Extended-Spectrum *β*-Lactamase (ESBL) Genotypes among Multidrug-Resistant Uropathogenic *Escherichia coli* from Clinical Isolates

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Abstract

Urinary tract infections (UTIs) are a major public health concern with Escherichia coli being the predominant uropathogen. The increasing emergence of multidrug-resistant (MDR) strains, particularly those producing extended-spectrum β-lactamases (ESBLs), complicates treatment strategies. This study aimed to detect and characterize ESBL genotypes among MDR uropathogenic E. coli isolated from clinical urine samples. Phenotypic confirmation of ESBL production was performed using standard disc diffusion methods. Molecular analysis was conducted to identify the presence of key ESBL genes including bla_{TEM}, bla_{SHV} *and* bla_{CTX-M}.

A high prevalence of ESBL producers was observed with bla_{CTX-M} *being the most common genotype detected. The isolates exhibited resistance to multiple antibiotic classes, while showing susceptibility to carbapenems. The findings highlight the urgent need for molecular surveillance and antibiotic stewardship to curb the spread of ESBL-producing uropathogens in clinical settings.*

Keywords: Extended-Spectrum β-Lactamase (ESBL), Uropathogenic *E. coli* (UPEC), Multidrug Resistance (MDR), Urinary Tract Infection (UTI), Polymerase Chain Reaction (PCR).

Introduction

Urinary tract infection (UTI) represents a wide variety of clinical entities involving microbial invasion of any tissue of the urinary system from the renal cortex to the urethral meatus. Every year, millions of people from all age groups are affected by UTI with a high risk of morbidity, mortality and significant healthcare costs. Etiological agents involved in urinary tract infection are much diverse and the most commonly encountered microorganisms are Gram-negative Enterobacteriaceae including *Escherichia coli*. The infections associated with these organisms are empirically treated with conventional antibiotics based on frequency of pathogens, local trends of antibiotic susceptibilities and the illness severity.

However, increasing rates of antibiotic resistance and high recurrence rates have greatly reduced the therapeutic options for UTI in recent years. Of particular concern, members of the family Enterobacteriaceae causing UTIs including *E. coli* and *K. pneumoniae*, harboring acquired plasmids encoding extended-spectrum *β*-lactamases (ESBLs) are rising globally. These plasmids rapidly spread resistance to third-generation cephalosporins as well as other antibiotics. First detected in 1983, more than 300 variants of ESBLs have been identified in various members of the family Enterobacteriaceae and other nonenteric organisms.

Among various genotypes, *CTX-M*, *SHV* and *TEM* have been described predominantly among the clinical strains of Enterobacteriaceae conferring broader antimicrobial resistance including *β*-lactams, fluoroquinolones and aminoglycosides[1]. Increased rate of multidrug-resistant uropathogenic *Escherichia coli* among urinary tract infections has been reported previously from Coimbatore, Tamilnadu zone and much of these studies were limited to phenotypic description of resistant bacteria.

However, reports describing molecular types of ESBL-producing *Escherichia coli* causing urinary tract infections among the patients and their epidemiology are largely unknown. In this perspective, we aimed to determine the incidence, bacterial etiology of urinary tract infections and genotypes of ESBL-producing multidrug-resistant *Escherichia coli* in a defined region, at the Coimbatore zone, Tamilnadu[2].

Material and Methods

Sample Collection and Isolation: Midstream urine samples were collected from tertiary hospitals for a period of one year from patients with suspected urinary tract infections and transported to laboratory for processing. A total of 1020 samples were collected.

Inclusion and Exclusion Criteria: Specimens representing the urinary tract infections among outpatients and inpatients attending tertiary hospitals at Coimbatore zonal level were included in the study. Midstream samples of urine, aseptically collected before initiation of antimicrobial therapy, were included in the study. However, repeated samples from the same patient and those not fulfilling the criteria are excluded[4].

