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Original Article

PREVALENCE AND ANTIBIOTIC RESISTANCE PATTERN OF ESBL-PRODUCING *ESCHERICHIA COLI* AMONG URINARY TRACT INFECTION PATIENTS IN A TERTIARY CARE HOSPITAL, DHAKA

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Abstract:

Background: *Extended-spectrum β-lactamases producing Escherichia coli (ESBL-EC) lend resistance to most β-lactam antibiotics. Because of limited treatment options, ESBL-EC infection generally more difficult to treat, leading to higher hospital costs, reduced rates of microbiological and clinical responses. This study aimed to determine the prevalence and antibiotic resistance pattern of ESBL-EC isolated from patients with urinary tract infection in a tertiary care hospital, Dhaka.*

Materials and methods: *This retrospective laboratory-based cross-sectional study was conducted at East West Medical College and Hospital, Turag, Dhaka from January 2024 to December 2024. A total of 1902 urine samples were collected from urinary tract infection patients and processed by standard microbiological methods. According to the CLSI guidelines In vitro susceptibility testing to different antibiotics of all identified isolates of E. coli was performed following Kirby–Bauer's disc diffusion method on Mueller–Hinton Agar.*

Results: *Among 1902 urine samples 535 (28.12%) were culture positive. Among culture positive isolates around 369 (68.97%) Gram negative isolates and 166 (31.03%) Gram positive isolates were found. E. coli and Staphylococcus aureus were the most isolated organism among Gram-negative and Gram-positive isolates respectively. Out of 227 isolates 64 (28.19%) were ESBL producers, of which 40 (62.5%) isolated from adult patients (over the age of fifty), Most of the ESBL producers was isolated from indoor patients 36 (56.25%). Among ESBL-producing E. coli, the highest antibiotic resistance was observed with ceftazidime (100%), ampicillin (98.43%), cefepime (100%), ceftrioxone (100%), aztreonam (98.43%) and ciprofloxacin (78.12%) and the highest antibiotic sensitivity was observed with carbapenem (100%), fosfomycin (98.43%), nitrofurantoin (93.75%) and gentamicin (55.56%).*

Conclusion: *The development of antibiotic resistance by ESBL-E. coli, the most frequent pathogen linked to urinary tract infections plays a crucial role in determining which antibiotic therapy is appropriate. This study highlights the importance of ongoing surveillance to guide empiric therapy and promote appropriate antibiotic use.*

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Uropathogenes, Extended-spectrum beta-lactamase, Antibiotic resistance, Urinary tract infection, Escherichia coli, Prevalence

Introduction

Antimicrobial resistance is a serious public concern, especially in developing countries, where easy access and inappropriate usage of antibiotics have increased the prevalence of resistance levels. According to the World Health Organization (WHO), antimicrobial resistance (AMR) stands out as a paramount global health and development challenge. Of the 4.95 million infection-related deaths worldwide, approximately 1.27 million have resulted directly from the predominance

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of bacterial AMR. [1] Urinary tract infection (UTI) is one of the most prevalent infectious diseases in humans and the second most common cause, following respiratory tract infections. The Enterobacterales group was a commonly isolated species in urine (85%), with *E. coli* as the predominant organism (72%).[2,3] As a member of the Enterobacteriaceae family, *Escherichia coli* is the most frequent bacterial cause of urinary tract infections, accounting for up to 80% of cases. [4,5] The extensive occurrence of *E. coli* and its persistent development of antibiotic resistance put humans and animals at significant risk of infection, with projections indicating a 67% increase in antibiotic usage by 2030, thereby imposing a financial strain on healthcare systems.[6]

