

Prevalence of extended-spectrum β -lactamases producing *Escherichia coli* in urinary specimens and their phenotypic detection by modified three-dimensional enzyme extract test: Comparison with the Phenotypic confirmatory disc diffusion test

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Abstract

Escherichia coli is the most common cause of UTI in community as well as in hospitalised patients. Detection of ESBLs producing organisms from samples such as urine may be important because this represents an epidemiologic marker of colonization, and therefore there is potential for transfer of such organisms to others.

Aims: The purpose of this study was to know prevalence of extended spectrum β -lactamases (ESBLs) in strains of *Escherichia coli* isolated from urine specimens and comparison of PCDDT and IMTDT of ESBL detection.

Settings and Design: Department of Microbiology, MGM Medical College, Indore. Cross sectional study.

Materials and Method: A total of 200 *Escherichia coli* strains isolated from 1500 consecutive non repeating urine specimens selected for the study. They studied for ESBL production phenotypic confirmatory disc diffusion test (PCDDT) and indirect modified three-dimensional enzyme extract test (IMTDT).

Statistical analysis used: Kappa statistics.

Results: 86% (171) of the isolates found to be resistant to atleast one of the 3rd generation cephalosporines tested (cefepodoxime, cefotaxime, ceftazidime and ceftriaxone). ESBL detected in 99 (58%) of the *Escherichia coli* by PCDDT and in 76% (130) by IMTDT.

Conclusions: Our study shows prevalence of ESBL producing *Escherichia coli* in urinary isolates. IMTDT found to be better than PCDDT for the detection of ESBL. The overall prevalence of ESBL producing uropathogenic *Escherichia coli* found to be 49.5% for our institute.

Keywords: ESBL, Three dimensional test, Uropathogenic *E. coli*, UTI

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections found in both indoor and outdoor patients.⁽¹⁾ *Escherichia coli* is the commonest cause of UTI in community as well as in hospitalised patients.⁽²⁾ Throughout the world the epidemiology of occurrence of the ESBL producers may vary profoundly with rapidly changing pattern over time. India has highest incidence (60%) of Urinary tract infections by ESBL producing *Escherichia coli*.⁽²⁾ Identification of all ESBLs producing organisms in clinical Microbiology laboratory is a major challenge. Some ESBL producers may appear susceptible to a third generation cephalosporins *in vitro*, due to the different affinity of these enzymes for different substrates and the inoculum effect. However, treatment of infections due to ESBLs producers, with third generation Cephalosporins may be ineffective in infections other than urinary tract.⁽³⁾

According to previous CLSI guidelines, isolates with positive phenotypic confirmatory test reported as resistant to all cephalosporins (except the Cephamycins) and aztreonam, irrespective of the MICs of that particular cephalosporin.⁽⁴⁾ When using the current interpretative criteria routine ESBL testing is not required before reporting results and it is no longer necessary to edit results for Cephalosporins, aztreonam,

or penicillin to resistant. ESBL testing is still useful for epidemiological or infection control purposes.⁽⁴⁾

Detection of ESBLs producing organisms from urine specimens may be important because it is an epidemiologic marker of colonization, and therefore there is a risk of infection to others. These infections significantly affect patient's morbidity, mortality and additional financial burden.⁽⁵⁾ The present study is aimed to know the prevalence of ESBL producing *Escherichia coli* in urinary isolates and to compare performance of phenotypic confirmatory disc diffusion test and indirect modified three dimensional enzyme extract test for ESBL detection.

Materials and Method

1500 consecutive non repeating urine specimens from patient's attending inpatient and outpatient Department of Maharaja Yashwant Rao Hospital (M.Y.H.), Indore, Madhya Pradesh with clinical symptoms of urinary tract infections received in the Department of Microbiology, MGM Medical College, Indore included in the study. Colony count of $\geq 10^5$ cfu/ml in semi quantitative urine culture was considered significant. The study period was of one year from July 2015 to June 2016. Study and data collection done after approval from the institute ethical committee.