Laboratory Procedure and Identification of Bacterial Uropathogens: Midstream urine specimens were processed by standard microbiological methods without delay in the bacteriology laboratory at Coimbatore. They were processed semi quantitatively by inoculating 0.001 µl of the specimen (using a calibrated wire loop) onto the Mac Conkey agar, Cystine Lactose Electrolyte Deficient (CLED) medium, Blood agar, Mueller Hinton agar and the inoculated plates were incubated for 24 hours at 37°C in aerobic environment.

Growth of single organism with a count of $\geq 10^5$ colony-forming units (CFU)/mL was considered to represent the infection and the organisms were identified using appropriate routine identification methods including colony morphology, Gram stain and an in-house set of biochemical tests. Among all isolates, the most predominant uropathogen, *Escherichia coli,* was further selected for the determination of antimicrobial susceptibility as well as detection of the multidrug-resistant (MDR) and extended-spectrum beta-lactamase- (ESBL-) producing strains[5].

Antimicrobial Susceptibility Testing: The antimicrobial susceptibility of *Escherichia coli* was determined by the disk diffusion method of modified Kirby–Bauer on the Mueller–Hinton agar (HiMedia Laboratories, India) following standard procedures recommended by the Clinical and Laboratory Standard Institute (CLSI). Antibiotics included in the testing panel were amoxicillin (AMX 10 μg), gentamycin (GEN 10 μg), cotrimoxazole (COT 25 μg), nitrofurantoin (NIT 300 μg), levofloxacin (LE 5 μg), amoxyclav (AMC 10μg), ceftazidime (CAZ 10 μg), piperacillin (PI 100 μg), piperacillin-tazobactam (PIT 100/10 μg), norfloxacin (NFX 10 μg), tobramycin (TOB 10 μg), imipenem (IMP 10 μg) and amikacin (AK 30 μg), ciprofloxacin (CPFX 30 μg) and ofloxacin (OF 5 μg). Interpretations of antibiotic susceptibility results were made according to the zone size interpretative standards of the CLSI. *Escherichia coli* MTCC 433 was used as a control strain for antibiotic susceptibility[6].

Multidrug-Resistant (MDR) *Escherichia coli* and Potential ESBL Producers: In this study, *Escherichia coli* isolates resistant to at least one agent of three different classes of commonly used antimicrobial agents were regarded as multidrug resistant (MDR). If the zone of inhibition (ZOI) was ≤25 mm for ceftriaxone, ≤22 mm for ceftazidime, and/or ≤27 mm for cefotaxime, the isolate was considered a potential ESBL producer as recommended by the CLSI and further tested by confirmatory methods[6].

Combination Disk Test for Phenotypic Detection of ESBL: Presumptive ESBL-producing isolates by initial screening were emulsified with 4–6 ml of peptone water to adjust the inoculum density equal to that of 0.5 McFarland turbidity standards. Combination disk test (CDT), as recommended by the CLSI, was performed on all *Escherichia coli* isolates presumed to be ESBL producers. In this test, the cefoperozone (30 μg) disk alone and in combination with sulbactum (cefoperozone + sulbactum, 30/10 μg) disk and piperacilum +tazobactam were applied onto a plate of Mueller–Hinton agar (MHA) which was inoculated with the test strain and then incubated in ambient air for 16–18 hours at 37°C. The isolate showing increase of ≥5 mm in the zone of inhibition of the combination discs in comparison to that of the cefotaxime disk alone was considered an ESBL producer[7].

Molecular Typing of ESBL Genes: All the phenotypic ESBL *Escherichia coli* isolates were subjected to molecular analysis for the confirmation of ESBL production. Molecular detection of *Escherichia coli* harboring ESBL genes (*Simplex NDM-1, CTXM-15 and OXA-48*) was carried out by conventional polymerase chain reaction (PCR) at Synbio Scientific Solution, Erode, Tamil Nadu.

