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Phenotypic Detection and Antibiotic Susceptibility Pattern of Extended-Spectrum β-Lactamase-Producing Uropathogenic *E. coli* Isolated in Sana'a City, Yemen

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Abstract

Background: Urinary tract infections pose a significant public health challenge in Yemen, exacerbated by the emergence of extended-spectrum -lactamase-producing uropathogenic *E. coli*. This study aimed to determine the prevalence and antimicrobial susceptibility profile of extended-spectrum -lactamase-producing uropathogenic *E. coli* in Sana'a City.

Methods: A cross-sectional study collected 359 urine samples from two major hospitals in Sana'a. Uropathogenic *E. coli* isolates were identified using standard methods, and ESBL production was confirmed phenotypically using the double-disk synergy test. Antimicrobial susceptibility was determined via Kirby-Bauer disk diffusion

Results: Of the samples, 155 (43.1%) were positive for uropathogenic *E. coli*, with 41.9% (65/155) confirmed as ESBL producers. These isolates exhibited high resistance to ampicillin, cephalosporins, and fluoroquinolones. Amikacin and meropenem remained the most effective antibiotics. Extended-spectrum -lactamase-producing uropathogenic *E. coli* infections showed significant associations with comorbidities including diabetes, heart disease, and urinary tract deformities ($p < 0.05$).

Conclusion: The high prevalence of multidrug-resistant extended-spectrum -lactamase-producing uropathogenic *E. coli* among Yemeni urinary tract infection in Sana'a city underscores the urgent need for routine susceptibility testing and antimicrobial stewardship programs to guide treatment and preserve effective antibiotics.

Article Info

Keywords:
Urinary Tract Infection (UTI), Uropathogenic E. coli (UPEC), Extended-Spectrum Beta-Lactamase (ESBL), Antimicrobial Resistance (AMR), Sana'a, Yemen.

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1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, leading to significant healthcare costs and morbidity [1]. Uropathogenic *Escherichia coli* (UPEC) is the main pathogen responsible for most community- and healthcare-associated UTIs [1]. However, the effectiveness of standard antibiotic regimens is increasingly compromised by the increase in antimicrobial resistance (AMR). A particularly concerning resistance mechanism is the production of extended-spectrum β-lactamases (ESBLs). ESBLs are enzymes produced by certain bacteria that confer resistance to important antibiotics, including penicillin, cephalosporins (such as third-generation antibiotics), and aztreonam [2] UPEC that produces ESBL (ESBL-UPEC) often harbors plasmids, which are small DNA molecules separated from chromosomal DNA, carrying genes for other resistance determinants, leading to multidrug resistance (resistance to multiple antibiotic classes). These infections are associated with higher treatment failure rates, longer hospital stays, and increased mortality [3]. Risk factors

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for ESBL-UPEC infections include previous antibiotic use, recurring UTIs, hospital admission, and underlying medical conditions, such as diabetes [4, 5].

The situation in Yemen was critical. A recent study conducted in Sana'a highlighted the severity of antimicrobial resistance (AMR), revealing that *E. coli* was identified in 27% of culture-positive clinical samples with a high multidrug resistance rate of 62.5% [6]. The same study documented extensive resistance to first-line antibiotics, including ampicillin (97.3%) and first-generation cephalosporins (90.2%), indicating a challenging therapeutic environment [6].

Although several studies in Yemen have documented extended-spectrum β-lactamase (ESBL)-producing strains in diverse clinical specimens, the roles of environmental and foodborne reservoirs in disseminating these resistant bacteria are also significant [7, 8].

To our knowledge, no study in Sana'a has investigated urinary tract infections (UTIs) caused by ESBL-producing uropathogenic *E. coli* (ESBL-UPEC). Therefore, this study is the first to determine the prevalence, identify risk factors, and characterize the antimicrobial resistance profiles of UPEC and ESBL-UPEC isolated from patients with UTI in major hospitals in Sana'a City, Yemen.

