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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 (cefpodoxime, 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 *disc*s of 3rd generation cephalosporin namely cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CTR) and cefpodoxime (CPD). Inhibition zone size of either of the four *disc*s, ≥22 mm for ceftazidime (30µg), ≥25 mm for ceftriaxone (30µg), ≥ 27 mm for cefotaxime (30µg) and ≥ 17 mm for cefpodoxime (10 µg) considered as probable ESBL production.[4]

Phenotypic confirmatory method

1. **Phenotypic confirmatory disc diffusion test (PCDDT):** In this test a *disc* of ceftazidime (30µg) and cefotaxime (30µg) alone, and in combination with clavulanic acid (30/10µg) 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 37^0C for 1 h. Then Crude enzyme extract from test bacterial strains prepared by repeated freeze–thawing.[7] 30µl of crude enzyme extract filled in the well. Plates then incubated at 37ºC 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 respectably.

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%)
E. coli	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 *E. coli*	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 Ananthakrishnan 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.

References

1. Schappert SM. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 1997. Vital Health Stat 13 1999; 143:i-iv, 1–39.
2. Hsueh PR, Hoban DJ, Carmeli Y, *et al*. Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region. J. Infect. 2011 Aug;63(2):114-23.
3. Nathisuwan S, Burgess DS, Lewis II JS. ESBLs: Epidemiology, Detection and Treatment. Pharmacotherapy 2001;21(8):920-928.
4. Performance Standards for Antimicrobial Susceptibility Testing, Twenty fourth Informational Supplement, CLSI Document M100-S24, Wayne, PA: Clinical and Laboratory Standards Institute, 2014.
5. Osterblad *et al*. (2000).A between species comparison of antimicrobial resistance in enterobacteria in fecal flora. J antimicrob.Chemother;44:1479-1484.
6. Chessbrough, M 2006, District laboratory practice in tropical countries, Part-2, New York, USA: Cambridge university. pp. 105-115.
7. M. Shahid, Abida Malik, Mithlesh Agrawal and Sanjay Singhal. Phenotypic detection of extended-spectrum and AmpC β-lactamases by a new spot-inoculation method and modified three-dimensional extract test: comparison with the conventional three-dimensional extract test. Journal of Antimicrobial Chemotherapy (2004) 54, 684–687.
8. Emery CL, Weymouth LA. Detection and clinical significance of ESbLs in a tertiary – care medical center. *J Clin Microbiol* 1997;35:2061-2067.
9. Nathisuwan S, Burgess DS, Lewis II JS. ESBLs: Epidemiology, Detection and Treatment. Pharmacotherapy 2001;21(8):920-928.
10. David L. Paterson and Robert A. Bonomo. Extended-Spectrum beta-Lactamases: a Clinical Update. Clinical microbiology reviews, Oct. 2005, p. 657–686.
11. Wiegand I, Geiss HK, Mack D, Sturenburg E, Seifert H. Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. J Clin Microbiol 2007;45:1167–1174.
12. Modi D, Patel D, Patel S, Jain M, Bhatt S, Vegad M. Comparison of various methods for the detection of extended spectrum beta lactamase in klebsiella pneumoniae isolated from neonatal intensive care unit, Ahmedabad. National journal of medical research 2012;2(3):348-53.
13. T Menon, D Bindu, CPG Kumar, S Nalini, MA Thirunarayan. Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. April 2006 Indian Journal of Medical Microbiology, (2006) 24(2):117-120.
14. Datta P, Thakur A, Mishra B, Gupta V. Prevalence of clinical strains resistant to various β-lactams in a tertiary care hospital in India. *Jpn J Infect Dis* 2004;57:146-9.
15. Manoharan A, Barla G S, Peter R, Sugumar M, Mathai D. Multidrug resistance mediated by co-carriage of extended-spectrum beta-lactamases, AmpC and New Delhi metallo-beta-lactamase-1 genes among carbapenem-resistant *Enterobacteriaceae* at five Indian medical centres. Indian J Med Microbiol 2016;34:359-61.