Chromosomal DNA Extraction and Amplification: For DNA extraction, a single colony of each ESBL-producing *Escherichia coli* was inoculated into Luria-Bertani broth and incubated till the logarithmic state. Extraction and purification of DNA of bacteria were carried out using a commercial kit following manufacturer's instructions. Purified DNA from bacterial isolates was used as a template to detect ESBL genotypes: Simplex NDM-1, CTXM-15 and OXA-48 β-lactamase genes. Primers for the amplification of ESBL genotypes (Simplex NDM-1, CTXM-15 and OXA-48) were designed and purchased from Eurofins, Bangalore. The sequences are as listed in table 1.

Polymerase chain reaction- (PCR-) based amplification of ESBL genes was carried out as per the method previously described. The targeted gene sequence is NDM-1, CTAM-15 and OXA-48. Amplification reactions were carried out in a DNA thermal cycler (CG) with the following thermal and cycling conditions: initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 45 seconds of 35 cycles, annealing at 50°C for 30 secs of 35 cycles (for NDM-1 and OXA-15) and 50°C for 30 secs of 35 cycles (for CTAM-15), extension at 72°C for 3 minutes of 35 cycles and final extension at 72°C for 2 minutes[9].

Table 1
Primer sequences for NDM-1, CTXM-15 and OXA 48

***β*-lactamase targeted Gene**	**PRIMERS (5’-3’)**	**Amplicon Size (bp)**
NDM-1	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621 bp
CTXM-15	F: AGAATAAGGAATCCCATGGTT R: ACCGTCGGTGACGATTTTAG	913 bp
OXA-48	F: TATATTGCATTAAGCAAGGG R: CACACAAATACGCGCTAACC	800 bp

Statistical Analysis: SPSS and GraphPad Prism were used for data analysis, Chi-square test (χ^2) has been used to identify the association between ESBL genes and antibiotic resistance. Logistic Regression was used to predict MDR status based on ESBL genotypes.

Results and Discussion

Out of the total 1020, urine samples, 834 urinary tract specimens from patients suspected of having UTI were selected for the study. Females-530 (64%) was the significant subgroup of patients affected with UTI and most of them belonged to the age group 21–30 years. Incidences of UTI varied with different age group, gender and type of patients are presented in table 2 and percentage details are given in figures 1, 2 and 3. The female urethra is anatomically shorter and located closer to the anus compared to males, making it easier for bacteria to enter the urinary tract, increasing the risk of infection. Improper perineal hygiene practices and hormonal changes during menstruation, pregnancy, or menopause can further predispose women to UTIs.

Bacterial Uropathogens: Eight hundred and thirty-four bacterial uropathogens were recovered from patients with suspected UTI. Gram-negative bacteria (72.4%) were more common and *Escherichia coli* (376)- 45.07%) remained the predominant pathogen associated with UTI in all age groups. Other pathogens isolated from UTI cases were *Klebsiella pneumoniae* (*38*-6.2%), *Proteus mirabilis* (29-4.8%), *Pseudomonas aeruginosa* (42-,7.0%), *Enterobacter SP (30-5.0%)*, *Acinetobacter* sp (54-8.9%) and *Klebsiella oxytoca* (35-5.7%).

