Extended Spectrum Beta - Lactamase Producing Escherichia coli at a Tertiary Care Hospital in Maharashtra, India: Phenotypic Detection and Antimicrobial Sensitivity Pattern

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Original Article

Abstract: Extended spectrum beta lactamases (ESBLs) are rapidly evolving plasmid mediated; TEM and SHV derived enzymes, capable of hydrolyzing oxyimino-cephalosporins and monobactams. Bacteria producing ESBLs remain an important cause for failure of therapy with cephalosporins and other antibiotics. ESBL testing is useful for epidemiological or infection control purposes.**Aims:** The present study was conducted to detect ESBLs in strains of *Escherichia coli* isolated from various clinical samples in a tertiary care hospital. **Material and Methods:** A total of 272 non enteric randomly chosen non repetitive *E.coli* isolates obtained over the period of one year from both outpatient and hospitalized patients were studied. Out of 272 isolates, 191 (70.22%) were screened as ESBL producing. They were further studied for ESBL production by phenotypic confirmatory disc diffusion test (PCDDT). **Results:** It was observed that not all screen positive isolates were confirmed as ESBL producers. Of the total 191 ESBL positive isolates, the PCDDT method detected 168 (87.95%) cases. Overall prevalence of ESBL in *E.coli* was found to be 61.76%. Only 4 strains (1.47%) were found resistant to imipenem and 11 strains (4.04%) were found resistant to meropenem. **Conclusions:** The present study shows that any of the three screening agents can be used to detect potential ESBL producers. The routine antibiotic sensitivity test may fail to detect ESBL mediated resistance. Therefore, screening for detection of ESBL and confirmation of the same should be carried out by PCDDT method as it is simple, reproducible, cost effective and sensitive method. ESBL detection studies help to formulate an empirical antibiotic policy to treat Gram negative infections in respective hospitals.

Key-words: *Escherichia coli*, ESBLs, oxyimino-cephalosporins, phenotypic confirmatory disc diffusion test

Introduction: *Escherichia coli*, the most significant species in the genus *Escherichia*, is recognized as an important potential human pathogen. In early 1960s, Temoniera (TEM-1) was the first plasmid mediated β lactamase described in a single strain of *E.coli*.[1] Extended spectrum β-lactamases (ESBLs) are plasmid mediated enzymes that efficiently hydrolyze oxyimino-cephalosporins conferring resistance to most of the β lactams, including third generation cephalosporins (3GC), penicillins and aztreonam. These are mutant forms of TEM-1, TEM-2 and Sulfhydril Variable (SHV-I) enzymes. The ability of these enzymes to spread to other bacteria through the plasmids has led to the dramatic increase in their prevalence in a very short span of time. The persistence and numerous outbreaks of infection with organisms producing ESBLs have been observed worldwide over the last 20 years with high prevalence in *E.coli* particularly in Indian subcontinent.[2, 3]

Organisms producing ESBLs remain an important cause for failure of therapy with cephalosporins and other classes of antibiotics. Failure to identify ESBL-producing organisms also contributes to their unnoticed spread. Therefore, their detection and reporting is an important responsibility of clinical Microbiologist, particularly in developing countries, where there is excessive use of antibiotics and lack of adequate antimicrobial resistance surveillance has been seen.[4]

The present study was carried out to detect ESBL producing *E.coli* from various

clinical isolates and to study their antimicrobial resistance.

Material and Methods:

A total of 272 non enteric randomly chosen non repetitive *E. coli* isolates obtained from cultures of various specimens such as urine (131), pus (118) and others (23) like blood (2), sputum (1), endotracheal aspirate (9), bronchoalveolar lavage (4) and ascitic/pleural/synovial fluid (7) were studied for ESBL production. The samples were obtained from both outpatients and inpatients between March 2010 and April 2011 and processed for isolation by standard methods.[5] The isolates were identified based on colony characters on Blood agar and MacConkey agar, by standard biochemical tests.[6] The antimicrobial susceptibility of the isolates was performed by standard Kirby-Bauer disc diffusion technique with commercially available discs (HiMedia, Mumbai, India) on Mueller Hinton agar (MHA) plates. The discs used were ciprofloxacin (5 µg), ofloxacin (5 µg), gatifloxacin (5 µg), levofloxacin (5µg), gentamicin (10 µg), amikacin (30 µg), cotrimoxazole (1.25/ 23.75 µg), tetracycline (30 µg), imipenem (10 µg), meropenem (10 µg), netilmicin (30 µg), piperacillin+tazobactam combination (100 µg+10 µg) and for urine samples norfloxacin (10 µg), nitrofurantoin (300 µg). For sensitivity to 3GC ceftazidime, ceftriaxone and cefotaxime each 30 µg disc was used.

