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Research Article

# STUDY ON PHENOTYPIC DETECTION OF ESBL IN GRAM NEGATIVE BACTERIAL ISOLATES IN A TERTIARY CARE HOSPITAL IN BANGALORE

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**Abstract- Background:** Extended spectrum β-lactamase (ESBL) producing gram negative organisms particularly the multi-drug resistant strains have become a major global health problem. **Aims:** The present study was aimed at determining the prevalence of extended spectrum β-lactamase (ESBLs) production in gram negative bacterial isolates obtained from various clinical isolates and to detect their antibiotic sensitivity pattern. **Material and Methods:** A total of 173 gram negative isolates obtained from various clinical specimens were processed by conventional methods and the ESBL detection was done by phenotypic confirmatory disc diffusion test along with the routine susceptibility testing recommended by CLSI. **Results:** 82 (47.4%) among the 173 gram negative isolates were ESBL producers. The most common ESBL producing organism was *E.coli* (53.08%). The isolates from pus (59.61%) showed the maximum ESBL production. Carbepenems were found to the most effective drug against ESBL producers. **Conclusion:** The high rate of ESBL production along with high degree of antibiotic co-resistance among the ESBL strains in our study emphasizes on the need for routine surveillance of ESBL among gram negative isolates using the phenotypic confirmatory test which can help the clinicians in early and effective disease management.

**Keywords-** Extended spectrum β-lactamase (ESBL), Gram negative bacteria, Phenotypic confirmatory test, Cephalosporins

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## Introduction

ESBLs are plasmid mediated enzymes that are capable of conferring resistance to penicillins, first, second and third generation cephalosporins and aztreonam (but not cephamycins and carbapenems) and render them ineffective. ESBL's are transmissible β-lactamases which are inhibited by clavulanic acid, tazobactum and sulbactum. They are derived from point mutation occurring in the genes encoding TEM and SHV enzymes. Other newly emerged β-lactamase enzymes are CTX-M, PER, VEB and GES [1,2]. So far about 400 different types of ESBL's have been recognized around the world [3]. They belong to Ambler molecular class A and Bush–Jacoby functional group 2be [4]. They have been detected in Enterobacteriaceae such as *E.coli*, *Klebsiella* spp, *Citrobacter* spp, *Enterobacter* spp, *Proteus* spp and non-lactose fermenters like *Pseudomonas aeruginosa*. The routine antibiotic susceptibility testing in many labs fail to detect the ESBL producers as they might exhibit false sensitive zone to any of the third generation cephalosporin like cefotaxime, ceftazidime, ceftriaxone [5]. ESBL producing organisms may also carry co- resistance genes for other non - β-lactam antibiotics such as aminoglycosides, fluroquinolones, trimethoprim- sulfomethoxazole, and tetracycline, leading to treatment failure. Major risk factors for colonization or infection with ESBL producing organisms are prolonged antibiotic exposure, prolonged ICU stay, nursing home residency, debilitating illness, unprecedented use of third generation cephalosporin and increased use of intravenous devices or catheters [6]. Thus treatment of ESBL producing gram negative bacilli has emerged as a major challenge worldwide and moreover a thorough knowledge of the resistance pattern of ESBL producing organisms is needed for appropriate treatment of these patients. Thus, the present study was conducted with the objective of estimating the prevalence of ESBL producing gram negative isolates from various clinical samples and to determine their antibiotic susceptibility pattern.

## Material and Methods

The prospective study was conducted over a period of 6 months (August 2017 to January 2018) at a tertiary care hospital in Bangalore. Samples were obtained from both out- patients and in- patients. The samples were processed and the isolates were identified by standard laboratory methods [7]. A total of 173 no repetitive gram negative isolates were obtained from various clinical samples such as urine, pus, sputum, tracheal aspirates and blood. The antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. Presence of ESBL was confirmed by the phenotypic confirmatory disc diffusion test recommended by CLSI. [8] Antibiotic discs were procured from HiMedia, Mumbai. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 (HiMedia Laboratories, Mumbai) were used as positive and negative controls.