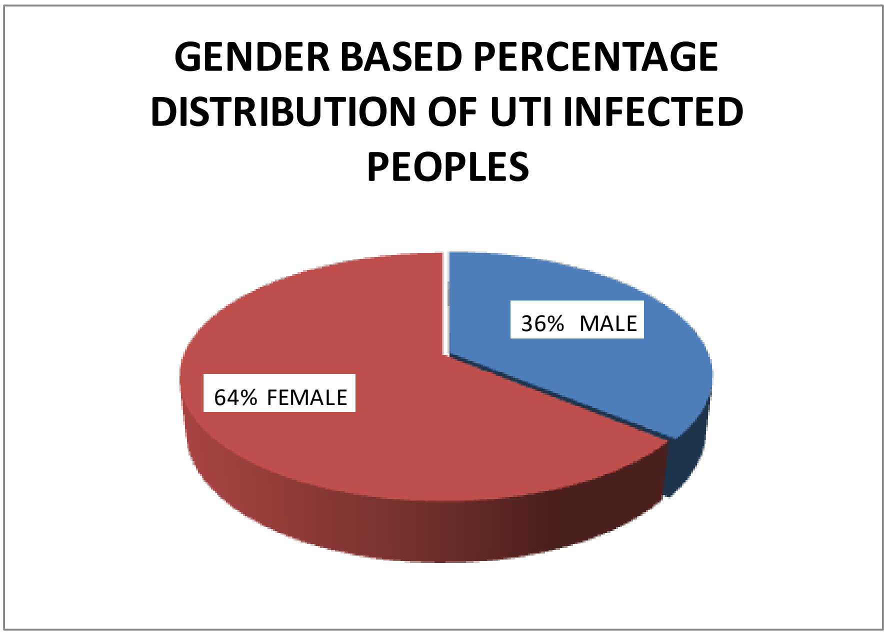

Chart 1: Gender based distribution of Urinary tract infection

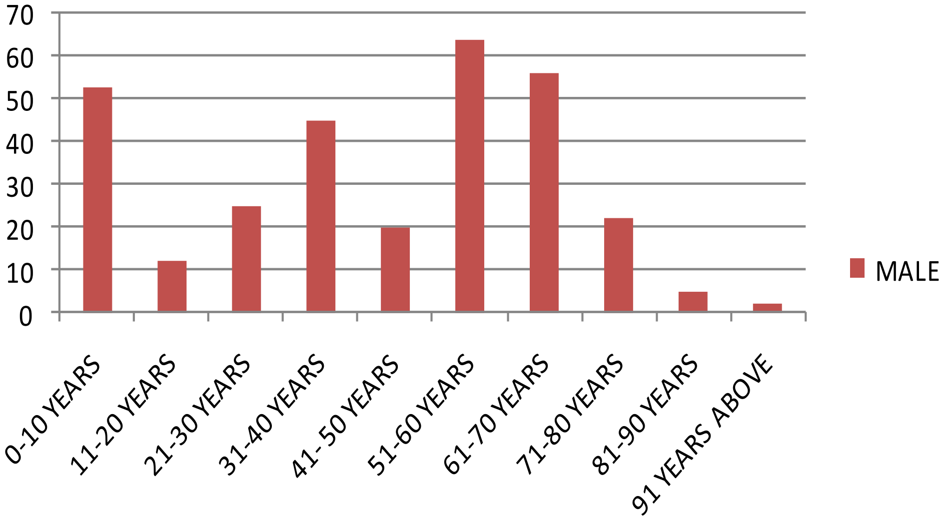

Chart 2: Age-wise distribution of urinary tract infections in male patients

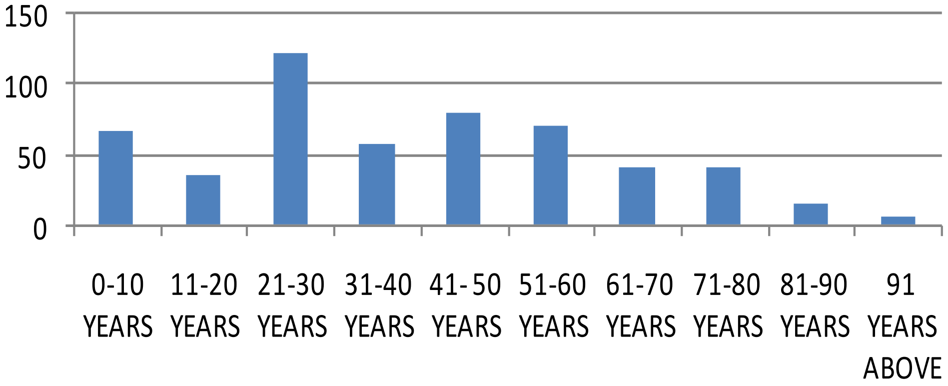

Chart 3: Age-wise distribution of urinary tract infections in male patients

Table 2
No. of Patients with urinary tract infection with different Age groups

Age Group (Years)	Male	Female
0-10 YEARS	53	67
11-20 YEARS	12	35
21-30 YEARS	25	123
31-40 YEARS	45	57
41- 50 YEARS	20	79
51-60 YEARS	64	70
61-70 YEARS	56	40
71-80 YEARS	22	40
81-90 YEARS	5	14
91 YEARS ABOVE	2	5
TOTAL	**304**	**530**