βlactam antibiotics are the most commonly prescribed and safest antimicrobial agents for urinary tract infections (UTIs).[7] They are a class of broad-spectrum antibiotics that contain a beta-lactam ring in their molecular structure.[8,9] But several studies prove the increased resistance of *E. coli* in UTIs to beta-lactam antibiotics.[10,11] The emergence of extended-spectrum beta-lactamase (ESBL) produces bacteria that hydrolyze the beta-lactam ring, inactivate the antibiotic, and reduce, consequently, the number of treatment options, which becomes another worry. [9, 12] Extended-Spectrum Beta-Lactamase (ESBL) *E. coli,* a significant multidrug-resistant bacterium, is responsible for severe infections in both hospital and community environments, particularly in lower-middle-income countries where hygiene and sanitation are lacking. As a result, hospital admissions, fatalities, illness prevalence, and healthcare costs increase.[13] The beta-lactam antibiotics, in particular carbapenems, have been widely regarded as the treatment of choice for ESBL-EC infections.[14] However, the worldwide emergence of beta-lactam antibiotic-resistant strains of Enterobacteriaceae poses a threat to the effectiveness of this class of antibiotics.[15,16] In addition, co-resistance to sulfonamides and fluoroquinolones occurs frequently, restricting the options for alternative treatments.[17,18]

Bangladesh experienced an increasing prevalence of UTIs. However, the magnitude of the UTI associated with ESBL-producing uropathogens, with particular concern to *E. coli,* needed more attention. Recognizing current regional data on the antimicrobial susceptibility patterns of common uropathogens is essential for guiding effective treatment decisions. Therefore, this study has been conducted to investigate the prevalence of ESBL-producing *E. coli* in patients with urinary tract-associated infections. Additionally, examine the magnitude of antimicrobial-resistant ESBL-producing *E. coli* from UTI patients. The findings of this study will be useful in the management of ESBL-producing *E. coli* associated with UTI and also improvement of treatment outcomes for patients with UTI in Bangladesh.

Materials and Methods

This is a retrospective cross-sectional laboratory-based study from January 2024 to December 2024. Data were collected during routine bacteriological culture at the East west Medical College Microbiological Laboratory. Here consecutive sampling method were used.

A total of 1902 urine samples were included in this study. Patients with clinically suspected UTI were included in the study. We included all individuals whose urine cultures were positive in this study. The exclusion criteria were negative microbiological cultures, a lack of culture requests, cases with other microbes identified, and urine specimens obtained after the initiation of empiric antibiotic therapy. Each isolate was categorized as either community-acquired or hospital-acquired. Community samples included those originating from outpatient rooms of the hospital or of external general practitioners in the surrounding area.

Sample collection and processing

Clean catch midstream urine was collected in a sterile urine container by the patient or by the caregiver of the patient (in case of children). Urine culture was done in the clinical microbiology lab of East-west Medical College Hospital following standard procedure[19]. Briefly, a loopful (~10μl) of urine samples were streaked on MacConkey agar (Oxoid Ltd., UK) and blood agar (Oxoid Ltd., UK) by a semi-quantitative method using a calibrated loop. Plates were incubated at 35°C aerobically and examined at 18–24 hours and they were further incubated for another 24 hours before a negative report was issued. Cultures were quantitated, and 'significant' bacteriuria was defined as a case of UTI in specimen containing bacterial

species $1.0x10^5$ CFU/ml of urine according to the standard guidelines.[20] Patients with non-significant bacteriuria (<1.0x105 CFU/ml) were excluded from further analysis.

Identification of bacterial species

Isolates were identified based on Gram's stain reaction, culture characteristics and biochemical properties.

Antimicrobial Susceptibility Testing

In vitro susceptibility testing to different antibiotics of all identified isolates of *E. coli* was performed following Kirby–Bauer's disc diffusion method on Mueller–Hinton Agar (Hi-Media, India) according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI 2020)[21]. Antibiotics tested in our study include the following: Ampicillin (10 µg), amoxicillin clavulanate (AMC; 20/10µg), cefoxitin (FOX; 30µg), piperacillin/tazobactam(TZP; 100/10µg), fosfomycin (FOS; 200 µg), gentamycin (CN; 10 µg), tobramycin (TOB; 10 µg), amikacin (AK; 30 µg), ciprofloxacin (5µg), levofloxacin (5µg), ceftriaxone (CRO; 30 µg), ceftazidime (CAZ; 30 µg), cefixime (CFM; 10 mg), cefuroxime (CXM; 30 µg), imipenem (IPM; 10 µg), meropenem (MEM; 10µg), co-trimoxazole (SXT; 1.25/23.75 µg), nitrofurantoin (F; 300µg), mecelinum (MEL;25µg), and colistin (CT; 10 µg) (Oxoid™, UK).