Table 1: Distribution pattern of ESBL producing E.coli isolated from various sites

Specimen	Total ESBLs	
	n	%
Urine (131)	81	61.83
Pus (118)	72	61.01
Others (23)	14	60.86
Total (272)	168	61.76

$p<0.0001$

Each strain was screened for possible ESBL production by Clinical Laboratory Standards Institute (CLSI) guidelines. 3GC were used as screening agents. All the positive strains by screening method were further subjected to confirmation by Phenotypic confirmatory disc diffusion test (PCDDT) recommended by CLSI.[7]

Phenotypic Confirmatory Disc Diffusion Test (PCDDT):

In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was swabbed onto MHA plate using sterile cotton swab. The ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/ 10 µg) were placed at a distance of 20 mm apart on the agar. Similarly, cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) were placed 30 mm apart. After incubating overnight at 37° C, a ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone was inferred as positive test and organism considered ESBL producer (Figure 1).

Klebsiella pneumoniae ATCC strain 700603 and *E. coli* ATCC strain 25922 were used as positive and negative controls respectively.

Chi-square test was used to analyze the data.

Table 2: Distribution of ESBL producers and non-producer in outpatients and Inpatients

Distribution	ESBL producers		Non-producers		Total	
	n	%	n	%	n	%
Inpatient	102	60.72	28	26.92	130	47.79
Outpatient	66	39.28	76	73.08	142	52.21
Total	168	100	104	100	272	100

Results:

In the present study, 272 isolates of *E.coli* were studied out of which 191 (70.22%) isolates were presumptively considered ESBL producers on the basis of their resistance to the three screening agents. All ESBL producers were uniformly resistant to the screening

agents used, indicating any of the three screening agent can be used to look for potential ESBL producers. ESBL was seen mostly in urine samples. Chi-square test was used to analyze the data (Table 1). Confirmation of ESBLs was done on these 191 isolates by PCDDT method. Not all screen positive isolates were confirmed as ESBL producers. Of the total 191 screen-positive isolates, PCDDT method detected 168 (87.95%) cases. Overall prevalence of ESBL production in *E.coli* was found to be 61.76%. Hospitalized patients (60.72%) showed more ESBL production in comparison to outpatients (39.28%) (Table2).

Table 3: Antimicrobial susceptibility pattern of E.coli (n=272) isolates

Antimicrobial agent	Sensitive n (%)	Resistant n (%)
Amikacin (30 µg)	173 (63.60)	99 (36.39)
Gentamicin (10 µg)	122 (44.85)	150 (55.14)
Ciprofloxacin (5 µg)	48 (17.64)	224 (82.35)
Ofloxacin (5 µg)	45(16.54)	227 (83.45)
Gatifloxacin (5 µg)	112 (41.17)	160 (58.82)
Levofloxacin (5 µg)	78 (28.67)	194 (71.32)
Co-trimoxazole (1.25 µg /23.75 µg)	148 (54.41)	124 (45.58)
Tetracycline* (30 µg)	28 (19.71)	114 (80.28)
Meropenem (10 µg)	261 (95.95)	11 (4.04)
Netilmicin (30 µg)	150 (55.14)	122 (44.85)
Piperacillin-tazobactam (100 µg +10 µg)	235 (86.39)	37 (13.60)
Imipenem (10 µg)	268 (98.52)	04 (1.47)
Nitrofurantoin† (300 µg)	88 (67.17)	43 (32.82)
Norfloxacin† (10 µg)	28 (21.37)	103 (78.62)
Ceftazidime (30 µg)	101 (37.13)	171 (62.86)
Ceftriaxone (30 µg)	95 (34.92)	177(65.07)
Cefotaxime (30 µg)	98 (36.02)	174 (63.97)

*Antimicrobial not tested against urinary isolates.
†Antimicrobials tested against urinary isolates only.

Most of the strains were sensitive to carbapenems (>95%) followed by piperacillin-tazobactam (86.39%) and amikacin (63.60%). Majority of the strains were resistant to fluroquinolones (FQs) like ofloxacin 227 (83.45%) and ciprofloxacin 224 (82.35%). Nitrofurantoin (67.17%) was found to be more effective than norfloxacin (21.37%) for urinary isolates. Imipenem showed resistance in 4 (1.47%) isolates of E.coli and meropenem showed resistance in 11 (4.04%) isolates. All these strains were from hospitalized patients (Table 3).