The *Escherichia coli* strains identified on the basis of colony colour on HiCrome UTI Agar, colony

morphology, staining characters, motility and other relevant biochemical tests.⁽⁶⁾

Screening test for ESBLs: Screening of ESBLs producing isolates carried out by standard *disc* diffusion criteria using four *discs* of 3rd generation cephalosporin namely cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CTR) and cefpodoxime (CPD). Inhibition zone size of either of the four *discs*, ≥ 22 mm for ceftazidime (30 μ g), ≥ 25 mm for ceftriaxone (30 μ g), ≥ 27 mm for cefotaxime (30 μ g) and ≥ 17 mm for cefpodoxime (10 μ g) considered as probable ESBL production.⁽⁴⁾

Phenotypic confirmatory method

1. **Phenotypic confirmatory disc diffusion test (PCDDT):** In this test a *disc* of ceftazidime (30 μ g) and cefotaxime (30 μ g) alone, and in combination with clavulanic acid (30/10 μ g) used. A ≥ 5 mm difference in zone diameter for either antimicrobial agent tested alone and in combination with clavulanic acid considered as ESBL producer.⁽⁴⁾

2. **Indirect modified three dimensional enzyme extract test (IMTDT):** MHA plates inoculated with strains of *Escherichia coli* ATCC 25922 adjusted to 0.5 McFarland standard. A disc of cefotaxime placed on the plate and a well of 4 mm diameter punched at a distance of 2 mm from the antibiotic disc. Approximately 15 mg of test strain scraped from the culture plate using a sterile wire loop and suspended in 0.5 ml of peptone water in a sterile microcentrifuge tube and incubated at 37 $^{\circ}$ C for 1 h. Then Crude enzyme extract from test bacterial strains prepared by repeated freeze–thawing.⁽⁷⁾ 30 μ l of crude enzyme extract filled in the well. Plates then incubated at 37 $^{\circ}$ C for 24 hours. Heart shaped distortion of zone of inhibition appearing behind the well and reaching the *disc* indicative of a positive test.

E. coli ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 used as a negative control and positive control respectively.

Results

We have isolated 200 strains of *Escherichia coli* from a total of 1500 urine specimens. (Table 1)

Table 1: Culture results of urine specimens

Specimens Isolates	IPD (%), n=856	OPD (%), n=644	Total (%), n=1500
Sterile/ insignificant growth/mixed growth	640 (75%)	520 (81%)	1160 (77.3%)
<i>E. coli</i>	106 (12%)	94 (14.5%)	200 (13.3%)
Others	110 (13%)	30 (4.5%)	140 (9.3%)

In all urinary isolates, *Escherichia coli* from IPD patients were 106 (49.07%) and from OPD patients were 94 (75.8%).

A total of 171 (86%) *Escherichia coli* strains were found screening positive for ESBL and results of their confirmation by PCDDT and IMTDT are given in Table 2.

Table 2: Testing for ESBLs production

No. of <i>E. coli</i>	Screening (%)	PCDDT(%)	IMTDT(%)
IPD patient, n=106	99 (93%)	46 (46%)	75 (76%)
OPD patient, n=94	72 (77%)	53 (74%)	55 (76%)
Total, n=200	171 (86%)	99 (58%)	130 (76%)

Discussion

Now a day antibiotics are being used extensively and newer antibiotics are continuously being added for the treatment of various infections. An extensive use of β -lactam antibiotics in hospital and community have created a major problem leading to increased morbidity, mortality and health care cost. Proper use of antibiotics is very important for various reasons. Emergence and re-emergence of antimicrobial resistance may leads to therapeutic failure especially of empirical therapy. With the emergence of ESBL strains the problem is substantially enhanced throughout the world.

We found that *Escherichia coli* in urinary isolates from inpatients were 49.07%, which is within the range found in other studies^(16,17,19) and from outpatients were 75.8%, which is also within the range found in different studies.⁽¹⁷⁻¹⁹⁾ In our study *Escherichia coli* causing urinary tract infections found to be the most common pathogen isolated from inpatients and outpatients departments.