2. MATERIALS AND METHODS

2.1. Study Design and Ethics

This cross-sectional study was conducted between 2023 and 2024.

Ethical Approval: This study was approved by the Committee of the Biological Department, Microbiology Branch, Faculty of Science, Sana'a University (no. 825-9). Written informed consent was obtained from all participants in their native Arabic language after a full explanation of the study's purpose. All the data were anonymized using coded identifiers and stored under secure conditions.

2.2. Study Population

A total of 359 clean-catch and midstream urine samples were collected from patients clinically diagnosed with UTIs at two major hospitals in Sana'a, Yemen, between December 2023 and December 2024: Al-Kuwait University Hospital and Al-Thwrah Hospital. The patients were provided with clear verbal and written instructions on the proper clean-catch technique to minimize contamination. The samples were immediately stored at 4 ℃, and cultures were performed within 24 h of collection. All the participants were interviewed using a structured questionnaire designed specifically for this study.

The questionnaire collected demographic (age and sex) and clinical data, including history of prior UTIs and comorbidities (e.g., diabetes and heart disease).

2.3. Bacterial Isolation and Culture

Urine samples were cultured on blood agar and MacConkey agar (HiMedia, India) using a calibrated loop, and incubated aerobically at 37 ℃ for 18–24 h. Significant bacteriuria was defined as bacteriuria $\geq 10^5$ CFU/mL [9].

2.3.1. Identification of Uropathogenic E. coli

Presumptive *E. coli* isolates were identified by pink, lactose-fermenting colonies on MacConkey agar and grey, often beta-hemolytic colonies on blood agar. Confirmation was achieved through Gram staining (gram-negative bacilli) and IMViC biochemical profile tests (indole positive, methyl red positive, Voges-Proskauer negative, citrate negative), consistent with the standard clinical methods [10]

2.4. Antimicrobial Susceptibility Testing and Screening for ESBL Producers

Antimicrobial susceptibility profiling for all UPEC isolates was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA), strictly following the Clinical and Laboratory Standards Institute (CLSI M100) guidelines [11]. *E. coli* ATCC® 25922 was used as the quality control strain for all Antimicrobial Susceptibility Testing (AST) procedures.

Antibiotic susceptibility was determined by measuring inhibition zone diameters and interpreting them as susceptible, intermediate, or resistant according to CLSI M100 (2020-2022) breakpoints [11]; specific criteria are listed in Table 1.

Isolates exhibiting resistance or reduced susceptibility to cefotaxime and/or ceftazidime were flagged as presumptive ESBL producers and were selected for phenotypic confirmation.

2.5. Phenotypic Confirmation of ESBL Production by Double-Disk Synergy Test

Presumptive ESBL-producing isolates identified as cephalosporin-resistant were subjected to confirmatory testing using the Double-Disk Synergy Test (DDST) as previously described [4, 11], with modifications.

A Mueller-Hinton agar plate was inoculated using a McFarland standard suspension (0.5 mL). A central amoxicillin-clavulanic acid disk was flanked by ceftazidime, cefotaxime, and aztreonam disks, placed 20 mm away (center-to-center).

The plates were incubated at 35±2 ℃ for 16-18 hours. A positive result for presumptive ESBL production was

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indicated by a "keyhole" effect—a synergistic zone of inhibition extending from a cephalosporin or aztreonam disk toward the central amoxicillin-clavulanic acid disk.

Table 1. CLSI Breakpoint Criteria for Disk Diffusion Test Interpretation for E. coli (adapted from CLSI [11]).

