A survey on antibiotic resistance of Extended-Spectrum beta-lactamase-producing *Escherichia coli* isolated from urinary tract specimens at Hue University of Medicine and Pharmacy Hospital

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Abstract

Background: Urinary tract infections caused by Extended-Spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) strains are a major concern and a key driver of β-lactam antibiotic group's resistance. Finding the frequency of ESBL-producing *E. coli* isolated from urine samples and analyzing their antibiotic resistance profiles were the objectives of this study. **Materials and methods:** A retrospective, cross-sectional study was conducted on urine samples at Hue University of Medicine and Pharmacy Hospital from January 2023 to April 2024. Bacterial culture, identification, and antibiotic susceptibility testing were performed according to standard microbiology laboratory procedures. **Results:** 104 ESBL-producing *E. coli* strains were isolated, accounting for 45.2% of patients with urinary tract infections caused by *E. coli*. This group was most common in patients over 60 years old, primarily from patients of the Urology outpatient clinic and the Department of General Internal Medicine - Endocrinology - Musculoskeletal and the prevalence was quite similar between males and females (46.9% and 44.6% respectively). The isolates showed high resistance to ampicillin, some cephalosporins, and quinolones, but still remained highly susceptible (>90%) to carbapenems and fosfomycin, and fully susceptible to nitrofurantoin. **Conclusion:** *E. coli* producing ESBL are a real burden in the treatment of urinary tract infections. Consequently, investigating the antibiotic resistance profiles of ESBL-producing *E. coli* in clinical settings is essential for informing physicians' decisions on appropriate antimicrobial therapy.

Keywords: *urinary tract infections, E. coli, ESBL, antibiotic resistance.*

1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections. This infection affects 150 million people worldwide annually and can especially cause recurrent infections in perimenopausal and menopausal women. The most common cause of UTIs is Gram-negative bacteria belonging to the *Enterobacteriaceae* family. Some common members of this family include *Escherichia coli* (*E. coli*), *Klebsiella*, *Enterobacter*, and *Proteus*. Moreover, the emergence of Gram-negative bacteria capable of producing extended-spectrum beta-lactamases (ESBLs) has contributed to the growing epidemiological concern of antibiotic resistance among enteric pathogens. This issue primarily arises from the ability of these bacteria to produce enzymes that hydrolyze and render ineffective a broad range of beta-lactam antibiotics, including third- and fourth-generation cephalosporins, penicillins, and aztreonam.The high antibiotic resistance associated with beta-lactams in *Uropathogenic Escherichia coli* (*UPEC*) strains, related to ESBL production, significantly reduces treatment effectiveness, limits treatment options, increases treatment costs, and raises mortality rates in the clinical management of UTIs [1].

There are several studies worldwide on ESBL-producing *UPEC* strains from various types of specimens, including urine. Shayan et al. (2015) reported that up to 62.7% of *E. coli* strains carried ESBLs with the presence of CIT, FOX, and TEM encoding genes [2]. Similar results were also reported from studies by Pootong et al. (2018) in a hospital in Central Thailand and by Sadeghi et al. (2021) in Iran, with ESBL-carrying *E. coli* rates of 38.7% and 46%, respectively [3, 4]. Recent data recorded in 2022 by Radera et al. studying the virulence genotype and multidrug resistance of *E. coli* isolated from community-acquired and hospital-acquired UTIs, showed that hospital-acquired *E. coli* strains were more resistant to more drugs than community-acquired strains, including carbapenem and ceftazidime [5]. Many other studies have also reported similar results on ESBL-producing Gram-negative bacilli or *Enterobacterales* [6, 7].

In Vietnam, the detection of ESBL-producing *E.*

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coli strains has received much attention from various studies. A study by Vo Thai Duong et al. (2022) on various clinical samples at Can Tho Central General Hospital, which recorded an ESBL production rate of 60.7% in *E. coli* [8]; or a study by Nguyen Thanh Tin et al. (2018) on blood, urine, sputum, pus, and lesion fluid samples at Bac Lieu Provincial Hospital, which showed that ESBL-producing *E. coli* accounted for 62.9% [9].

