ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 6, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

STUDY OF MAGNITUDE OF UTI CAUSED BY ESBL-PRODUCING *ESCHERICHIA COLI* AND ASSOCIATED RISK FACTORS

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Received: 19 October 2021, Revised and Accepted: 15 April 2022

ABSTRACT

Objective: Globally, urinary tract infections (UTIs) caused by *Escherichia coli* that produce extended-spectrum lactamase (ESBL) have become more common. Our study determined the magnitude of UTI occurring due to ESBL-producing *E. coli* and associated risk factors. Different methods for their phenotypic detection were also compared.

Methods: Uropathogenic *E. coli* isolated in significant numbers were assayed microbiologically. *E. coli* isolates (n=247) that were found significant in number tested for ESBL production using three different phenotypic methods: Phenotypic combined disk diffusion test (PCDDT), double-disk approximation test (DDAT), and E-test for ESBL production. An antibiotic susceptibility test was performed for different antibiotics. Various risk factors associated with UTIs were correlated with ESBL- and non-ESBL-producing *E. coli*.

Results: We found that diabetes mellitus type 2 was the most common risk factor for UTI caused due to ESBL-producing *E. coli* (25%). Pregnant females and patients having recurrent UTI showed less positivity for ESBL production. DDAT detected 32 ESBL-positive isolates and PCDDT detected 37 positive isolates. E-test was taken as the gold standard for ESBL detection which detected 49 isolates as ESBL producers. The highest sensitivity (71.2%) and specificity (75%) were shown by PCDDT.

Conclusion: According to the study conducted, it was concluded that PCDDT was the most reliable and economic method for phenotypic detection of ESBL.

Keywords: UTI, Risk factors, ESBL, PCDDT, DDAT, E-test, Sensitivity, Specificity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i6.43478. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Urinary tract infections (UTIs) are the most common of all the community- and hospital-acquired infections around the globe. The clinical pictures of UTI can range from asymptomatic bacteriuria to sepsis-induced severe pyelonephritis [1,2]. The primary pathogen causing UTI is *Escherichia coli*. Multidrug resistance is growing in isolates from community-onset and hospital-acquired illnesses, exacerbating the problem.

Extended-spectrum lactamase (ESBL)-producing organisms from *Enterobacteriaceae* are now common in outdoor patients without recognized risk factors [3]. Therefore, the identification of ESBL-producing microorganisms has become a concerning issue for patients. Different phenotypic methods have been carried out to detect ESBL production by *Enterobacteriaceae* [4,5].

Therefore, a better perception of UTIs caused due to ESBL-positive *E. coli* will help physicians to choose suitable empirical therapy. Furthermore, it will lead clinicians to take measures to bring down risk factors for these resistant infections. This study was made to compare the phenotypic methods applied in most microbiological laboratories to discriminate between ESBL-positive and non-ESBL strains of *E. coli.*

METHODS

This study was done between October 2016 and September 2017 in cases of UTI caused by *E. coli*. Urine samples from clinically suspected UTI patients have been analyzed for significant bacteriuria. The urine samples received were cultured by semi-quantitative method on MacConkey agar and blood agar media by calibrated loop technique [6,7] and were incubated aerobically for 24 h at 37°C. The most common symptoms of UTIs include fever >38°C, higher frequency, suprapubic tenderness, urgency, or dysuria.

A sample showing *E. coli* ≥10^5 CFU/ml was considered for the study [8]. Growth with three or more types of colonies in a sample was considered to be contamination and a repeat sample has been advised.

Characteristics of UTI due to ESBL- and non-ESBL-producing *E. coli* have been collated demographically, associated with underlying diseases and risk factors for ESBL-producing *E. coli* were identified. The processed samples have been incubated and studied according to the standard laboratory protocol.

Detection of ESBL

The phenotypic combined disk diffusion test

- A broth culture of the test strain with 0.5 McFarland opacity standard suspension has been plated on an MHA plate.
- Cefotaxime (30 g) and cefotaxime plus clavulanic acid (30/10 g) and ceftazidime (30 g) and ceftazidime plus clavulanic acid (30/10 g) disks have been added to Mueller-Hinton agar plates and were incubated. The bacterium was classified as an ESBL producer by phenotypic combined disk diffusion test (PCDDT) if the inhibitory zone width of the clavulanate disk exceeded that of the antibiotic disk alone by 5 mm [9].

Double-disk approximation test (DDAT)

Similarly, a broth culture of the test strain with 0.5 McFarland opacity standard suspension has been inoculated on a Mueller-Hinton agar plate [10].