Antibiotic (Disk Potency)	R (mm)	I (mm)	S (mm)
Amikacin (30 µg)	≤ 14	15-16	≥ 17
Amoxicillin-Clavulanic Acid (20/10 µg)	≤ 13	14-17	≥ 18
Ampicillin (10 µg)	≤ 13	14-16	≥ 17
Aztreonam (30 µg)	≤ 21	22-26	≥ 27
Cefepime (30 µg)	≤ 24	-	≥ 25
Cefotaxime (30 µg)	≤ 22	23-25	≥ 26
Ceftazidime (30 µg)	≤ 19	20-22	≥ 23
Chloramphenicol (30 µg)	≤ 12	13-17	≥ 18
Ciprofloxacin (5 µg)	≤ 15	16-20	≥ 21
Gentamicin (10 µg)	≤ 12	13-14	≥ 15
Imipenem (10 µg)	≤ 19	20-22	≥ 23
Meropenem (10 µg)	≤ 19	20-22	≥ 23
Nalidixic Acid (30 µg)	≤ 13	14-18	≥ 19
Nitrofurantoin (300 µg)	≤ 14	15-16	≥ 17
Piperacillin-Tazobactam (100/10 µg)	≤ 17	18-20	≥ 21
Tetracycline (30 µg)	≤ 11	12-14	≥ 15
Tobramycin (10 µg)	≤ 12	13-14	≥ 15

Note: R: Resistant, I: Intermediate, S: Susceptible

2.6. Statistical Analysis:

Data were analyzed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies and percentages. Associations between risk factors and ESBL production were assessed using chi-square tests, with statistical significance set at $P < 0.05$. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to determine the strength of the association.

3. RESULTS

Table 2. Demographic and Clinical Characteristics of Patients with Urinary Tract Infections (n=359)

Characteristic	Category	UTI Samples (n=359)	Percent %
Age Group(years)	< 20	**70**	**19.5 %**
	21 – 40	**193**	**53.8 %**
	41 – 60	**56**	**15.6 %**
	> 60	**40**	**11.1 %**
Sex	Male	**160**	**44.6%**
	Female	**199**	**55.4%**

3.1. Demographic and Clinical Characteristics of the Study Population

A total of 359 urine samples were collected from patients with clinically diagnosed UTIs. The patient population was predominantly female (55.4%) and in the 21-40 years age group (53.8%), as shown in **Table 2.**

3.2. Prevalence and Distribution of Uropathogenic *E. coli*

Of the 359 isolates, 155 (43.1%) uropathogenic *E. coli* (UPEC) were identified (**Figure 1**). Out of 155 UPEC, 90/155 (58.1%) non-ESBL UPEC and 65/155 (41.9%) extended-spectrum beta-lactamases uropathogenic *Escherichia coli* were isolated, which was confirmed by DDT, as shown in **Figure 2.**

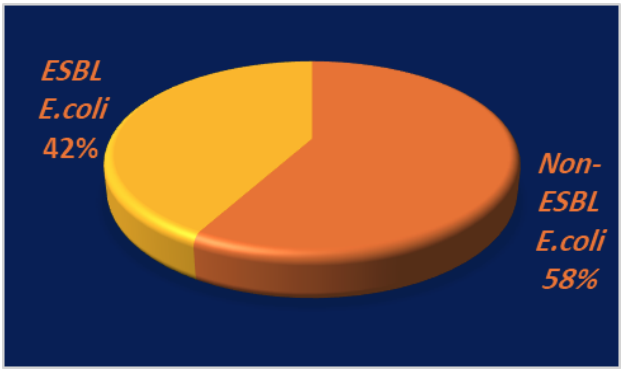


Figure 1. Prevalence of ESBL Production among Uropathogenic *E. coli* (UPEC) isolates (n=155).

The blue arrow indicates a positive test, showing keyhole indentation in the inhibition zone of the ceftazidime (CAZ, 30 µg) disk towards the amoxicillin-clavulanate (AMC, 20/10 µg) disk, confirming ESBL production.