In Thua Thien Hue, information on ESBL-producing *E. coli* strains has also been reported and recorded. The study by Mai Van Tuan et al. at Hue Central Hospital in 2006 recorded an ESBL production rate of 30.4% in Gram-negative bacilli, of which *E. coli* accounted for the highest proportion (27 strains) [10]. A study by Nguyen Thi Tuyen et al. at Hue University of Medicine and Pharmacy Hospital on 246 *E. coli* strains from various specimen sources identified 91.5% as multidrug-resistant strains, of which 50% carried ESBLs [11].

However, studies on *E. coli* strains in urine specimens are quite limited and not well documented. We found a study by Ngo Duc Ky (2022) on type 2 diabetic patients with UTIs, with an *E. coli* isolation rate of 65.3%, of which ESBL-carrying strains accounted for 47.4% and resistance to quinolones was 42.1 - 57.9% [12].

Therefore, we conducted the study: "A survey on antibiotic resistance of Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* strains isolated from urinary tract specimens at Hue University of Medicine and Pharmacy Hospital" with two objectives:

1. To determine the rate of ESBL-producing *E. coli* strains isolated from urinary tract specimens at Hue University of Medicine and Pharmacy Hospital.

2. To identify some factors associated with ESBL-producing *E. coli* strains isolated from urinary tract specimens at Hue University of Medicine and Pharmacy Hospital.

2. SUBJECTS AND RESEARCH METHODS

2.1. Research Subjects

- Inclusion criteria: All urine specimens indicated for culture, identification, and antibiotic susceptibility testing at the Department of Microbiology, Hue University of Medicine and Pharmacy Hospital from January 2023 to April 2024.

- Exclusion criteria: Urine samples were included those with the presence of many vaginal epithelial cells and presence of multiple bacterial morphotypes (≥ 3 bacterial morphotypes) on Gram-stained slides from specimens or with ≥ 3 bacterial colony types on culture plates. All second urine cultures on the same patient with the same *E. coli* isolation results were also not included in the study.

2.2. Study Location and Time

The study was conducted at the Department of Microbiology, Hue University of Medicine and Pharmacy Hospital from January 2023 to April 2024.

2.3. Study Design

The study was designed as a cross-sectional study combined with retrospective data retrieval stored at the Department of Microbiology.

2.4. Methods of Data Collection

- Retrieval of electronic data stored at the Department of Microbiology from all patients who came for examination and treatment at Hue University of Medicine and Pharmacy Hospital diagnosed with UTIs from January 2023 to April 2024 and met with the inclusion and exclusion criteria.

- Urine specimens were primarily midstream urine for patients who can self-collect. Other urine specimens were obtained via bladder catheters and drainage tubes or directly from the kidneys, ureters, and bladder through surgical procedures. To ensure accurate culture, isolation, and bacterial identification results, all urine specimens intended for culture must be collected in sterile containers to prevent external contamination.

- The process of culturing and identifying urine specimens was carried out according to the standard procedures of the Department of Microbiology, Hue University of Medicine and Pharmacy Hospital. Briefly, after being evaluated macroscopically and microscopically (via wet mount slide), the urine sample underwent quantitative culture on Chromagar medium (CHROMagar™, Kanto Chemical Co., Inc group, Japan). After 24 hours of incubation at an appropriate temperature, the urine sample was considered to indicate a urinary tract infection caused by bacteria when the bacterial count in the quantitative culture exceeds 10^5 CFU/ml (CFU = colony forming unit). However, when the bacterial count was below 10^4 CFU/mL or within the equivocal range of 10^4 - 10^5 CFU/mL, further evaluation was conducted - taking into account the sampling site, white blood cell count, and the presence of definitive UTI symptoms to determine the likelihood of a true infection. Suspected colonies were isolated and identified based on biochemical properties.