16. Weber G, Riesenberg K, Schlaeffer F, Peled N, Borer A, Yagupsky P. Changing trends in frequency and antimicrobial resistance of urinary pathogens in outpatient clinics and a hospital in southern Israel, 1991–1995. Eur J Clin Microbiol Infect Dis 1997;16:834–8.
17. Grude N, Tveten Y, Kristiansen B-E. Urinary tract infections in Norway: bacterial etiology and susceptibility. A retrospective study of clinical isolates. Clin Microbiol Infect 2001;7:543–7.
18. Gupta K, Sahm DF *et al*. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. Clin Infect Dis 2001;33:89–94.
19. Bouza E, San Juan R, Munoz P, Voss A, Kluytmans J. A European perspective on nosocomial urinary tract infections I. Report on the microbiology workload, etiology and antimicrobial susceptibility (ESGNI- 003 study). Clin Microbiol Infect 2001;7:523–31.
20. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res 2004;120:553-6.
21. Aruna K, Mobashshera T. Prevalence of extended spectrum betalactamase production among uropathogens in South Mumbai and its antibiogram patterns. EXCLI J 2012;11:363-72.
22. Gururajan G, Kathireshan A, Kaliyaperumal, Balagurunathan R. Prevalence of extended spectrum beta lactamases in uro-pathogenic Escherichia coli and Klebsiella species in a Chennai suburban tertiary care hospital and its antibiogram pattern. Res J Microbiol 2011;6:796-804.
23. Padmini S B, Raju B A, Mani K R. Detection of Enterobacteriaceae producing CTX-M extended spectrum β-lactamases from a tertiary care hospital in south India. Indian J Med Microbiol 2008;26:163-6.
24. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian J Med Microbiol 2006;24:208-11.
25. Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL & Amp-C betalactamases & susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res 2008;127:85-8.
26. Bajpai T, Pandey M, *et al*. Prevalence of extended spectrum beta-lactamase producing uropathogens and their antibiotic resistance profile in patients visiting a tertiary care hospital in central India: Implications on empiric therapy. Indian J Pathol Microbiol 2014;57:407-12.
27. Metri Basavaraj C., Jyothi P., Peerapur Basavaraj V. The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India. Journal of Clinical and Diagnostic Research. 2011 June, Vol-5(3):470-475.
28. Babypadmini S, Appalaraju B. Extended-spectrum β-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol 2004;22:172-4.
29. Khurana S, Taneja N, Sharma M. Extended spectrum beta-lactamase mediated resistance in urinary tract isolates of family Enterobacteriaceae. Indian J Med Res 2002;116:145-9.
30. Ananthakrishnan AN, Kanungo R, Kumar A, Badrinath S. Detection of extended spectrum β-lactamase producers among surgical wound infections and burn patients in JIPMER. Indian J Med Microbiol 2004;18:160-5.
31. Singhal S, Mathur T, Khan S, Upadhyay DJ, *et al*. Evaluation of methods for AmpC beta-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 2005;23:120-4.
32. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in Escherichia coli isolated from extraintestinal infections. Indian J Med Microbiol. 2007 Oct;25(4):369-73.
33. B. Sasirekha, R. Manasa, P. Ramya and R. Sneha. Frequency and Antimicrobial Sensitivity Pattern of Extended Spectrum β-Lactamases Producing *E. Coli* And Klebsiella Pneumoniae Isolated In A Tertiary Care Hospital. Al Ameen J Med Sci (2010) 3(4):265-271.
34. Kesavaram P, Krishnan P, Sikhamani R. Detection of Extended-spectrum beta lactamases and AmpC beta-lactamase production in Escherichia coli causing urinary tract infection among HIV and non-HIV patients. Am Med J 2011;2:54-8.
35. Pourakbari B, Ferdosian F and Mahmoudi *et al*., (2012). Increase resistant rates and ESBL production between *E. coli* isolates causing urinary tract infection in young patients from Iran. Brazilian Journal of Microbiology 43(2):766-769.
36. Singh S., Kumar S., Sandhu R., *et al*. Prevalence of drug resistance in ESBL producing Escherichia coli causing UTI in rural tertiary care hospital from Haryana, India. International Journal of Basic and Applied Medical Sciences 2015 Vol. 5(3) September-December, pp. 1-7.
37. Ravinder Pal Singh, Sonali Jain, *et al*. Comparative study of ESBL producing Escherichia coli in OPD and IPD patients of urinary tract infections. Int. J. Curr. Microbiol. App. Sci (2013) 2(12):45-50.

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