) of bacteria was resistant to cefepime and the least number of the bacteria (8.33%) to piperacillin/tazobactam and carbapenems. The amikacin was found to be 100% effective. *E. coli* isolates resisting second line drugs; 7 *E. coli* from non-diabetic patients and 3 *E. coli* from diabetic patients showed 100% susceptibility towards colistin, polymyxin B and tigecycline (Table 4).

**ESBL producing MDR E. coli**

Out of 150 *E. coli*, 44% isolates were MDR, all of which were screened as suspected ESBL producers. Out of 66 ESBL suspected isolates, 56 were confirmed as ESBL producer *E. coli*. The combination disk using ceftazidime; CAZ (30 µg) with ceftazidime plus Clavulanic acid [CAZ (30 µg) + CV (10 µg)] confirmed 52 as ESBL producers while Cefotaxime, CTX (30 µg) with cefotaxime with clavulanic acid [CTX (30 µg) + CV (10 µg)] showed 56 ESBL producing *E. coli* (Table 5). A total of 37.33% (56/150) was considered as the ESBL producers including 92.3% (12/13) from diabetic patents and 83.0% (44/53) were from non-diabetic patients. Similarly, 87.5% ESBL producing *E. coli* were isolated from outpatients whereas 12.5% were isolated the inpatients (Table 7).

**Antimicrobial resistance pattern among the non-ESBL and ESBL E. coli**

Among the non-diabetic patients, amoxicillin was the most resisted antibiotic whereas 2.4% non-ESBL producing *E. coli* and 9.1% ESBL producing *E. coli* showed the least resistance towards nitrofurantoin. Similarly,
among the diabetic patients, the non-ESBL *E. coli* were 100% resistance to the gentamicin and nitrofurantoin while the cephalosporins were the most sensitive antibiotics. The ESBL producing *E. coli* showed 100% resistance to amoxicillin and the nitrofurantoin was observed to be the most effective antibiotic. The non-ESBL MDR strains among the non-diabetics showed the highest resistance to the amoxicillin-clavulanic acid and cefperazone/sulbactam whereas the ESBL strains showed the highest resistance to the amoxicillin-clavulanic acid (Table 6).

**Detection of ESBL genes**

Out of the 56 phenotypically confirmed ESBL producing isolates of *E. coli*, $bla_{TEM}$ gene was detected in 53.57% isolates during the amplification process by PCR. Similarly, the $bla_{CTX-M}$ gene was detected in 87.5% isolates. Both $bla_{TEM}$ and $bla_{CTX-M}$ genes were detected in 50%. Two (3.57%) strains harboured only $bla_{TEM}$ gene while 37.5% harboured only $bla_{CTX-M}$. Both the genes were absent in 8.93% isolates. Although, the occurrence of ESBL producers were higher among diabetic patients, screening of ESBL genes showed comparatively less detection than that of non-diabetic patients (Table 7, Fig. 3).

**Discussion**

The prevalence of UTI was found to be 17.20% which was lower than the studies by Kattel et al., and Rijal et al. [25, 26]. MSU specimens being collected from the patients under treatment, infections due to slow-growing bacteria or those which might not be able to grow in routine culture media used in the study might be the possible causes of a low rate of culture positivity [27].

The prevalence of UTI in female was 77.98% which was significantly higher than in male (22.08%). The results were in agreement with the findings by many other investigators [28–30]. The close proximity of the vaginal and anal opening in the female and the absence of the prostatic fluid which has the bactericidal property in the female may make them more susceptible to the UTIs [29, 31]. The patients of age group (21–40) showed the highest significant growth i.e. 44.49% which was similar to the study by Yadav and Prakash 2017, and Khan et al., 2013 [8, 29]. The age group (1–20) showed the least significant growth i.e. 13.30%. Dash et al., 2014 and Thapa et al., 2013 concluded the incidence of UTI is greater in the sexually active female of reproductive age [32, 33].

The overall prevalence of UTI was 32.29% in diabetic patients and 15.97% in non-diabetic patients. A similar prevalence rate i.e. 34.5% in diabetic patients and 26.7% in non-diabetic patients was found in the study conducted in the Dhulikhel Hospital- Kathmandu University Hospital [34]. Similarly, another study found the prevalence of UTI in diabetic and non-diabetic patients to be 32% and 13% respectively [35]. Mubarak et al., 2012 reported higher prevalence of UTI among diabetic patients [36]. It might be due to the diabetic nephropathy and incomplete bladder emptying in hyperglycaemic condition of diabetic patient [37]. Every 10 years of diabetes duration increases 1.9-fold prevalence of bacteriuria [38]. However, the culture positivity among diabetic and non-diabetic patients were not much different accounting for 43.8% and 42.9% respectively in a study conducted in Bangladesh [39]. The prevalence of UTI in female diabetic patients was 46% which was higher compared to prevalence in diabetic male patients 43% [40]. These variations in the results may be due to
the differences in the sample size and clinical conditions among the study population as mentioned in many studies.