Table 3
Antibiotic susceptibilities of Uropathogenic *E. coli* isolates
TOTAL=834 ESBL producers (*n* = 154)

Antibiotics	Disc concentration	Resistant (%)
Piperacillin	100µg	26%
Amoxicillin	25µg	40%
Levofloxacin	5µg	37%
Amoxyclav	10µg	54.50%
Piperacillin/Tazobactem	100/10µg	37%
Norfloxacin	10µg	50%
Tobramycin	10µg	68%
Amikacin	30µg	44%
Ciprofloxacin	30µg	78%
Ceftriazone	30µg	44%
Ceftazidime	30 µg	45%
Gentamycin	10µg	58%
Cotrimoxazole	25 µg	68%
Nitrofurantoin	300µg	75%
Ofloxacin	5µg	65%
Imipenem	10µg	58%

Table 4
Distribution of ESBL genotypes among uropathogenic *Escherichia coli* (*n* = 154)

ESBL Genotypes	Frequency	%
NDM-1	65	42.2%
CTXM-15	68	44.2%
OXA-48	21	13.6%

Antibiogram of *Escherichia coli*: Diverse pattern of antimicrobial susceptibilities was observed among the *E. coli* isolates. Nitrofurantoin (92.2%) was the most effective first-line therapeutic regimens for uropathogenic *E coli* isolates. Almost half of the isolates were resistant to gentamycin and nettlin. Moreover, 64.9% of *Escherichia coli* were found multidrug resistant. In combination, about 21% of the isolates were resistant to beta-lactam, Cephotaxime and norfloxacin12% were resistant to beta-lactam. Tobramycin and imipenem 9% were resistant to beta-lactam, cefazidime. These results are similar to the previous studies[13].

ESBL *Escherichia coli*: About 18.4% (154/376) of *Escherichia coli* isolates were confirmed as ESBL producers. ESBL-producing *Escherichia coli* isolates were significantly more resistant to antibiotics as compared to non-producers of ESBL. These results are also are consistent with the previous reports[14]. The antibiogram is presented in table 3.

Genotype Distribution among ESBL *E coli*: One Hundred fifty four isolates of *E. coli* were confirmed by plate assay. Molecular identification was used for selected isolates for NDM, of ESBL genes Among the ESBL genotypes, NDM-1 (42.2%) was more common, followed by CTXM-15 (44.1%) and OXA-48 (13.6%). More than half (86.3%) of the ESBL-producing *E.coli* isolates were possessing NDM-1 *and* CTXM-15 genes. The frequency of distribution of selected ESBL genotypes are presented in table 4. Figure 4 shows the PCR amplification of NDM-1 genes in the selected isolates.

Urinary tract infection (UTI) continues to be the common clinical entity among the patients of the inpatient and outpatient departments. However, the reported incidences and their epidemiology in Coimbatore are not consistent enough to reveal the actual scenario regarding the etiological spectrum and antimicrobial susceptibilities. In this laboratory-based study, we examined the organisms causing urinary tract infections and their antibiograms along with the production of extended-spectrum beta-lactamase enzymes by phenotypic and genotypic approaches. Overall incidence of UTI in our study was quite low, when compared to the previous reports from similar studies in Coimbatore, Tamilnadu.