Discussion:

Infections due to ESBL producers range from uncomplicated urinary tract infections (UTIs) to life threatening sepsis. Risk factors for colonization or infection with ESBLs include older age, history of hospitalization, treatment with cephalosporins, penicillins and quinolones. In the recent years, several new ESBLs of the non-TEM and the non-SHV types emerged, such as the enzymes of the CTX-M, PER, VEB and the GES lineages.[8] Enterobacteriaceae, mostly *E.coli* producing the CTX-M enzymes have been identified predominantly from the community acquired UTIs. Various reports suggest that the CTX-M-type ESBLs may now actually be the most frequent ESBL type worldwide.[8]

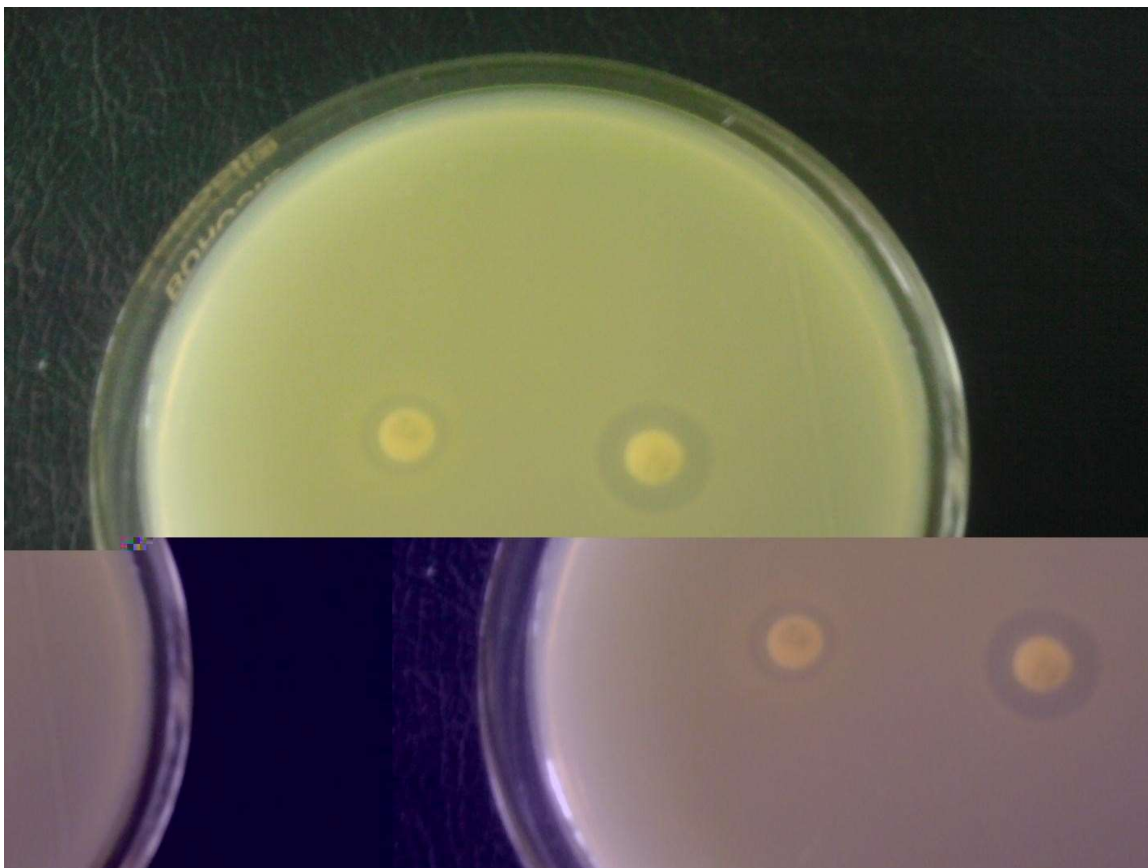

Figure 1: Phenotypic Confirmatory Disc Diffusion Test (PCDDT) Proposed by CLSI: ESBL production confirmed by an increase in zone diameter of ≥5mm for ceftazidime (CA) and ceftazidime – clavulanic acid (CAC).