Among the heterogeneous bacterial etiological agents of UTI, member of Enterobacteriaceae family remains the predominant pathogens. The members of Enterobacteriaceae, being the normal flora of the human intestine, can easily invade and attach the uro-epithelium causing UTI infections. The total Gram-negative and Gram-positive bacteria involved in the UTI were found to be 89.14% and 10.86% respectively. Among the total Gram-negative bacteria, *E. coli* and *Klebsiella pneumoniae* were the most predominant ones. Similar results were present in other studies as well [40–42]. However, 19.64% of *E. coli* was involved in bacteriuria followed by 2.7% *K. pneumoniae* in the study conducted in International Friendship Children Hospital [43], out of which 33.33% were MDR isolates. Similarly, 7.04% *E. coli* and 2.3% *K. pneumoniae* were isolated from significant bacteriuria in the study conducted by Chander and Shrestha [44].

The bacteria involved in UTI were similar in both diabetic and non-diabetic patients. *E. coli* was the predominant pathogen involved with 71.86% in diabetic patients and 67.2% in non-diabetic patients followed by *Klebsiella pneumoniae* i.e. 15.63% in diabetic patients and 14.80% in non-diabetic patients which was in accordance to a study by Jankhwala et al. [35]. Bonadia et al., 2006 found that *E. coli* constituted 32.5% and 31.4% followed by *Enterococcus* spp. 9.4% and 14.5% respectively among diabetic and non-diabetic patients [45]. They have also reported *Pseudomonas* spp. as the third most frequently isolated bacteria in diabetic and non-diabetic male patients with occurrence of 8.5% and 17.2% respectively. Similar to the results in male, the female patients were found to be infected with 54.1% and 58.2% *E. coli* followed by *Enterococcus* spp. 8.3% and 6.5% and *Pseudomonas* spp. 3.9% and 4.7% respectively with and without diabetes mellitus. Akbar 2001 showed that though *E. coli* was the most common bacteria in both CA-UTI and hospital-acquired UTI in non-diabetics, *Pseudomonas* spp. was the most common bacterium among the hospital-acquired UTI in diabetic patients [46].

Regarding the antimicrobial susceptibility testing of *E. coli* isolates, most of them are resistant to amoxicillin and third-generation cephalosporins which could be due to the production of Beta lactamases [47]. The highest number of bacteria was sensitive to nitrofurantoin. Amikacin was another most effective drug against the MDR isolates. Nearly half of *E. coli* isolates was found to be MDR strains out of which 84.85% of the screened ESBL producers were confirmed to possess either single or both of the bla_{TEM} and bla_{CTX-M} genes. The incidence of ESBL producing *E. coli* among the outpatients (89.1%) was higher than inpatients (63.64%) which were in contrast to the findings [43], which revealed that incidence of ESBL producing *E. coli* among the inpatients was higher than in outpatients. In this study, the ESBL producing *E. was* found comparatively higher in number than in the studies by Parajuli et al. and Seyedjavadi et al. [48, 49]. Pokhrel et al., 2014, and Chander and Shrestha 2013 [44, 50] revealed even less ESBL producing *E. coli*. Diabetic patients showed an increase in number of infections as compared to non-diabetic patients. The number, however, was not statistically significant. The emergence of more and more ESBL producing *E. coli* isolates form UTI patients in the recent years in comparison to the previous study can be related with disease chronicity and long antibiotic therapy among the patients suffering from diabetic, heart diseases and other non-communicable complications. These resistant strains are rapidly spreading in different clinical setting. These ESBL producing strains are highly resistant to the oxy-imino-cephalosporins. However, certain β-lactams may not be totally resisted by these strains which create the problem in the treatment procedures.
The prevalence of $bla_{\text{CTX-M}}$ gene was found to be higher than that of the $bla_{\text{TEM}}$ gene which was in accordance with the study by Pokhrel et al., 2014 and Parajuli et al., 2016 [48, 50]. Many other studies have also shown that the genes responsible for the production of CTX-M-type was more prevalent among the tested strains in comparison to the genes encoding other ESBL like SHV-type or TEM-type $\beta$-lactamases. Ojdana et al., Vidhya and Sudha 2013 and Valenza et al., too found the higher prevalence rate of the $bla_{\text{CTX-M}}$ genes [51–53]. However, in contrast to this study higher prevalence of the $bla_{\text{TEM}}$ genes was detected in $E. \text{coli}$ followed $bla_{\text{CTX-M}}$ by Rezai et al. in the North of Iran [54]. The repression of the genes in the bacteria might cause a variation in their prevalence rate. However, the presence of genes is not associated with diabetic condition of patients.