The lower incidence in this study might be due to the sample numbers and the region of sampling. In addition, more outpatients were found with UTI than inpatients. Concomitantly, significantly more females (64%) were found with UTI. The higher occurrence of UTI in females of the reproductive age group in this study has been well supported by other studies[16,17]. Furthermore, elderly males were found more affected by UTI in this study, as they might have bladder outflow obstruction and other chronic comorbid conditions[18].

We observed that Gram-negative bacteria were the most predominant (72.4%) organisms associated with the cases of UTI and *Escherichia coli* (62.4%) was the major pathogen. Members of Enterobacteriaceae have been well described as the primary agents for UTI than other organisms in several studies.

Higher incidence of *E. coli* seen in our study also resembled the results of previous studies from Coimbatore[19]. Although very low number of Gram-positive bacteria and yeasts were isolated in this study, they are also responsible for UTI in various studies[19]. Antimicrobial resistance among uropathogenic bacterial species is one of the major findings of this study. *Escherichia coli*, the major uropathogen, was highly resistant to commonly used therapeutic drugs (beta-lactams, gentamycin and ceftazidime). Out of 834 *E. coli* isolates, 23% were resistant to ciprofloxacin, 17.1% resistant to ofloxacin, 16.3% to norfloxacin, 15.7% to levofloxacin and -14.6 % to amoxyclav.

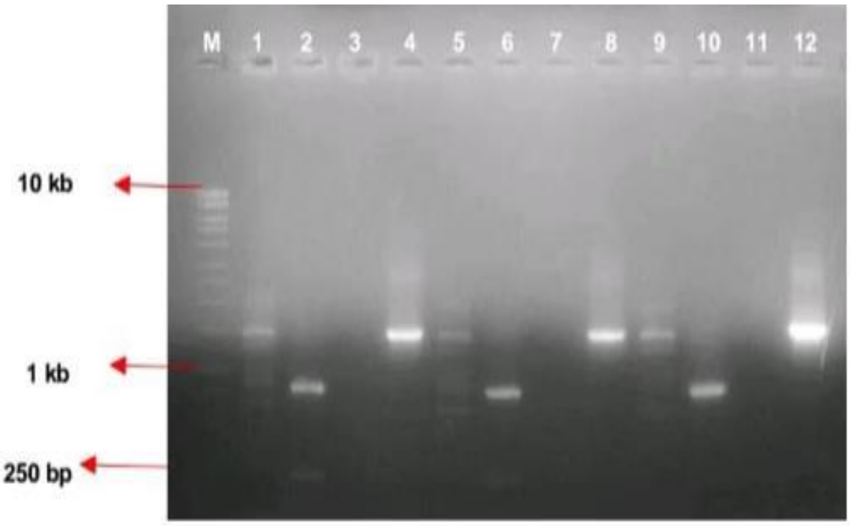

Figure 1: Analysis of PCR products on 1% agarose gel electrophoresis

As the efficacy is less, hence should be assessed before using as an empirical therapy. In addition to this, susceptibility findings of isolates against cephalosporins and quinolones show a substantial increase in their resistance, as reported by others[14].

However, nitrofurantoin (28%) and gentamycin (30.2%) were effective against uropathogenic *E coli* strains. As stated by others too, these can be considered as the first-line therapeutic regimen for UTI. Carbapenems including imipenem would be useful as secondary therapy for multidrug-resistant and complicated UTIs. However, in the recent years, the emergence of urinary isolates with carbapenem resistance is further complicating the treatment of UTIs[19].

In this study, we found a high proportion of *E. coli* (62.2%) isolates to be multidrug resistant (MDR). Our findings on MDR bacteria in UTI cases are compatible with the reports from different parts of the world including Coimbatore, Tamilnadu. Furthermore, the most common MDR pattern among *E. coli* isolates was resistance towards beta-lactams, cefuroxime and norfloxacin (21%), which may be due to the production of hydrolytic enzymes (*β*-lactamases) by the bacteria. Our finding suggests that the antibiotic treatment options for UTIs caused by *E. coli* have been severely challenged due to the resistance to commonly used antibiotics, leading to the situation relying only on certain reserve antibiotics[20].