- Phenotypic tests for detecting extended-spectrum beta-lactamase (ESBL) production

were also performed according to CLSI M100 recommendations (13) using two types of antibiotic discs from Liofilchem srl (Roseto degli Abruzzi, Italy) or CTX (Cefotaxime) and CTL (Cefotaxime + clavulanic acid) or using two discs CAZ (Ceftazidime) and CAL (Ceftazidime + clavulanic acid) to determine ESBL-producing bacteria. These two discs were placed opposite each other. If the bacteria produced ESBL, the diameter of the combined disc was ≥ 5mm compared to the single disc (Figure 1).

- The antibiotic susceptibility testing was processed using the disc diffusion method on agar plates performed according to the M02 - revision 13 guidelines - Standard procedure for antibiotic susceptibility testing using the disc diffusion method. The results of bacterial susceptibility to antibiotics were evaluated by comparing the diameter of the inhibition zone in millimeters (mm) with the breakpoint values in the CLSI M100 standard - 2020 (Clinical and Laboratory Standards Institute, 2020) or EUCAST (European Committee for Antimicrobial Susceptibility Testing) documents for each antibiotic to determine susceptibility (S=Susceptible), intermediate (I=Intermediate), and resistance (R=Resistant).

2.5. Data Processing and Analysis Methods: All data were statistically processed using Excel software, and then the data were encoded as variables, and the collected data were processed using SPSS 20.0 statistical software.

Figure 1. The phenotypic test for detecting extended-spectrum beta-lactamase (ESBL) production is performed according to CLSI M100 recommendations (13).

3. RESULTS

During the study period from January 2023 to April 2024, 2616 urine samples were cultured at the Department of Microbiology, Hue University of Medicine and Pharmacy Hospital. Among them, the rate of positive cultures was 463, accounting for 17.7%, and the main causative agent was *Escherichia coli*, accounting for 47.9%, followed by *Pseudomonas aeruginosa* (24.4%) and *Enterococcus* spp. (11%).

3.1. Rate and characteristics of urinary tract infections caused by ESBL-Producing *E. coli* strains isolated at Hue University of Medicine and Pharmacy Hospital

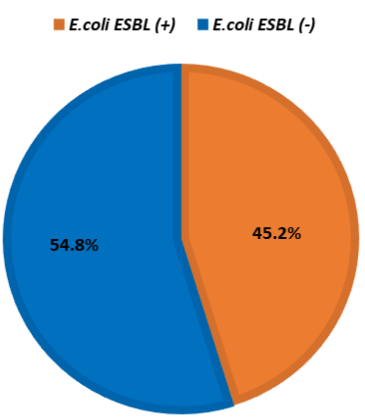

Chart 1. Percentage of ESBL-Producing *E. coli* isolated from urinary specimens

Out of 230 *E. coli* strains isolated from urine specimens, 104 strains produced ESBLs, accounting for 45.2%.

Table 1. Distribution of ESBL-Producing *E. coli* strains isolated from urine samples by departments

Clinical departments	Total positive urine samples by culture		Positive for ESBL-Producing *E. coli*	
	n	Percentage (%)	n	Percentage (%)
Urology - Neurology	99	21.4	18	18.2
General Internal Medicine - Endocrinology - Musculoskeletal	37	8	9	24.3
Urology Outpatient Clinic	252	54.4	62	24.6
Other Departments	75	16.2	15	20
Total	463	100	104	22.5

Among patients with urinary tract infections (UTIs), the rate of infection caused by ESBL-producing *E.coli* is 22.5%. Among them, the number of UTI patients infected with ESBL-producing *E.coli* is concentrated in the Urology outpatient clinic (24.6%) and the Department of General Internal Medicine - Endocrinology - Musculoskeletal (24.3%). The results of positive urine cultures are highest in the Urology clinic at 54.4% and lowest in the Department of General Internal Medicine - Endocrinology - Musculoskeletal (8%).