**Conclusions**

Diabetes mellitus is one of the most rapidly increasing non-infectious diseases among the older age groups (> 40 years). Opportunistic infections including urinary tract infections is another burden for diabetic patients. Moreover, increasing antimicrobial resistance of bacterial uropathogens is causing a huge problem in disease management leading to high rate of mortality and morbidity of diabetic patients. Identifying multidrug resistant causative agents and proper diagnosis in UTI among diabetic patients are crucial for disease management. However, few routinely used techniques such as disk diffusion are not sufficient to confirm such MDR strains including ESBL. So, to avoid treatment failure due to misdiagnosis, new approaches of diagnosis including molecular tools have to be adopted.

**Limitations**

Due to limited molecular resources and time constraint, all the isolates could not be confirmed for production of ESBL enzymes by CD test and those ESBL producers were not screened for all possible ESBL genes due to lack of primers and limited PCR master mix.

**Abbreviations**

AST: Antibiotic Susceptibility testing; CA-UTI: Community Associated Urinary Tract Infection; CD: Combined Disc; CFU: Colony Forming Unit; CLED: Cysteine Lactose Electrolyte Deficient; CLSI: Clinical Laboratory Standard Institute; ESBL: Extended Spectrum Beta Lactamases; KMH: Kathmandu Model Hospital; MDR: Multi-Drug Resistance; MSU: Mid-Stream Urine; NUTI: Nosocomial Urinary Tract Infection; PCR: Polymerase Chain Reaction; UTI: Urinary Tract Infection

**Declarations**

**Ethics approval and consent to participate**

The study was reviewed and approved by Institutional Review Committee (IRC), of the Public Health Concern Trust, Nepal (phect-NEPAL), Kathmandu Model Hospital, Kathmandu, Nepal on June 14, 2018. A copy of information letter and consent form was given to participants to obtain written consent before enrollment in the research and taking sample. In case of children under 16 years, a written consent was obtained from a parents
or guardian attending hospital along with the participant. For an illiterate participant, information was provided by reading the consent form in presence of witness using local language.

**Consent for publish**

All authors are read and reviewed the final manuscript and give consent for publication.

**Availability of data materials**

Raw data in the excel file was provided.

**Competing interests**

The authors declared no competing interests.

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**Authors’ contributions**

TN with BS developed the concept of this study. The laboratory work was done by TN and AG at KMH under the supervision of BS, BM and RDJ. The molecular work was done by TN under direct supervision of UTS and MKU at laboratory of GoldenGate International College. The disease diabetes was confirmed by Dr. RDJ and data were analyzed by TN and MKU. The first draft of manuscript was prepared by TN, AG, MKU and BL. The final draft and English language correction were done by UTS. All the authors have read and reviewed the manuscript thoroughly.

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**Tables**

**Table 1**: Bacterial growth profile based on gender and age groups
### Table 2: Bacterial growth profile based on medical status (diabetic and out/in patients)

| Medical status       | Growth          | Total number | p value |
|----------------------|-----------------|--------------|---------|
|                      | Not-significant (%) | Significant (%) |         |
| Non-diabetic         | 984 (84.03)     | 187 (15.97)  | 1171    | < 0.001 |
| Diabetic             | 65 (67.71)      | 31 (32.29)   | 96      |
| Total                | 1049            | 218          | 1267    |

| Medical status       | Growth          | Total number | p value |
|----------------------|-----------------|--------------|---------|
| Outpatients          | 966 (83.35)     | 193 (16.65)  | 1159    | 0.087   |
| Inpatients           | 83 (76.85)      | 25 (23.15)   | 108     |
| Total                | 1049            | 218          | 1267    |