Screening and Confirmation of ESBL Producers

Conventional microbiological method was used to identified the ESBL-producing *E. coli* isolate. Moreover, screening of ESBL-producing *E. coli* was carried out through a zone of inhibition of ≤25mm for ceftriaxone and/or ≤22 mm for ceftazidime and or ≤17 mm cefuroxime and or 27mm for cefotaxime considered as positive ESBL producer[20]. ESBL-producing *E. coli* was confirmed phenotypically using the combined disc method. Discs containing ceftazidime (30µg) and a combination of discs containing clavulanic acid (30µg + 10µg) were placed at 25mm apart. The isolate was considered an ESBL producer when an increase in zone of the diameter of ≥5mm in the zone of inhibitor for ceftazidime+ clavulanic acid compared to ceftazidime alone was confirmed as ESBL-producing *E. coli* according to CLSI guideline[21]. For quality assurance, the reference strains of *E. coli* (ATCC 26122) were used in quality control for culture and susceptibility tests as well as for the detection of ESBL detection.

Statistical Analysis

Data were seized and analyzed by using IBM SPSS Statistics for Macintosh, Version 23.0 (IBM Corp, Armonk, NY, USA) and presented in percentage base distribution. The statistical analysis of ESBL and Non-ESBL variables (age, sex and origin) was analyzed by Chi-square Test. $P<0.05$ was considered statistically significant.

Results:

During the study period, among 1902 urine samples 535 (28.12%) were culture positive (Figure 1). Among the culture positive uropathogens, 369 (68.97%) were Gram negative isolates and 166 (31.04%) were Gram positive isolates. Among Gram negative isolates, *E. coli* was most common organism 227 (61.52%) followed by *Enterobacter* 82 (22.22%) (Figure 3). On the other hand, *Staph. aureus* was the most prevalent organism among Gram positive isolates 92 (55.42%) followed by *Enterococci* 78 (46.99%) (Figure 4). Out of 227 *E. coli* isolates, 64 (28.19%) were ESBL producer (Figure 2) of which most 40 (62.50%) isolates were isolated from adult patients (fifty or more than fifty). Most ESBL producers were isolated from indoor patients 36 (56.25%). For gender, females have higher prevalence of UTI caused by ESBL producing *E. coli* compared to men (table I). Out of 64 sample of ESBL producing *E. coli*, 38 (59.37%) sample were isolated from female patients. The calculated p-value for sex (p = 0.412), for age (p=0.614) and for origin (p= 0.873) as displayed in Table 1 shows that the risk of contracting ESBL-EC is similar between the two groups studied.

The resistance observed in ESBL-EC sample detailed in Table 2. Analysis of â-lactam and cephalosporin family shows that all strains of *E. coli* producing ESBL were resistant to ceftazidime, cefepime, ceftriaxone, 98.44% resistant to ampicillin, cefuroxime, aztreonam, and 82.81% were resistant to ciprofloxacin, 68.75% were resistant to levofloxacin, 48.44% and 46.88% were resistant to tetracycline and co-trimoxazole respectively.

However, the analysis shows that the carbapenems are very effective against ESBL-EC with a sensitivity (100%) followed by Fosfomycin (98.44%) and nitrofurantoin (93.75%).

Table-I

Some key risk variables in urine E. coli isolates from January, 2024 to December, 2024

Factor	Non-ESBL *E. coli* isolates (N =163)	ESBL-*E.coli* isolates (N =64)	Total (N=227)	P*
Sex				
Male	55 (33.74%)	26 (40.63%)	81 (35.68%)	0.412
Female	108(66.26%)	38 (59.37%)	146 (64.32%)	
Age				
0-5	12(7.36%)	03(4.69%)	15 (6.61%)	
6-18	06 (3.68%)	01(1.56%)	07 (3.08%)	0.614
19-49	56 (34.35%)	20 (31.25%)	76 (33.48%)	
≥ 50	89 (54.60%)	40 (62.50%)	129(56.83%)	
Origin				
Inpatients	88 (53.99%)	36 (56.25%)	36 (56.25%)	0.873
Outpatients	75 (46.01%)	28 (43.75%	28.43.75%)	

“p value” obtained by the chi-square test provides to determine the existence of link between ESBL-EC and various risk factors.