Over the time, incidence and epidemiology of MDR and ESBL-producing uropathogenic *E. coli* have been continuously changing and higher rates are reported from developing countries. Alongside, we observed diverse genotypes of ESBL among *E coli* isolates. In this study, *NDM-1* (42.2%) was the most predominant genotype of ESBL among *E coli* isolates. However, the *NDM-1*gene has been described as the most common genotype of ESBL among enterobacteriaceae in literature. We found the dominance of the *NDM-1* gene among ESBL-producing enterobacteriaceae from various clinical specimens.

Moreover, multiple occurrences of genes in the same organism were also noted, where *NDM-1* + *CTAM-15* (86.4%) was common. These genes are usually present on the large plasmids accompanied with the genetic determinants conferring resistance towards various antimicrobials. In this study, ESBL-producing isolates were more resistant to ceftazidime, amoyclave and norfloxacin. However, nitrofurantoin and sulbactum proved to be the optimal first-line drug in the cases of UTI caused by ESBL *E coli* in our study.

Infections caused by ESBL-producing organisms are a global problem. Mobile genetic elements contained in the bacterial species are easily transferable to other organisms in the vicinity. Timely detection of the resistant strains along with their antimicrobial susceptibilities is very important for the effective management of UTI in the endemic regions. However, limited facilities of detection and poor understanding of such antimicrobial resistance in bacteria and over the counter medicines are influencing factors responsible for global dissemination of such pathogens[21].

Conclusion

High burden of antimicrobial resistance and increased prevalence of ESBL-producing *Escherichia coli* associated with UTI are the major findings of this study. Diverse genotypes of ESBL *E. coli* along with resistance towards common antibiotics were observed. Nitrofurantoin and sulbactum were found as the most useful first-line drugs to be used in the cases of UTI in our setting. In this perspective, regular national-wide epidemiological surveillance of bacterial pathogens causing UTIs and their antimicrobial resistance would be useful in developing the treatment guidelines in our country[23-25].

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References

1. Acharya A., Gautam R. and Subedee L., Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal, *Nepal Medical College Journal: NMCJ*, **13(1)**, 30–33 **(2011)**

2. Baral P., Neupane S., Marasini B.P., Ghimire K.R., Lekhak B. and Shrestha B., High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal, *BMC Research Notes*, **5P**, 5-38 **(2012)**

3. Chen Y.H., Ko W.C. and Hsueh P.R., Emerging resistance problems and future perspectives in pharmacotherapy for complicated urinary tract infections, *Expert Opinion on Pharmacotherapy*, **14(5)**, 587–596 **(2013)**

4. Chin B.S. et al, Risk factors of all-cause in-hospital mortality among Korean elderly bacteremic urinary tract infection (UTI) patients, *Archives of Gerontology and Geriatrics*, **52(1)**, e50–e55 **(2011)**

5. Flores-Mireles A.L., Walker J.N., Caparon M. and Hultgren S.J., Urinary tract infections: epidemiology, mechanisms of infection and treatment options, *Nature Reviews Microbiology*, **13(5)**, 269–284 **(2015)**

6. Foxman B., Urinary tract infection syndromes, *Infectious Disease Clinics of North America*, **28(1)**, 13 **(2014)**

7. Hicklinsg D.R. and Nitti V.W., Management of recurrent urinary tract infections in healthy adult women, *Reviews in Urology*, **15(2)**, 41–48 **(2013)**

8. Isenberg H.D., Clinical Microbiology Procedure Handbook, 2nd, Washington, DC, USA, American Society for Microbiology **(2004)**

9. Kattel H.P., Acharya J., Mishra S.K., Rijal B.P. and Pokhrel B.M., Bacteriology of urinary tract infection among patients attending Tribhuvan University teaching hospital Kathmandu Nepal, *Journal of Nepal Association for Medical Laboratory Sciences*, **9(1)**, 25–29 **(2008)**