### Table 3: Distribution of bacterial isolates from specimens of diabetic and non-diabetic

| Age groups (yrs) | Gender | Total Bacterial Growth (%) | p-value |
|------------------|--------|-----------------------------|---------|
|                  | Male (%) | Female (%) |                       |         |
| 1-20             | 10 (34.48) | 19 (65.52) | 29 (13.30) |         |
| 21-40            | 10 (10.30) | 87 (89.70) | 97 (44.49) | 0.001   |
| 41-60            | 9 (23.07)  | 30 (76.93) | 39 (17.9)  |         |
| >60              | 19 (35.85) | 34 (64.15) | 53 (24.31) |         |
| Total            | 48 (22.02) | 170 (77.98) | 218      |         |
| Bacterial isolates        | Non-diabetic patients | Diabetic patients | Total number of bacterial isolates |
|---------------------------|-----------------------|-------------------|-----------------------------------|
|                           | Number (%)            | Number (%)        |                                   |
| **Acinetobacter spp.**    | 0                     | 1 (3.13)          | 1 (0.45)                          |
| **Enterobacter aerogens** | 2 (1.06)              | 0                 | 2 (0.9)                           |
| **Enterobacter cloacae**  | 6 (3.17)              | 0                 | 6 (2.71)                          |
| **Enterococcus faecalis** | 6 (3.17)              | 1 (3.13)          | 7 (3.17)                          |
| **Escherichia coli**      | 127 (67.2)            | 23 (71.86)        | 150 (67.87)                       |
| **Klebsiella oxytoca**    | 1 (0.52)              | 0                 | 1 (0.45)                          |
| **Klebsiella pneumoniae** | 28 (14.81)            | 5 (15.63)         | 33 (14.93)                        |
| **Proteus vulgaris**      | 1 (0.52)              | 0                 | 1 (0.45)                          |
| **Proteus mirabilis**     | 1 (0.52)              | 0                 | 1 (0.45)                          |
| **Pseudomonas aeruginosa**| 2 (1.06)              | 0                 | 2 (0.9)                           |
| **S. epidermidis**        | 1 (0.52)              | 0                 | 1 (0.45)                          |
| **S. saprophyticus**      | 14 (7.40)             | 2 (6.25)          | 16 (7.24)                         |
| **Total**                 | 189                   | 32                | 221                               |

Table 4: Antibiotic susceptibility testing of E. coli isolates from urine samples
| Antibiotic category                          | Antibiotics used               | Non. of Resistant *E. coli* isolates (%) from |
|---------------------------------------------|--------------------------------|---------------------------------------------|
|                                             |                                | Non-diabetic patients | Diabetic patients |
|                                            |                                | (n=127)                      | (n=23)            |
| **First Line Drugs**                        |                                |                             |                  |
| Penicillin + β-lactamase inhibitors         | Amoxicillin                    | 83 (65.35)                  | 15 (65.22)       |
| Extended spectrum cephalosporins; 3rd and 4th generation cephalosporins | Cefixime                       | 53 (41.74)                  | 13 (56.51)       |
|                                             | Cefotaxime                     | 53 (41.74)                  | 13 (56.51)       |
|                                             | Ceftriaxone                    | 53 (41.74)                  | 13 (56.51)       |
|                                             | Ceftazidime                    | 53 (41.74)                  | 13 (56.51)       |
| Folate pathway inhibitors                   | Cotrimoxazole                  | 59 (46.46)                  | 12 (52.17)       |
| Aminoglycosides                             | Gentamicin                     | 14 (11.11)                  | 3 (13.04)        |
| Fluoroquinolones                            | Levofoxacin                    | 48 (37.8)                   | 11 (47.83)       |
|                                             | Ofloxacin                      | 51 (40.16)                  | 12 (52.17)       |
|                                             | Norfloxacin                    | 55 (43.3)                   | 13 (56.51)       |
| Nucleic acid synthesis inhibitors           | Nitrofurantoin                 | 6 (4.72)                    | 2 (8.7)          |
| **Second Line Drugs**                       |                                |                             |                  |
| Aminoglycosides                             | Amikacin                       | 6 (13.64)                   | 0                |
| Penicillin + β-lactamase inhibitors         | Amoxicillin-clavulanic acid    | 38 (86.36)                  | 7 (58.33)        |
| Extended spectrum cephalosporins; 3rd and 4th generation cephalosporins | Cefperazone/Sulbactam          | 28 (63.64)                  | 5 (41.67)        |
|                                             | Cefepime                       | 31 (70.45)                  | 8 (66.67)        |
| Tetracyclines                               | Doxycycline                    | 13 (29.55)                  | 4 (33.33)        |
| Carbapenems                                 | Imipenem                       | 6 (13.64)                   | 1 (6.33)         |
|                                             | Meropenem                      | 6 (13.64)                   | 1 (6.33)         |
| β-lactamase inhibitors                      | Piperacillin/Tazobactam        | 8 (18.18)                   | 1 (6.33)         |
| **Third/last Line Drugs**                   |                                |                             |                  |
| Polymyxins                                  | Colistin                       | 0                           | 0                |
|                                             | Polymyxin B                    | 0                           | 0                |
| Glycylcyclines                              | Tigecycline                    | 0                           | 0                |