The prevalence of ESBLs in *E.coli* among clinical isolates varies from place to place and rapidly changes over a period of time. There have been varied prevalence reports of ESBLs from Indian hospitals, ranging from 31.7%-81%[9-22] (Table 4). In the present study, ESBL mediated resistance was seen in 61.76% strains of *E.coli*. These reports are in accordance with the recent studies from Giriyapur *et al*[13] who have reported prevalence of ESBLs in E.coli to be 62.19% but not in agreement with Umadevi *et al*[16] who have reported higher ESBL producers (81%) in their study, however Manohar *et al* and Basavaraj *et al* have reported less prevalence of 46.28% and 31.7% respectively in *E.coli* strains in recent years which could be due to the different antibiotic policies implemented in hospitals.[15-16]

Though fluroquinolones (FQs) can be used for the treatment of uncomplicated UTIs, when found susceptible, the increasing *in-vitro* resistance as seen in our study will limit the role of these drugs in the future.

In earlier studies, there were reports of less and less sensitivity to co-trimoxazole

drug.[17-18] This drug has become nearly obsolete from the hospitals. But the recent year studies reported better sensitivity to the co-trimoxazole drug. Shobha *et al*[19] have reported the maximum sensitivity of 75%. Agrawal *et al*[20] and Shiju *et al*[21] have also reported sensitivity of 68.35% and 49% respectively to co-trimoxazole. The present study also showed the sensitivity of 54.41% to this drug. The upcoming better sensitivity reports indicate the “re-emergence” of co-trimoxazole to treat most of the Gram negative infections. This reversal could be due to the non-use of this drug for many years in hospitals and this inexpensive drug might have better future in recent times.

Combination of piperacillin-tazobactam and aminoglycosides showed the highest activity after carbapenems. The carbapenems are known to be stable against ESBL enzymes and effective in the treatment of infections caused by ESBL-producing bacteria.

Table 4: Comparative studies across India

Authors	Year published	*E.coli* (%)
Jain, *et al.*[8]	2003	63.6
Babypadmini, *et al*[9]	2004	41
Singhal, *et al*[10]	2005	62
Kumar, *et al*[3]	2006	63.7
Rao, *et al*[11]	2007	62.9
Sinha, *et al*[12]	2008	64.80
Aggarwal, *et al*[17]	2009	40
Giriyapur, *et al*[13]	2011	62.19
Umadevi *et al*[14]	2011	81.00
Manoharan, *et al*[15]	2011	46.28
Basavaraj *et al*[16]	2011	31.7
Present study	**2011**	**61.76**

Many researchers reported no resistance to carbapenem group of antibiotics. But in the present study, 1.47% and 4.04% strains were found to be resistant to imipenem and meropenem respectively. Gupta *et al*[22] have also reported 2.1% and 3.5% resistance to imipenem and meropenem respectively. Basavaraj *et al*[16] have also reported 8% resistance to carbapenems. These reports indicate the usage of carbapenem antibiotics in the wake of increasing resistance rates to β-lactam and non-β-lactam antibiotics. This carbapenems resistance could be because of the conjugational transfer of R-plasmids bearing the bla_{IMP} gene.[23] Emerging resistance to carbapenems indicates the need to emphasize on the rational use of antimicrobials and these drugs should be considered as “reserve drugs”.

These results of present study indicate that the routine susceptibility testing done by Clinical Microbiology Laboratories may fail to detect ESBL positive strains and can sometimes erroneously detect isolates to be sensitive to any of the 3GC leading to therapeutic failures. Therefore, the regular detection of ESBL by PCDDT method should be carried out by adding just two more drugs to the routine panel of antimicrobial drugs. Every laboratory should perform this technically simple PCDDT method, where molecular methods can not be performed, as this method is highly sensitive and specific compared to genotypic methods.[24]

In conclusion, to detect ESBL isolates, besides PCDDT, many other methods like E Tests, Vitek system, Double Disc Synergy Test (DDST) and Microscan panels are commercially available. But these are expensive and can not be used routinely. Whereas, PCDDT method is reproducible, technically easy, cost effective and sensitive,

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thus useful in busy diagnostic Microbiology laboratories.

It is further concluded that, such institutional studies help to formulate an empirical antibiotic policy to treat Gram negative infections in a hospital. The knowledge of the resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use, as well.

Acknowledgment:

The authors are thankful to Dr. Nagoba BS. Assistant Dean (R & D), MIMSR Medical College, Latur. India for guidance and support.

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