10. Keerthi K., Lohith E.A., Vasantha T., Siva Kumar K. and Jyothi N.V.V., Determination of heavy metals concentration in commercial green tea samples by using inductively coupled plasma optical emission spectrometer (ICP-OES), *Res. J. Chem. Environ*., **28(4)**, 1-6 **(2024)**

11. Kumari N., Ghimire G., Magar J.K., Mohapatra T.M. and Rai A., Antibiogram pattern of isolates from UTI cases in Eastern part of Nepal, *Nepal Medical College Journal: NMCJ*, **7(2)**, 116–118 **(2005)**

12. Lalueza A. et al, Risk factors for bacteremia in urinary tract infections attended in the emergency department, *Internal and Emergency Medicine*, **13(1)**, 41–50 **(2018)**

13. Livermore D.M., Current epidemiology and growing resistance of gram-negative pathogens, *The Korean Journal of Internal Medicine*, **27(2)**, 128–142 **(2012)**

14. Magiorakos A.P. et al, Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, *Clinical Microbiology and Infection*, **18(3)**, 268–281 **(2012)**

15. Maharjan A. et al, Ugly bugs in healthy guts! Carriage of multidrug-resistant and ESBL-producing commensal Enterobacteriaceae in the intestine of healthy Nepalese adults, *Infection and Drug Resistance*, **11**, 547–554 **(2018)**

16. Najar M.S., Saldanha C.L. and Banday K.A., Approach to urinary tract infections, *Indian Journal of Nephrology*, **19(4)**, 129–139 **(2009)**

17. Nickel J.C., Urinary tract infections and resistant bacteria: highlights of a symposium at the combined meeting of the 25th international congress of chemotherapy (ICC) and the 17th European congress of clinical microbiology and infectious diseases (ECCMID), march 31-april 3, 2007, Munich, Germany, *Nature Reviews Urology*, **9(2)**, 78–80 **(2007)**

18. Orenstein R. and Wong E.S., Urinary tract infections in adults, *American Family Physician*, **59(5)**, 1225–1234 **(1999)**

19. Parajuli N.P., Maharjan P., Joshi G. and Khanal P.R., Emerging perils of extended spectrum β-lactamase producing enterobacteriaceae clinical isolates in a teaching hospital of Nepal, *BioMed Research International*, https://doi.org/10.1155/2016/1782835 **(2016)**

20. Paterson D.L. and Bonomo R.A., Extended-spectrum-lactamases: a clinical update, *Clinical Microbiology Reviews*, **18(4)**, 657–686 **(2005)**

21. Picozzi S.M. et al, Extended-spectrum beta-lactamase-positive Esscherichia coli causing complicated upper urinary tract infection: urologist should act in time, *Urology Annals*, **6(2)**, 107–112 **(2014)**

22. Pitout J.D. and Laupland K.B., Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern, *The Lancet Infectious Diseases*, **8(3)**, 159–166 **(2008)**

23. Raza S., Pandey S. and Bhatt C.P., Microbiological analysis of isolates in Kathmandu medical college teaching hospital, Kathmandu, Nepal, *Kathmandu University Medical Journal (KUMJ)*, **9(4)**, 295–297 **(2012)**

24. Shakya R., Amatya R., Karki B.M., Mandal P.K. and Shrestha K.K., Spectrum of bacterial pathogens and their antibiogram from cases of urinary tract infection among renal disorder patients, *Nepal Medical College Journal: NMCJ*, **16(1)**, 75–79 **(2014)**

25. Wayne P.A., Performance Standards for Antimicrobial Disk Susceptibility Tests, 12th, Wayne, PA, USA, Clinical and Laboratory Standards Institute **(2015)**

26. Yadav K.K., Adhikari N., Khadka R., Pant A.D. and Shah B., Multidrug resistant Enterobacteriaceae and extended spectrum beta-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal, *Antimicrobial Resistance and Infection Control*, **4(1)**, 42 **(2015)**.

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