- Amoxicillin/clavulanic acid (20/10 μg) and cefotaxime (30 μg) were placed at a distance of 15 mm and incubated.

- The cefotaxime inhibitory zone of the ESBL generating bacteria has been extended toward the clavulanic acid disk.

The E-test

The Biomerieux E-test ESBL CT/CTL strips made of a non-porous, inert plastic container (5×60 mm). The MIC reading scales in g/mL were calibrated on one side of the strip, while two predetermined exponential gradients applied to the reverse surface. CTL stands for cefotaxime (0.016–1 g/mL) plus 4 g/mL clavulanic acid and CT for cefotaxime (0.25–16 g/mL) gradient. Although the setup followed normal E-test protocols for Gram-negative aerobes, an inhibition ellipse may develop at each end of the strip.

In the presence of clavulanic acid, if the MIC of CT reduced by ≥3 log2 dilutions, either there is a formation of the phantom zone or CT ellipse is deformed, the ESBL production has been confirmed.

E-test ESBL strip had been applied to the inoculated agar surface with a pair of forceps placing the MIC scale upward. The incubation of agar plates was done in an inverted position at 35±2°C for 16–20 h.

The plates were been examined after incubation. Where the inhibition ellipses intersected the MIC strip, CT and CTL MIC values have been obtained. A circular zone (phantom zone) was occasionally visible beneath the CTL gradients, but no ellipse was visible around the CT end. Due to the synergy between CT and clavulanic acid diffusing across the CTL sections, the presence of a phantom zone or ellipse deformation also signals ESBL synthesis.

Statistical analysis

Each phenotypic method's diagnostic ability was assessed by comparing its sensitivity, specificity, and positive and negative predictive values. E-test was taken as the gold standard test.

RESULTS

We identified 247 UTI cases caused by *E. coli*. Out of these, ESBL production was confirmed in 49 (20%) cases with phenotypic detection. Mostly, the patients were male. Benign prostate hypertrophy was more common amongst males above 40 years of age (28%) and catheterization was among young patients, that is, below 40 years of age (12%) (Table 1).

Diabetes mellitus type 2 accounts for almost equal in both age groups. It is the most common risk factor for UTI caused due to ESBL-producing *E. coli.* Positivity for ESBL production was lower in pregnant females and patients with recurrence of UTI (Table 3).

ESBL production has been detected by two phenotypic methods – DDAT and PCDDT. DDAT detected 32 ESBL-positive isolates and PCDDT detected 37 positive isolates (Table 4). Out of 49 samples positive for ESBL production, considering E-test as the gold standard, PCDDT detected 35 true-positive isolates and 14 true negatives for ESBL production resulting in a sensitivity of 71.4% and specificity of 75%. Similarly, DDAT detects only 29 positive strains resulting in the sensitivity and specificity of 59.2% and 62.5%, respectively. This method showed a maximum number of false positives (n=3) (Table 5).

DISCUSSION

Microbial invasion and subsequent multiplication of the microorganism in the urinary system causes UTI [11]. The etiology of UTIs and the antibiotic susceptibility of UTI causing bacteria have changed throughout time in both community and hospital settings [12,13]. ESBLs are now a major problem among community-onset or hospital-acquired UTIs. Prevalence rate of ESBL varies greatly worldwide and in different geographic areas.

UTI can be seen in association with several risk factors. Recent urological procedures remove the protective, local immunity of the urinary tract, and increase the risk of infection. UTI is seen chiefly in association with diabetes mellitus type-2, catheterization, renal calculi, benign prostate hypertrophy, and immunocompromised patients [14]. UTIs are more common in diabetes patients with a high socioeconomic position [15]. Patients with diabetes have lower cytokine secretion in the urinary tract and hence more deficient leukocytes, which are the most essential first-line host defense. As per Table 1, we found that the most expected risk factor associated with UTI is diabetes 54/247 (22%). Other authors have reported different associations with diabetes such as Acharya *et al.* [16]

Table 1: Age-wise distribution of risk factors associated with UTI patients in the study

Risk factor	Age		Total
	≤40	>40	
DM	26 (10.52)	28 (11.33)	54 (21.9)
Catheterization	31 (11.74)	18 (7.29)	49 (19.03)
BPH	4 (1.62)	28 (11.34)	32 (12.96)
Recurrent UTI	24 (8.91)	9 (3.64)	33 (12.55)
Renal calculi	25 (9.72)	5 (2.02)	30 (11.74)
Immunocompromised	11 (4.05)	12 (4.45)	23 (8.50)
Pregnancy	20 (8.10)	--	20 (8.10)
No risk factor	6	0	6
Total	147	100	247