### Phenotypic confirmatory disc diffusion test

Presence of ESBL among the isolates was detected by using both ceftazidime/ceftazidime-clavulanic acid (CAZ/CAC) (30/10 µg) and cefotaxime/ cefotaxime-clavulanic acid (CTX/CEC) (30/10 µg) disks. An increase in zone diameter by ≥5 mm in the presence of clavulanic acid than cephalosporin alone was interpreted as positive [8] [Fig-1].

## Results

Among the 173 gram negative isolates, 81(46.82%) were *E.coli*, 54 (31.21%)

*K.pneumoniae*, 21 (12.14%) *Pseudomonas* spp, 14 (8.1%) *Proteus* spp, and 3 (1.73%) were *Citrobacter* spp. ESBL production was confirmed in 82 (47.4%) of the isolates. *E.coli* (53.08%) was the most common ESBL producing organism followed by *K.pneumoniae* (50%), *Pseudomonas* (33.33%) and *Citrobacter* spp (33.33%) [Table-1]. ESBL production was more common among isolates obtained from pus 31(59.61%) followed by urine 43 (51.8%) [Table-2]. The ESBL detection rate was more in inpatients 54(46.55%) compared to outpatients 18(31.57%) [Table-3].

**Table-1** *Distribution of ESBL producers among different gram negative isolates:*

| Organism | Total | ESBL confirm positive (%) |
|---|---|---|
| *E.coli* | 81 | 43 (53.08%) |
| *K.pneumoniae* | 54 | 27 (50%) |
| *Pseudomonas spp.* | 21 | 7 (33.33%) |
| *Proteus spp* | 14 | 4 (28.57%) |
| *Citrobacterspp* | 3 | 1 (33.33%) |
| Total | 173 | 82 (47.4%) |

97% of the isolates showed resistance to at least one of the third generation cephalosporin (ceftazidime, cefotaxime, ceftriaxone) and 83% showed resistance to all the three third generation cephalosporin. All the ESBLisolates showed 100% sensitivity to imipenem, meropenem and Piperacillin-tazobactum while they were highly resistant to amoxicillin–clavulanic acid, ciprofloxacin, trimethoprim–sulfamethoxazole and gentamicin. Nitrofurantoin remained the most effective drug among the ESBL urinary isolates [Table-4].

**Table-2** *Sample wise distribution of ESBL producers:*

| Sample | No. of isolates | ESBL positive |
|---|---|---|
| Urine | 83 | 43 (51.8%) |
| Pus | 52 | 31 (59.61%) |
| Sputum | 28 | 6 (21.43%) |
| Tracheal aspirates | 6 | 1 (50%) |
| Blood | 4 | 1 (25%) |
| Total | 173 | 82 (47.4%) |

**Table-3** *Distribution of ESBL producers among In-patients and Out-patients:*

| | In-patients | Out-patients |
|---|---|---|
| ESBL positive | 54(46.55%) | 18(31.57%) |
| ESBL negative | 62 (53.44%) | 39 (68.42%) |
| Total | 116 | 57 |

**Table-4** *Antibiotic resistance pattern of ESBL positive isolates:*

| S. no. | Antibiotics | Percentage of resistance (%) | | | | |
|---|---|---|---|---|---|---|
| | | *E.coli* | *K.pneumoniae* | *Pseudomonas* spp. | *Proteus* spp. | *Citrobacter* spp. |
| 1. | Amikacin | 39 | 43 | 71 | 46 | 37 |
| 2. | Gentamicin | 63 | 77 | 67 | 55 | 62 |
| 3. | Amoxycillin-clavulanic acid | 91 | 94 | - | 88 | 89 |
| 4. | Piperacillin-tazobactum | 0 | 0 | 0 | 0 | 0 |
| 5. | Cefoperazone-sulbactum | 1 | 2 | 2 | 0 | 0 |
| 6. | Ciprofloxacin | 85 | 81 | 86 | 78 | 72 |
| 7. | Norfloxacin* | 82 | 79 | 79 | 71 | 69 |
| 8. | Cotrimoxazole | 75 | 82 | 83 | 76 | 65 |
| 9. | Imipenem | 0 | 0 | 0 | 0 | 0 |
| 10. | Meropenem | 0 | 0 | 0 | 0 | 0 |
| 11. | Nitrofurantoin* | 15 | 18 | - | - | 9 |