Table 2. Distribution of ESBL-producing *E.coli* strains isolated from urine samples by patient's gender

Gender	*E.coli* ESBL (+) (n,%)	*E.coli* ESBL (-) (n,%)	p
Male	30 (46.9%)	33 (53.1%)	0.767
Female	74 (44.6%)	92 (55.4%)	

The rate of men with urinary tract infections caused by ESBL-producing *E.coli* was quite similar with that of women (47.6% and 44.6%). The difference between two groups was not statistically significant (p>0.05).

Table 3. Distribution of ESBL-producing *E.coli* strains isolated from urine samples according to patients' age group

Age group	*E.coli* ESBL (+) n (%)	*E.coli* ESBL (-) n (%)	p
≤ 20 years old	1 (33.3%)	2 (66.7%)	
21 - 40 years old	8 (32%)	17 (68%)	
41 - 50 years old	9 (47.4%)	10 (52.6%)	0.703
51 - 60 years old	32 (46.4%)	37 (53.6%)	
> 60 years old	54 (47.4%)	60 (52.6%)	

The groups of patients >60 years old and 41-50 years old had the highest rate of UTIs caused by ESBL-producing *E.coli* (47.4%) and the lowest in the groups ≤40 years old. However, there was no association between the rate of UTI caused by ESBL-producing *E.coli* and age groups (p>0.05).

3.2. Antibiotic resistance characteristics of ESBL-producing *E.coli* isolated from urine specimens

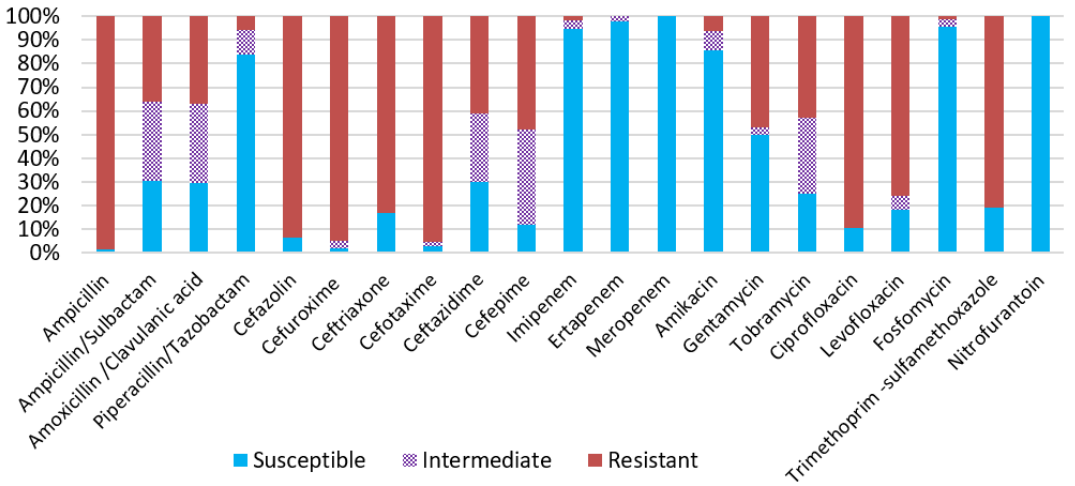

Chart 2. Antibiotic susceptibility profile of ESBL-Producing *E. coli* strains isolated from urine samples

ESBL-producing *E. coli* strains remained highly susceptible to nitrofurantoin (100%), fosfomycin (95.7%) and the carbapenem group with susceptibility rates >90%, including meropenem, imipenem, and ertapenem. Within the aminoglycoside group, ESBL-producing *E. coli* strains remained highly susceptible to amikacin (85.7%) but susceptibility to gentamicin and tobramycin were ≤50%. Among combination antibiotics, ESBL-producing *E. coli* strains remained highly susceptible to piperacillin/tazobactam (84%) while they exhibited low susceptibility to antibiotic groups such as penicillins (1.4%), 1st, 2nd, 3rd, and 4th generation cephalosporins (cefazolin - 6.5%; cefuroxime - 1.7%; ceftriaxone, cefotaxime, and ceftazidime with susceptibility rates <31%; cefepime - 12%). The same pattern couls be found in the quinolone antibiotic group, including ciprofloxacin and levofloxacin, with susceptibility rates <20%.