In our study 49.5% *Escherichia coli* found to be ESBL producers by PCDDT which is near to that of Tankhiwale *et al.* (48.3%),⁽²⁰⁾ Aruna and Mobashshera *et al.* (49.32%),⁽²¹⁾ Gururajan *et al.* Chennai (47%)⁽²²⁾ and is higher than that found in the studies of Padmini S B *et al.* (41%),⁽²³⁾ Kumar *et al.* (39%),⁽²⁴⁾ Taneja *et al.* (40.2%),⁽²⁵⁾ Bajpai T *et al.* (41.6%),⁽²⁶⁾ Metri Basavaraj C *et al.* (32%),⁽²⁷⁾ Babypadmini *et al.* (41%),⁽²⁸⁾ Khurana *et al.* (26.6%)⁽²⁹⁾ and is lower than that found in the studies of Ananthkrishnan AN *et al.* (58.06%),⁽³⁰⁾ Singhal *et al.* (62%),⁽³¹⁾ Sharma S *et al.* (51%),⁽³²⁾ B. Sasirekha *et al.* (71.7%),⁽³³⁾ Kesavaram *et al.* (59.1%).⁽³⁴⁾

The prevalence of ESBL producing *Escherichia coli* among indoor patients found in our study was near to that of Pourakbari and coworkers (42%)⁽³⁵⁾ and less than that of Singh S. and Kumar *et al.* (55.55%)⁽³⁶⁾ and Ravinder Pal *et al.* (76%).⁽³⁷⁾

The prevalence of ESBL producing *Escherichia coli* among outdoor patients found in our study was more than that found in Pourakbari and coworkers

(32%)⁽³⁵⁾ and Singh S. and Kumar *et al.* (44.44%)⁽³⁶⁾ and is less than that found in Ravinder Pal *et al.* (63%)⁽³⁷⁾

Though PCDDT is easy to perform and interpret, it can be useful in the those isolates which produce only ESBL but are not useful when AmpC enzyme also present along with ESBL.⁽¹²⁾ In our study prevalence of ESBL producing *Escherichia coli* confirmed by PCDDT was more in OPD patients than IPD patients. This lower detection rate of ESBL producing *Escherichia coli* in IPD patients probably due to presence of co-carriage of ESBLs, Amp C and New Delhi metallo-beta-lactamase-1 genes in IPD isolates of *E. coli*. In a study, the majority of the (42.9%) *Escherichia coli* isolates carried two or more of the beta-lactamase genes.⁽¹⁵⁾

Sensitivity and specificity of phenotypic confirmatory tests are more than the genotypic confirmatory tests. However, phenotypic confirmatory tests may be falsely positive or negative. Organisms that lack ESBLs may give rise to false-positive confirmatory tests due to hyper production of SHV-1.⁽¹⁰⁾

Previous reports clearly suggested that the prevalence of infection by ESBL producing organisms vary greatly geographically and rapidly changing over time and which is probably due to the risk factors variation and extent of antibiotic use.

Sensitivity of IMTDT reported is 98% to 100%. Thus it is found to be most sensitive test among other available tests such as PCDDT, double disc synergy test and modified direct three dimensional test.⁽¹²⁻¹⁴⁾ In our study 76% strains gave positive result with IMTDT which is lower than that found in a study done in Chennai by T Menon *et al* at in 2003 (87.5%)⁽¹³⁾ and in study of Dhara Modi *et al.* 2012 (100%).⁽¹²⁾ In the present study, IMTDT found to be better than PCDDT in the detection of ESBLs. Dhara Modi *et al.* 2012⁽¹²⁾ also reported IMDTD superior to PCDDT. This test determined to be very sensitive in detecting ESBLs, but it is more technically challenging and labour intensive than other methods.

While each of these tests has its merit, none of these methods can accurately detect all strains producing ESBLs. In present study on the basis of kappa value (-0.058), statistically we can say that there is no agreement between PCDDT and IMTDT for the detection of ESBL.

Conclusion

Considering various findings of the present study, it can be concluded that Extended spectrum β lactamases are gradually increasing in India with co-resistance to some other classes of antibiotics is very alarming. We found detection rate of 58% of ESBL by PCDDT and 76% by IMTDT. IMTDT found to be better than PCDDT for the detection of ESBL. The

overall prevalence of ESBL producing uropathogenic *Escherichia coli* found 49.5% for our institute.

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