Table 5: Confirmatory of ESBL by combination disks
| Combination disk assay | No. of suspected ESBL | No. of confirmed cases | Total confirmed cases |
|------------------------|-----------------------|------------------------|-----------------------|
| CAZ (30 µg) and CAZ (30 µg) + CV (10 µg) | 66 | 52 | 56 |
| CTX (30 µg) and CTX (30 µg) + CV (10 µg) | 66 | 56 |  |

Table 6: Antibiotic resistivity pattern of non-ESBL and ESBL E. coli

| Antibiotics used | Resistance pattern in non-diabetic (%) | Resistance pattern in Diabetic (%) |
|------------------|----------------------------------------|-----------------------------------|
|                  | non ESBL producers | ESBL producers | non ESBL producers | ESBL producers |
| First line drugs (n=150) | (n=83) | (n=44) | (n=11) | (n=12) |
| Amoxicillin | 39 (46.99) | 44 (100) | 3 (27.27) | 12 (100) |
| Cefixime | 9 (10.84) | 44 (100) | 1 (9.09) | 12 (100) |
| Cotrimoxazole | 34 (40.97) | 25 (56.82) | 4 (36.36) | 8 (66.67) |
| Gentamicin | 9 (10.84) | 5 (11.36) | 0 | 3 (25) |
| Levofloxacin | 19 (22.89) | 29 (65.9) | 4 (36.36) | 7 (58.33) |
| Ofloxacin | 20 (24.1) | 31 (70.45) | 4 (36.36) | 8 (66.67) |
| Norfloxacin | 23 (27.71) | 32 (72.73) | 5 (45.45) | 8 (66.67) |
| Nitrofurantoin | 2 (2.4) | 4 (9.1) | 0 | 2 (16.67) |

| Second line drugs (n=56) | (n=11) | (n=33) | (n=2) | (n=10) |
|--------------------------|-------|-------|------|-------|
| Amikacin | 5 (45.45) | 1 (3.03) | 0 | 0 |
| Amoxicillin-clavulanic acid | 8 (72.78) | 30 (90.91) | 1 (50) | 6 (60) |
| Cefperazone/Sulbactam | 8 (72.78) | 20 (60.61) | 1 (50) | 4 (40) |
| Cefepime | 7 (63.67) | 24 (72.73) | 1 (50) | 7 (70) |
| Doxycycline | 6 (54.55) | 7 (21.21) | 1 (50) | 3 (30) |
| Imipenem | 6 (54.55) | 0 | 1 (50) | 0 |
| Meropenem | 6 (54.55) | 0 | 1 (50) | 0 |
| Piperacillin/Tazobactam | 6 (54.55) | 2 (6.06) | 1 (50) | 0 |

Table 7: Status of ESBL and representative ESBL genes among uropathogenic E. coli from diabetic and non-diabetic patients
| Methods used       | ESBL parameters | Number (%) in | p value |
|-------------------|-----------------|---------------|---------|
|                   |                 | Diabetic      | Non-diabetic |
| Phenotypic, n=66  | ESBL producer   | 12 (92.3)     | 44 (83.0) | > 0.05 |
| (CD test)         | ESBL non producer | 1 (7.7)       | 9 (17.0)  |         |
| Total             |                 | 13            | 53       |
| Genotypic, n=56   | blaTEM-1 gene detected | 5 (41.7) | 25 (56.8) | > 0.05 |
| (blaTEM-1 screening) | blaTEM-1 gene not detected | 7 (58.3) | 19 (43.2) |         |
| Total             |                 | 12            | 44       |
| Genotypic, n=56   | ctxM gene detected | 10 (83.3)     | 39 (88.6) | > 0.05 |
| (blactxM screening) | ctxM gene not detected | 2 (16.7) | 5 (11.4)  |         |
| Total             |                 | 12            | 44       |

**Figures**

**Figure 1**

Percentage of MSU specimens with significant bacterial growth (Significant growth 17.20%)
Figure 2

Phenotypic confirmation of ESBL by CD method (A: Ceftazidime; B: Ceftazidime + clavulanic acid / C: Cefotaxime; D: Cefotaxime + clavulanic acid (ESBL positive result)
Figure 3

Electrophoresis of PCR products of blaTEM gene (459 bp) (a) and blaCTX-M gene (521 bp) (b) of E. coli on an agarose gel. Lanes 1 and 9 is DNA ladder (100 bp). Lanes 2, 3, 4, 5, 6, 7 and 8 are bands of positive PCR products.

Supplementary Files

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