4. DISCUSSION

In our study, *E. coli* was the leading causative agent of urinary tract infections, accounting for 47.92%. This rate is comparable to the 2020 statistics of the Ministry of Health at 16 hospitals participating in the antibiotic resistance surveillance network, with a leading UTI rate due to *E. coli* of 43.8% [14].

Furthermore, the research results showed that the rate of ESBL-producing *E. coli* strains among the *E. coli* strains isolated from urine specimens during the period from January 2023 to April 2024 at Hue University of Medicine and Pharmacy Hospital in our study accounted for 45.2%. This result, although slightly lower compared to studies by other colleagues in Vietnam and abroad [2, 3, 8, 11, 12, 15]. A study by Truong Thi Thu Suong (2023) on 299 bacterial strains isolated from urine specimens diagnosed with UTIs at Da Nang C Hospital from January 2022 to December 31, 2022, reported an ESBL-producing *E. coli* rate of 51.5% [16]. This difference may be due to the characteristics of the different cultured samples; from different patient sources and may also be due to different geographical characteristics as well as medical treatment facilities.

Our results showed that the number of patients with UTIs due to ESBL-producing *E. coli* was concentrated in the Urology Outpatient Clinic (24.6%) and the General Internal Medicine - Endocrinology - Musculoskeletal department (24.3%). Although the General Internal Medicine - Endocrinology - Musculoskeletal department had a positive urine culture rate of only about 1/8 compared to the rate in the Urology Outpatient Clinic, the rate of positive urine cultures with ESBL-producing *E. coli* in these two departments/wards is approximately the same.

In this study, we also wanted to examine the distribution of ESBL-producing *E. coli* strains (written as *E. coli* ESBL (+)) and non-ESBL-producing *E. coli* strains (written as *E. coli* ESBL (-)) by gender and age. The results showed that the rate of urinary tract infections due to ESBL-producing *E. coli* was quite similar between males and females (46.9% and 44.6%, respectively), and the patient groups >60 years and 41-50 years had the highest rates of UTIs due to ESBL-producing *E. coli* with 47.4%. However, we did not find any association between the rate of infection with ESBL-producing *E. coli* strains with either gender or age group. This result was similar to the research results of author Vo Thai Duong

(2022), who conducted a cross-sectional descriptive study on 392 *E.coli* strains collected from cultured specimens of infected patients at Can Tho Central General Hospital from July 2021 to May 2022. This study recorded 61.5% of *E. coli* in males producing ESBL, higher than in females at 56.9%, and the highest ESBL production rate of *E. coli* in the patient group over 65 years old with a recorded rate of 59.6% [8].

Regarding the characteristics of ESBL-producing *E. coli* strains to antibiotics, we have presented the following results: ESBL-producing *E. coli* strains remain highly susceptible to the following antibiotics: nitrofurantoin (100%) and fosfomycin (95.7%); the carbapenem group with susceptibility rates >90%, including meropenem, imipenem, and ertapenem; highly susceptible to the combination antibiotic piperacillin/tazobactam (84%). This is a very noteworthy characteristic in oral treatment for specific urinary tract infections, especially the two antibiotics, although not very common today, still have very high susceptibility over 90% for ESBL-producing *E. coli*, which are nitrofurantoin and fosfomycin. This view has also been shared with a similar study from author Ki-Sup Park et al. on the urinary specimens of children [17].