*Tested for urinary isolates only

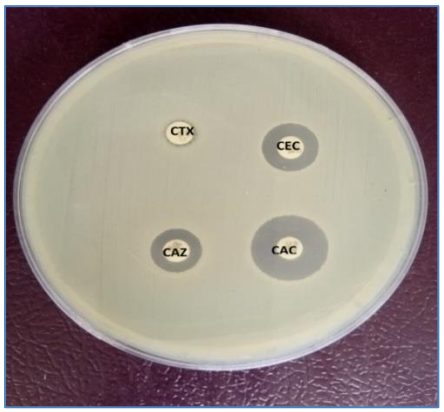


**Fig-1 Phenotypic confirmatory disc diffusion test**

## Discussion

Infections caused by ESBL producing gram negative bacteria are increasing at alarming rate posing a major problem in clinical therapeutics in community and hospital settings [9]. This could be attributed to the association of multi drug resistance in ESBL producing isolates as they carry co-resistance genes for other non - β-lactam antibiotics and thus limiting the therapeutic options [10].

The ESBL detection in our study was 47.4% which was in concordance with other studies [11-13]. ESBL production was highest in *E.coli* isolates (53.08%) followed by *K.pneumoniae* 27 (50%) which was similar to studies done by Shiju, *et al.,* and Linda, *et al*.[9,14] 33.33% of the *Pseudomonas* spp. were ESBL producers in our study. A study by Dutta, *et al.,* had also reported similar rates [15].

The isolates from pus showed high rate of ESBL production (59.61%) followed by urinary isolates (51.8%) and tracheal aspirate (50%). Rudresh, *et al.,* from Bangalore also reported higher production of ESBL among isolates obtained from exudates.[16] The ESBL production was more among isolates from in-patients compared to out- patients which were similar to Kumar *et al* and Linda *et al* study [4,14]. This could be attributed to the longer hospital stay, prolonged antibiotic use, recent invasive procedure and the use of catheters among hospitalised patients which pose as important risk factors in rapid dissemination of ESBL producing strains in hospital settings. In our study all the ESBL isolates were 100% sensitive to imipenem, meropenem and Piperacillin-tazobactum but showed high resistance to fluoroquinolones, amino glycosides and cotrimoxazole. Similar antibiogram has been reported in other studies done on ESBL strains [9,16,17]. This could be due to the carriage of multidrug resistance genes in the plasmids which encode for ESBL. The ESBL isolates from urine showed comparatively low degree of resistance to nitrofurantoin but were highly resistant to norfloxacin. Shashwati *et al* also reported nitrofurantoin to be more effective than norfloxacin in urinary ESBL isolates [11]. Thus in our study, Carbapenems, Piperacillin-tazobactum and Cefoperazone-sulbactum served as the most effective therapeutic option for treating infections caused by ESBL producing organisms.

## Conclusion

This study emphasizes on the need for routine monitoring of ESBL production using phenotypic confirmatory test as it is simple, cost effective and less time consuming. We also recommend the use of carbapenems as the drugs of choice for ESBL producers, owing to the increased rate of multi-drug resistance exhibited by them towards the conventional β -lactam and also the non β–lactam antibiotics. However, antimicrobial susceptibility testing should be performed for each ESBL strain before prescribing antibiotics and the carbapenems should be kept as reserve drugs only for multi-drug resistant strains.

**Application of research:** Restricted use of antibiotics especially the third generation cephalosporin, along with implementation of proper infection control

measures can help in preventing the spread and emergence of ESBL resistance.

**Abbreviations:** Extended spectrum β-lactamase (ESBL), ceftazidime (CAZ), ceftazidime-clavulanic acid (CAC), cefotaxime (CTX), cefotaxime-clavulanic acid (CEC), Clinical Laboratory Standard Institute (CLSI)

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