Table-II

Antimicrobial susceptibility pattern of ESBL-producing E. coli from the urine samples of patients suffering from urinary tract infections (N=64).

Antibiotic susceptibility	Sensitive	Resistance
Ceftriaxone (CRO)	*00*	*64 (100%)*
Ceftazidime (CAZ)	*00*	*64 (100%)*
Cefuroxime (CXM)	*01(1.56%)*	*63 (98.44%)*
Cefepime (FEP)	*00*	*64 (100%)*
Cefoxitin (FOX)	*64 (100%)*	*00*
Ampicillin (AMP)	*01 (1.56%)*	*63 (98.44%)*
Amoxyclav (AMC)	*55 (85.94%)*	*09 (14.03%)*
Piperacillin-tazobactam (TZP)	*51 (76.69%)*	*13 (20.31%)*
Trimethoprim/sulfamethoxazole(SXT)	*34 (53.12%)*	*30 (46.88%)*
Tetracycline (TE)	*33(51.56%)*	*31 (48.44%)*
Ciprofloxacin (CIP)	*11 (17.19%)*	*53 (82.81%)*
Tobramycin (TOB)	*50 (78.13%)*	*14(21.87%)*
Mecelinum (MEL)	*57 (89.06%)*	*07(12.28%)*
Gentamycin (CN)	*57 (89.06%)*	*07(12.28%)*
Nitrofurantoin (F)	*60 (93.75%)*	*04 (6.25%)*
Levofloxacin (LEV)	*20 (31.25%)*	*44(68.75%)*
Amikacin (AK)	*44 (68.75%)*	*20 (31.25%)*
Imipenem (IPM)	*64(100%)*	*00*
Meropenem (MEM)	*64 (100%)*	*00*
Aztreonam (ATM)	*01 (1.56%)*	*63 (98.44%)*
Fosfomycin (FOS)	*63(98.44%)*	*01 (1.56%)*

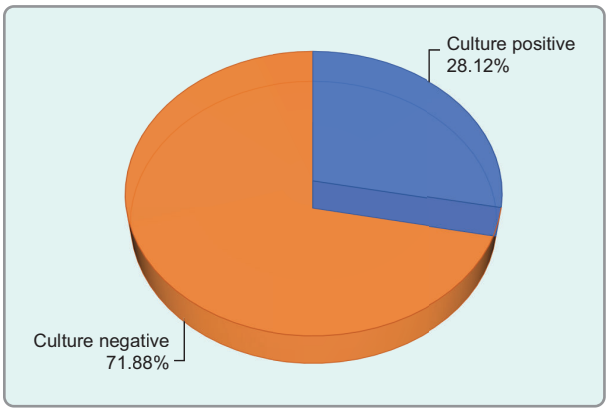

Figure 1: *Proportion of culture-positive urine samples identified during the study period (28.12%).*

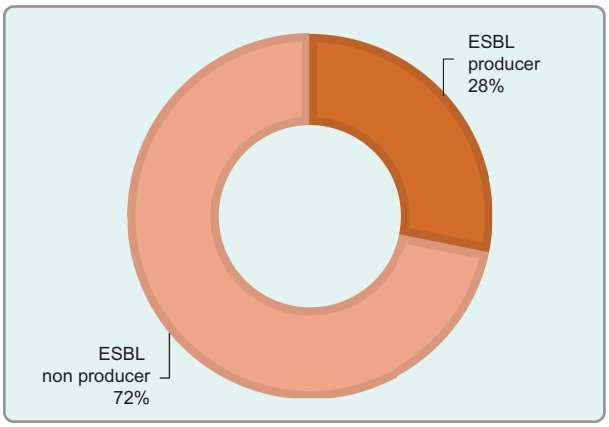

Figure 2: *Percentage of ESBL-producing Escherichia coli isolates among the total samples analyzed (n = 227).*