The low susceptibility of ESBL-producing E. coli isolates in this study to multiple antibiotic classes—including penicillins (1.4%), first- to fourth-generation cephalosporins (e.g., cefazolin: 6.5%; cefuroxime: 1.7%; ceftriaxone, cefotaxime, and ceftazidime: <31%; cefepime: 12%), and quinolones such as ciprofloxacin and levofloxacin (<20%) - was anticipated and highlights the high level of antimicrobial resistance in these strains. This finding serves as a critical warning for clinicians regarding the treatment challenges posed by ESBL-producing E. coli and other multidrug-resistant organisms.The results on the antibiotic resistance situation in the *E. coli* bacteria group isolated in the urinary tract in this study were quite similar to the antibiotic surveillance report in Vietnam in 2020 published by the Ministry of Health in 2023 [14] and some other local studies [15, 16, 18]. Nguyen Nhut Thang and colleagues (2024) studied on *E. coli* strains collected from different patient samples at the Laboratory Department of Can Tho City General Hospital. They found that the bacteria were resistent to 9 out of 15 tested antibiotics, with rates ranging from 55.4% for ampicillin/sulbactam to 98% for ampicillin. However, these strains were still susceptible to a few antibiotics such as imipenem (93.2%), ertapenem (91.9%) and a small proportion from 0 - 24.4% of resistant strains at an intermediate level [18]. A study closely aligned with ours, conducted by Nguyen Thi Van (2023) on patients with complicated urinary tract infections at Bac Ninh Provincial General Hospital, also reported high levels of antibiotic resistance in *E. coli* isolates. The findings revealed complete resistance to ampicillin (91.8%) and piperacillin (71.7%), resistance rates ranging from 47.4% to 67.2% for third- and fourth-generation cephalosporins, and a 52.4% resistant rate to gentamicin. Additionally, resistance to tobramycin and amikacin was 17.5%, while fluoroquinolones exhibited resistance rates exceeding 70% [15].

The characteristics of ESBL-producing *E. coli* strains related to antibiotic groups recorded from the study by Santosh Pantha et al. from ESBL-producing *E. coli* strains at a level 3 pediatric hospital in Nepal during the period from August 2016 to February 2017 also gave remarkable results with high susceptibility to nitrofurantoin (84.2%) and recorded 100% susceptibility to imipenem [19]. However, according to a systematic and comprehensive assessment by Zelalem Asmare et al. in 2024 providing information on antibiotic resistance in UPEC strains in Ethiopia showed drug resistance rates, ranging from 3.64% for amikacin to 85.32% for ampicillin. The highest drug resistance was ampicillin (85.32%), followed by amoxicillin at 82.52%, tetracycline at 60.67%, and trimethoprim/sulfamethoxazole at 57.17%. In contrast, lower resistance rates were for amikacin at 3.64% and meropenem at 5.26% [20]. This once again confirms the importance of conducting antibiotic resistance studies separately in each medical hospital and not using the research results of other hospitals to apply to the current one.

5. CONCLUSION

The research results provide important data on the distribution characteristics as well as the antibiotic resistance situation of ESBL-producing *E. coli* strains in urine specimens at Hue University of Medicine and Pharmacy Hospital during the period from 01/2023 to 04/2024. There are 104 ESBL-producing *E. coli* strains, accounting for 45.2% of *E. coli* strains isolated from urinary tract specimens. This bacterial group is most common in the age group over 40; mainly from patients of the Urology Outpatient Clinic and the General Internal Medicine - Endocrinology - Musculoskeletal Department; found in males (46.9%) and in females (44.6%). The bacterial strains in the study are quite resistant to ampicillin, cephalosporin antibiotics, and quinolone groups; however, they are still quite susceptible

(>x90%) to carbapenem, fosfomycin antibiotics and completely susceptible to nitrofurantoin. Futher study on the genetic characteristics of these resistent strains should be better for clear understanding the molecular pattern of those antibiotic resistance.

REFERENCES