Table 2: Sex-wise distribution of risk factors associated with UTI patients in the study

Risk factor	Sex		Total
	Male	Female	
DM	31 (12.5)	23 (9.31)	54 (22)
Catheterization	22 (8.91)	27 (10.12)	49 (19.03)
BPH	32 (12.96)	--	32 (12.96)
Recurrent UTI	13 (5.26)	20 (7.29)	33 (12.55)
Renal calculi	20 (8.10)	10 (3.64)	30 (11.74)
Immunocompromised	11 (4.45)	12 (4.05)	23 (8.50)
Pregnancy	--	20 (8.10)	20 (8.10)
No risk factor	4	2	6
Total	133	114	247

Table 3: DDAT and PCDDT results (n=247)

Test	ESBL producer	Percentage
DDAT	32	13
PCDDT	37	15

Table 4: Comparison of all phenotypic tests for ESBL production

Test method for ESBL detection	ESBL positive by E-test (n=49)		ESBL negative by E-test (n=8)	
	Positive (TP)	Negative (FN)	Positive (FP)	Negative (TN)
PCDDT	35	14	2	6
DDAT	29	20	3	5

Table 5: Performance parameter of all phenotypic tests for ESBL production (in percentage)

TEST	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
PCDDT	71.4	75	94.5	12.2	72
DDAT	59.2	62.5	90.6	10.2	59.6
E-test	100	100	100	100	100

(34.5%) and Vata *et al.* [17] (17.7%). We also found that catheterized patients with age ≤40 years of age are more prone to UTI 31/247 (12%). Catheterization is also a well-known risk factor for UTI. The risk of UTI rises due to inexperienced sterilizing techniques used during catheter insertion or contamination of the catheter's collecting system. In this study, we found that a total of 49 (19%) catheterized patients developed UTIs similar to Mahesh *et al.* [18] (14.09%). Among catheterized patients, non-ESBL producers were higher in number as most of the samples recorded in our study were from OPD patients (153/247).

In our study, UTI was more common in senior males (above 40 years) (12%), due to prostatic hypertrophy. Other writers have also mentioned this risk factor, claiming that prostate disease in men is to blame for the rise in UTI cases. These results are similar with Kumar *et al.* [19] and Sujatha *et al.* [20]. While comparing risk factors with the production of ESBL, diabetes has been recorded as the highest number among ESBL producers (25%).

In the present study, E-test was taken as gold standard. Forty-nine strains were positive by E-test. Out of 49 E-test positive isolates, 37 were positive by PCDDT and 32 strains were positive by DDAT. PCDDT showed a sensitivity of 71.4% and specificity of 75% whereas DDAT showed sensitivity of 59.2% and specificity of 62.5%. Similarly, Singh and Singh [21] also reported PCDDT with the highest sensitivity among other six phenotypic methods for ESBL production. Several phenotypic methods are available for the detection of ESBL producing organisms. According to our study, E-test is the most accurate test for ESBL detection, but it is an expensive method. Hence, laboratories should adopt a cost-effective, economical, and precise detection procedure for ESBL detection. In the present study, PCDDT showed a higher sensitivity for ESBL detection. Hence, PCDDT can be adopted as a routine test for ESBL detection. Effective management of patients suffering from UTI caused by ESBL *E. coli* depends mainly on the diagnosis and selection of an effective antimicrobial agent for the organism.

CONCLUSION

Keeping in mind the challenging nature of these resistant isolates, analyzing these phenotypic methods were highly recommended. Our results suggest PCDDT to be the most sensitive test for ESBL detection. The primary risk factor among ESBL-positive organisms was found to be diabetes followed by BPH. Diagnosis of UTI with ESBL-producing organisms requires good cooperation between the clinician and the microbiologist and a proper laboratory testing protocol.

ACKNOWLEDGMENT

This work has been carried out in the Department of Microbiology, SMS Medical College, Jaipur, India. The authors would like to thank the staff and the HOD of the microbiology department for providing the necessary support and a research-oriented environment.

AUTHORS' CONTRIBUTIONS

Adya Chaturvedi – The writer, collected the data, and conceived and designed the analysis. Bhavana Gupta – Contributed to analysis tools. Ashutosh Chaturvedi – Helped in data collection. Rashmi Sisodiya – Helped in structuring and editing the paper. Rajni Sharma* – Structured and designed the analysis.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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