3.3. Comparative Antibiotic Susceptibility Profiles

Susceptibility profiles differed markedly between the non-ESBL and ESBL-UPEC isolates (**Table 3**). Non-ESBL isolates were largely susceptible to carbapenems (>91%) and amikacin (88.9%) but resistant to ampicillin (74.4%). In contrast, ESBL-UPEC exhibited extensive

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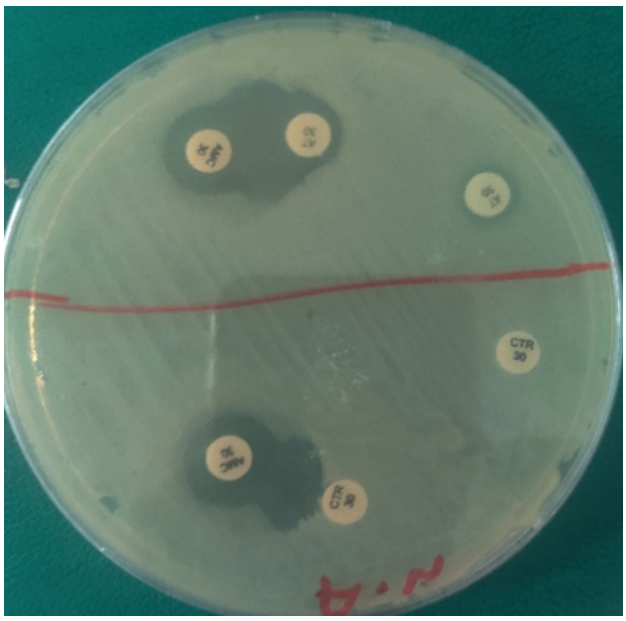


Figure 2. Double-Disk Synergy Test for ESBL Detection.

resistance to third-generation cephalosporins (≥95%). Among these isolates, amikacin (87.7%) and carbapenems (73.8-80.0%) remained the most effective agents, while nitrofurantoin showed limited activity (46.2%).

3.4. Risk Factors and Clinical Characteristics Associated with ESBL-UPEC Infections

Among the 359 samples, 155 UPEC isolates were identified, predominantly from female patients (94%) and individuals aged 21-41 years (48.4%). The highest disease burden was observed in the 60-75 age group. Clinically, most UPEC infections are community acquired (69.0%) and are most frequently diagnosed with cystitis. A key finding was the significantly higher prevalence of recurrent UTIs.

In addition, analysis of clinical risk factors revealed a strong, significant association between ESBL-UPEC infection and ICU admission (OR = 12.5, 95% CI: 3.0–51.8, $p < 0.001$), as shown in **Table 5**.

Furthermore, the presence of underlying comorbidities, including urinary tract deformity (OR = 2.7, 95% CI: 1.6–4.6, $p < 0.001$), heart disease (OR = 2.4, 95% CI: 1.5–4.0, $p < 0.001$), and diabetes (OR = 2.0, 95% CI: 1.4–3.0, $p < 0.001$), was also significantly associated with infection. In contrast, history of surgery and prostatic disease showed no significant association with ESBL-UPEC infection ($p = 0.200$ and $p = 0.987$, respectively).

4. DISCUSSION

This study provides critical insights into the epidemiology of ESBL-producing UPEC in Sana'a, Yemen and reveals an alarming resistance burden that demands immediate intervention.

The high ESBL prevalence of 41.9% among UPEC isolates places Yemen among regions with severe resistance problems, which is comparable to reports from India [12] and other high-endemicity settings. This finding is particularly concerning given that most ESBL-UPEC infections (58.5%) were community-acquired, indicating widespread dissemination beyond hospital environments.

Our susceptibility data reveal a potential therapeutic crisis. Near-complete resistance to third- generation cephalosporins and high fluoroquinolone resistance (61.5% to ciprofloxacin) eliminate conventional oral options for empirical therapy.

In this context, amikacin has emerged as the most consistently effective agent, with 87.7% susceptibility to ESBL producers. This aligns with findings from Ethiopia [13] and Egypt [14], supporting its potential role in tailored empiric regimens.