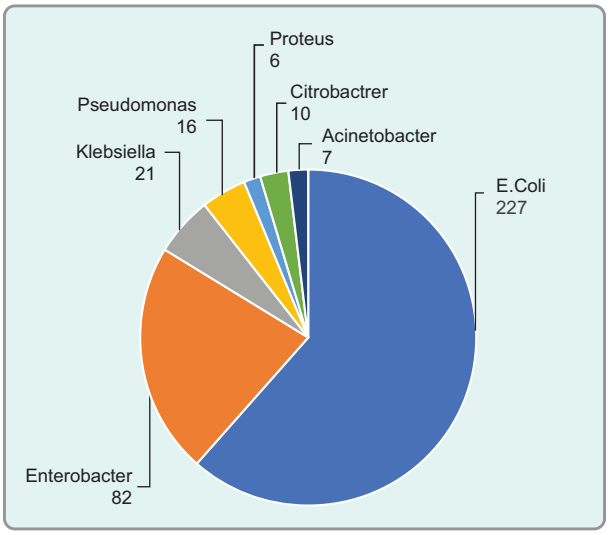

Figure 3: *Distribution of Gram negative uropathogens isolated from urine culture (n = 369).*

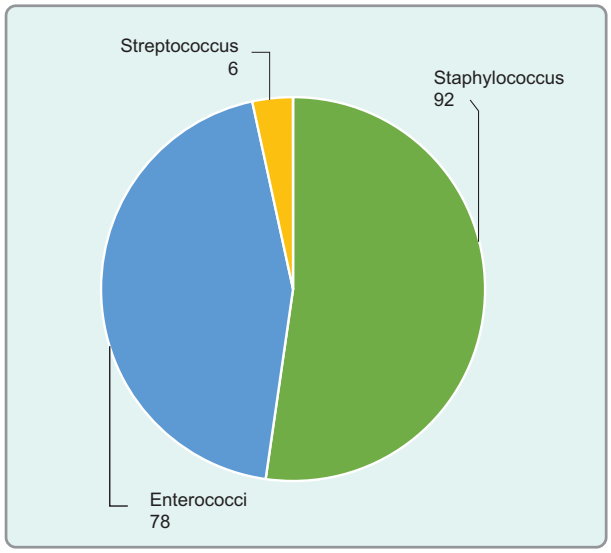

Figure 4: *Distribution of Gram positive uropathogens isolated from urine culture (n = 166).*

Discussion

The emergence and rapid dissemination of drug-resistant strains of Enterobactericeae particularly *E. coli* causing urinary tract infection leads to frequent treatment failure and complications. Since the 2000s, ESBL-EC have been considered as serious pathogens both in nosocomial and community infections around the world, and their virulence varies by region. [5] Drug-resistant urinary tract infection becomes a concerning health issue, especially in developing country like Bangladesh. However, there is continuous changes in etiology, burden of infection and antibiotic susceptibility pattern in different regions. Understanding the local epidemiology and its changes over time is essential for selecting an effective first-line antibiotic treatment that is appropriate for each region. We are aware of the significant burden we carry in our community due to various factors intertwined with the characteristics of medical practice, legislation, and the health care system. [22,23]

The overall isolation rate of uropathogens in our study was found to be 28.12% which is comparable with previous research of Ethiopia. [24,25] This study showed gram-negative bacteria were more frequent (68.97%) than gram-positive bacteria (31.03%). Similar finding had seen reported by several studies. [24, 26-27]

Current study shows that *E. coli* was the most predominant bacterium (61.52%) followed by *Enterobacter spp* and *Klebsiella spp* among gram-negative bacteria and *Staphylococcus aureus* was the