The demographic findings, which showed a higher incidence of ESBL-UPEC in females and in the 21-40 years age group, align with well-established global trends attributed to anatomical factors and sexual activity [15–17]. This shift from healthcare-associated to community-associated ESBL spread is a worrying trend observed in other countries such as Saudi Arabia [18] and China [19].

Significant associations with ICU admission, diabetes, and urinary tract abnormalities highlight patient populations that warrant enhanced surveillance and preemptive testing. These risk factors create a cycle of recurrent infections and antibiotic exposure, which drives resistance selection [4, 5, 20–22].

Within Yemen's broader AMR landscape, our ESBL-UPEC findings represent a critical component of the escalating crisis. Recent reports on carbapenem-resistant *Klebsiella pneumoniae* [23] underscore the parallel emergence of other multidrug-resistant pathogens, while calls for innovative approaches [24] highlight the diminishing therapeutic arsenal.

These findings necessitate urgent implementation of antimicrobial stewardship programs in Yemeni healthcare facilities. Routine ESBL screening and susceptibility testing should guide therapeutic decisions and preserve carbapenems for confirmed cases, while considering amikacin for serious infections. Public health authorities should prioritize the surveillance of community-associated ESBL strains to ensure their spread.

5. CONCLUSION

This study demonstrated an alarming prevalence of ESBL-producing UPEC in Sana'a, Yemen, with rates exceeding 40% among clinical isolates. Extensive multidrug resistance patterns severely limit treatment options, particularly oral therapies. Our findings suggest

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Table 3. Comparative Antibiotic Susceptibility Profiles of ESBL-Producing and Non-ESBL Uropathogenic *E. coli* Isolates

Antibiotic	ESBL Isolates (n=65)			Non-ESBL Isolates (n=90)		
	S (%)	I(%)	R(%)	S(%)	I(%)	R(%)
Aminoglycosides						
Amikacin	57 (87.7)	3 (4.6)	5 (7.7)	80 (88.9)	2 (2.2)	8 (8.9)
Gentamicin	43 (66.1)	3 (4.6)	19 (29.2)	71 (78.9)	8 (8.9)	11 (12.2)
Tobramycin	33 (50.8)	5 (7.7)	27 (41.5)	70 (77.8)	5 (5.6)	15 (16.7)
Penicillin						
Ampicillin	1 (1.5)	0 (0.0)	64 (98.5)	3 (3.3)	20 (22.2)	67 (74.4)
Amoxicillin-Clavulanate	27 (41.5)	2 (3.1)	36 (55.4)	52 (57.8)	0 (0.0)	38 (42.2)
Cephalosporins						
Cefepime(4th generation)	2 (3.1)	1 (1.5)	62 (95.4)	76 (84.4)	9 (10.0)	5 (5.6)
Cefotaxime(3rd generation)	1 (1.5)	0 (0.0)	64 (98.5)	42 (46.7)	9 (10.0)	39 (43.3)
Ceftazidime(3^{rd} generation)	2 (3.1)	1 (1.5)	62 (95.4)	25 (27.8)	27 (30.0)	38 (42.2)
Monobactams						
Aztreonam	3 (4.6)	1 (1.5)	61 (93.8)	32 (35.6)	37 (41.1)	21 (23.3)
Carbapenems						
Imipenem	48 (73.8)	4 (6.2)	13 (20.0)	82 (91.1)	0 (0.0)	8 (8.9)
Meropenem	52 (80.0)	1 (1.5)	12 (18.5)	87 (96.7)	0 (0.0)	3 (3.3)
Fluoroquinolones						
Ciprofloxacin	20 (30.8)	5 (7.7)	40 (61.5)	37 (41.1)	23 (25.6)	30 (33.3)
Levofloxacin	29 (44.6)	6 (9.2)	30 (46.2)	71 (78.9)	2 (2.2)	17 (18.9)
Quinolones						
Nalidixic Acid	3 (4.6)	2 (3.1)	60 (92.3)	39 (43.3)	17 (18.9)	34 (37.8)
Other Agents						
Chloramphenicol	20 (30.8)	6 (9.2)	39 (60.0)	45 (50.0)	10 (11.1)	35 (38.9)
Nitrofurantoin	30 (46.2)	7 (10.8)	28 (43.1)	73 (81.1)	6 (6.7)	11 (12.2)
Piperacillin-Tazobactam	25 (38.5)	7 (10.8)	33 (50.8)	59 (65.6)	16 (17.8)	15 (16.7)
Tetracycline	14 (21.5)	2 (3.1)	49(75.4)	54(60.0)	26(28.9)	10 (11.1)