leading cause of UTI among gram-positive isolates. The isolation rates of *E. coli*, which is the most common isolate, and other pathogens in this study were comparable to the rates documented previously and also strongly agreed with similar research [28-30]. However, two studies carried out with patients attending tertiary level hospitals outside of Dhaka, Bangladesh (in Rajshahi and Mymensingh), reported that *Staphylococcus saprophyticus* was the second leading cause of UTI after *E. coli* . [31,32] Out of 227 *E. coli* strains recovered from urine samples, 64 (28.19%) produced ESBLs, whereas 71.80% did not. Parallel results were previously reported regarding the occurrance of ESBL-*E. coli* isolates recovered from patients with UTIs, such as 37.11% [9] and 46.87%.[33] Adil Abalkhail et al. found 33.49% uropathogenic ESBL producing *E. coli*. [34] In the present study, among the positive *E. coli* cases, the prevalence rate of UTIs was found more in females, 108 (66.26%) than males, 55 (33.74%) for non-ESBL-EC. As for positive ESBL-EC cases, the prevalence rate was 38 (59.37%) in females and 26 (40.63%) in males. This finding is inconsistent with result reported by Sauda Arabia and Morocco. [34,35] Because of the architecture of their sexual organs, women are more commonly affected by UTIs, that is, the compact size and proximity of the urethra to the rectum encourage the spread of ESBL-*E. coli*. [35] Furthermore, most of ESBL-E. coli was detected in people 50 years old or above (62.5%) which is in parallel with the finding in Morocco. [35] Many studies confirm that advanced age (usually over 65 years) is a UTI risk factor for ESBL-EC (16,36-38]. This could be linked to the fact that women in these groups may experience vaginal prolapse after menopause, which can heighten the risk of bacteriuria. This is due to an increase in vaginal pH resulting from a reduction in lactobacillus in the birth canal, creating an opportunity for other uropathogens to establish themselves and making these patients' immune systems more susceptible to infections.

Our findings regarding the prevalence of ESBL-EC in the community (43.75%) and hospital (56.25%) settings are likely underestimated, which leads us to recommend systemic surveillance.

Precious study showed that ESBL-*E. coli* is more prevalent among women than men, and in hospitalizations, it accounts for a large proportion of all hospital-acquired infections and thus seems to be very dangerous. [39]

According to the result of the antibiogram, the highest antibiotic resistance among ESBL-EC was observed with ampicillin (98.43%), cefixime (100%), ceftrioxone (100%), Cefuroxime (98.43%), ceftazidime (100%), aztreonam (98.43%) and ciprofloxacin (82.81%). The highest antibiotic sensitivity was observed with carbapenems (100%), cefoxitin (100%), fosfomycin (98.43%) and nitrofurantoin (93.75%). Our findings are parallel with those of certain former investigations. [35,38,40] In our country, beta-lactamase and third generation cephalosporin groups of antibiotics used as an empirical antibiotic that can be easily purchased from a retail pharmacy. Moreover, the incomplete duration of drug treatments, the easy availability of drugs, self-medication practices, lack of strict laws of on drugs that punishes for drug misuses hence all this contributed to high emerging rate of drug resistance. [41] The development of resistance to beta-lactamase and cephalosporin groups that we detected in the current study indicated that these drugs may no longer be appropriate for symptomatic treatment. The standard recommendations state that an antibiotic must have been tested >90% sensitive on the community's pathogenic *E. coli* in order to be used as an empirical antibiotic. [42]

Conclusion

The current study demonstrates that the therapeutic prospects of beta-lactam antibiotics pose a significant issue and a major obstacle for physicians, as in recent years, ESBL-*E. coli*-induced UTIs have increased in frequency. Therefore, in both community and hospital settings, early diagnosis of infections caused by this organism is essential for timely treatment. Additionally, it is important to routinely measure and analyze antibiotic resistance, especially that caused by ESBL-*E. coli*, at different intervals. These results further imply that earlier and accurate approaches for detecting ESBL-producing bacteria should be employed to prescribe relevant antibiotics. Future studies should focus on improving the accuracy of empirical antibiotic prescriptions, optimizing sensitivity testing protocols, and addressing gender-based disparities in resistance patterns to reduce treatment failure and complications of antimicrobial resistance

Limitations of the study

Several limitations should be considered. First, as a retrospective analysis based on electronic medical records, some data were missing, and certain variables might have led to the outcomes not being consistently

documented. Notably, data on the timing of infection onset were not available, limiting our ability to distinguish between hospital acquired and pre-existing infections. Second, the study was conducted within a single institution, meaning the findings may not fully reflect practices or outcomes at other hospitals, potentially limiting their generalizability to different settings or regions.

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Institutional Review Board Statement Ethical approval granted from corresponding authorities.

Informed Consent Statement
Patient consent was waived because this was a retrospective laboratory data-based study, as approved by the ethical committee.

Conflict of interest:
The authors declare no competing interests.

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Abbreviations
The following abbreviations are used in this manuscript:
UTI Urinary Tract Infection
E. coli Escherichia coli
ESBLs Extended-Spectrum Beta-Lactamases

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