Note: Susceptibility testing was performed and interpreted according to Clinical and Laboratory Standards Institute (CLSI M100) guidelines. S, Susceptible; I, Intermediate; R, Resistant.

that amikacin is the most effective agent *in vitro* against resistant pathogens in vitro. These results highlight the critical need for comprehensive antimicrobial stewardship programs, enhanced laboratory capacity for routine susceptibility testing, and coordinated national surveillance to combat the escalating AMR crisis in Yemen.

5.1. Limitations and Future Work

"This study has some limitations. This study focused on the phenotypic detection and prevalence of ESBL-producing uropathogenic *E. coli*. Genetic characterization of resistance mechanisms, including genes encoding AmpC β-lactamases and carbapenemases (e.g., KPC, NDM, VIM, OXA-48) or plasmid-mediated colistin resistance (*mcr*), was not performed. Future studies should build on these findings by employing molecular techniques to characterize the prevalent ESBL genes (e.g., *bla*blaCTX-M , *bla*TEM, *bla*SHV), investigate their plasmid-mediated transmission networks, and analyze the virulence profiles of these resistant strains to fully understand their pathogenic potential.

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Table 4. Demographic and Clinical Characteristics of Patients with UPEC Infections

Characteristic	Category	All UPEC (n=155)	ESBL-UPEC (n=65)	Non-ESBL UPEC (n=90)	P. Value	Chi-Square
Age Group (years)	< 20	25 (16.1%)	8 (12.3%)	17 (18.9%)	0.542	2.15
	21 – 40	75 (48.4%)	35 (53.8%)	40 (44.4%)		
	41 – 60	40 (25.8%)	17 (26.2%)	23 (25.6%)		
	> 60	15 (9.7%)	5 (7.7%)	10 (11.1%)		
Sex	Male	61 (39.4%)	23 (35.4%)	38 (42.2%)		
	Female	94 (60.6%)	42 (64.6%)	52 (57.8%)	0.390	0.74
Patient Type	Inpatient	48 (31.0%)	27 (41.5%)	21 (23.3%)		
	Outpatient	107 (69.0%)	38 (58.5%)	69 (76.7%)	**0.015**	5.87

Note: Chi-square $\geq$ 3.84 (significant), P-value: Probability value $\leq$ 0.05 (significant)

Table 5. Association between Clinical Risk Factors and ESBL-UPEC Infection

Risk Factor	Non-ESBL UPEC (n=90) n (%)	ESBL UPEC (n=65) n (%)	p-value	Odds Ratio (95% CI)
Urinary Deformity	**15 (16.7%)**	**29 (44.6%)**	**<0.001**	**2.7 (1.6 - 4.6)**
Diabetes	**27 (30.0%)**	**39 (60.0%)**	**<0.001**	**2.0 (1.4 - 3.0)**
Heart Disease	**17 (18.9%)**	**30 (46.2%)**	**<0.001**	**2.4 (1.5 - 4.0)**
ICU Admission	**2 (2.2%)**	**18 (27.7%)**	**<0.001**	**12.5 (3.0 - 51.8)**
Surgery	**14 (15.6%)**	**15 (23.1%)**	**0.200**	**1.5 (0.8 - 2.9)**
Prostatic Disease	**11 (12.2%)**	**8 (12.3%)**	**0.987**	**1.0 (0.4 - 2.4)**

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