# PREVALENCE OF EXTENDED SPECTRUM β-LACTAMASE PRODUCING ENTEROBACTERIACEAE IN A TERTIARY CARE CENTRE

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**ABSTRACT**

**Background :**

Antimicrobial resistance is an emerging problem in health care settings. One of the common mechanisms of antimicrobial resistance is production of Extended-Spectrum Beta-Lactamase (ESBL) by Gram Negative Bacilli. Knowledge about the prevalence of ESBL producing strains in the local area must be obtained for proper therapeutic interventions in infections caused by them.

**Aims and Objectives :**

The purpose of the study was to detect the prevalence of Extended-Spectrum Beta-Lactamase producing Enterobacteriaceae isolates from various infections in a Tertiary Care Hospital, which guides in therapeutic management of such infections.

**Materials and Methods :**

A total of 2456 various clinical samples from hospitalized patients were recovered over a period of five months (Nov.2009 to March 2010). Clinical specimens were cultured and isolates were identified by conventional biochemical reactions. A total of 778 isolates were obtained, out of which 480 isolates were Enterobacteriaceae. The isolates were subjected to antimicrobial susceptibility test by standard methods. The Enterobacteriaceae isolates with reduced susceptibility to any of the third generation Cephalosporins (3GC) were selected for further study. Phenotypic detection of ESBL production was done by Double Disc Synergy Test and confirmed by MIC by agar dilution method.

**Results:**

In our study, among the 480 isolates of Enterobacteriaceae, 91 (18.95 %) were positive for ESBL by Double Disc Synergy Test (DDST) and confirmed by Minimum Inhibitory Concentration (MIC). Among the 91 ESBL producing isolates, 65 (26.42 %) were Escherichia coli and 26 (16.6 %) were Klebsiella spp. Eighty two isolates (90.10%) showed multidrug resistance.

**Conclusions:**

The ESBL production is associated with resistance to other antibiotics in our study. Prudent use of antibiotics and continuous surveillance programmes are mandatory to curb the dissemination of multidrug resistant strains in the hospital set up.

***Key Words : ESBL, Enterobacteriaceae, Hospitalized patients.***

**INTRODUCTION:**

Over the last two decades, many new beta lactam antibiotics have been discovered that were able to resist the hydrolytic action of beta-lactamases. With every new class of antibiotic, new beta-lactamases emerged that caused resistance to that class of drug[1]. The introduction of the Third generation Cephalosporins was considered to be a major breakthrough to overcome the resistance exhibited by the beta lactamases[2]. However, resistance to these extended- spectrum beta-lactam antibiotics emerged quickly. Because of their increased spectrum of activity, especially against oxyimino-cephalosporins, these enzymes were called extended-spectrum beta-lactamases (ESBLs)[1]. The first publication about plasmid-encoded beta - lactamases capable of hydrolyzing the extended spectrum Cephalosporins was in 1983 from University of Frankfurt[3]. Widespread mechanisms of antimicrobial resistance are posing a continuous problem in therapeutic interventions of infectious diseases. Production of extended spectrum β-Lactamase is an important mechanism of antimicrobial resistance exhibited by Enterobacteriaceae[2]. In our study we have made an attempt to detect the prevalence of ESBL producing Enterobacteriaceae isolates from various clinical samples from a tertiary care hospital which would assist in therapeutic interventions of infections caused by them.

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### Materials and Methods

A total of 2456 clinical samples from hospitalized patients were recovered over a period of 5 months (Nov. 2009 to March 2010) and processed at a tertiary care hospital. The approval of ethical committee has been obtained from our institution for the study. The samples were plated on MacConkey and Blood agar media and incubated aerobically at 37ºC overnight. Samples that showed growth after overnight incubation were subjected to conventional biochemical tests for proper identification. A total of 778 isolates were obtained, out of which 480 isolates were Enterobacteriaceae.

### Antimicrobial Susceptibility Test

The susceptibility of the 480 Enterobacteriaceae isolates to Third Generation Cephalosporins (3 GC) Ceftriaxone, Ceftazidime, Cephotaxime and to the other antibiotics such as Amikacin, Ampicillin, Ciprofloxacin, Cefipime, Gentamicin, Cotrimoxazole, and Imipenem ( Hi Media, India) was determined by Disc Diffusion Method[4]. The results were interpreted as per CLSI guidelines[5]. Escherichia coli ATCC 25922 strain was used for quality control. Enterobacteriaceae isolates with reduced susceptibility to Ceftriaxone (‹ 21 mm), Ceftazidime (‹ 18 mm) and Cephotaxime (‹ 20 mm) were selected for the study.

### ESBL detection by Double Disc Synergy Test ( DDST)

In the DDST, synergy was determined between a disc of Augmentin (20 mcg of amoxycillin & 10 mcg of clavulanic acid) and 30 mcg disc of each of the 3 GC antibiotics which are placed at a distance of 30 mm apart on a lawn culture of the resistant isolate under test on MHA. (Hi Media, India)[6] The test organism was considered to produce ESBL if the zone size around the test antibiotic is enhanced towards the Augmentin disc. This increase occurs because the Clavulanic acid present in the Augmentin disc inactivates the ESBL produced by the test organisms[7].

### ESBL Confirmation by MIC

MIC assay was performed on all strains that showed zone reduction for one or more of the 3GC antibiotics used in ESBL screening test. MIC was determined by agar dilution method against Ceftriaxone, Ceftazidime and Cephotaxime as described by CLSI[8].

### Result

**Table 1 Distribution of Enterobacteriaceae Isolates in Various Samples**

| S.No | Sample | Total no. of Isolates (n= 480) | ESBL positive (n = 91) |
|---|---|---|---|
| 1. | Urine | 271 | 44 |
| 2. | Blood | 73 | 21 |
| 3. | Pus | 87 | 19 |
| 4. | Others | 49 | 07 |

**Table 2 – No. of isolates positive for 3GC Screening & ESBL**

| S. No. | Organism | Total Nos. | 3 GC Screening Resistance | ESBL by DDST |
|---|---|---|---|---|
| 1. | E-Coli | 246 (51.3 %) | 66 (26.8 %) | 65 (26.42 %) |
| 2. | Klebsiella spp | 157 (32.7 %) | 26 (16.6 %) | 26 (16.6 %) |
| 3. | Proteus spp | 77 (16 % ) | 0 | 0 |
| **4.** | **Total Isolates** | **480** | **92 (19.16 %)** | **91 (18.95 %)** |

Of the 480 isolates, 85 (34.6 %) isolates were resistant to 4th generation cephalosporins and only 11 (2.3 %) were resistant to Imipenem. 82 (90.10%) ESBL producers were resistant to Ampicillin, Gentamicin, Cotrimoxazole, Ciprofloxacin and

Amikacin.

MIC of 3GC antibiotics for ESBL producing Enterobacteriaceae is shown in Table-3.

**Table 3 – MIC of 3GC antibiotics for E.coli & Klebsiella**

| Cephotaxime (µg/ml) | | Ceftriaxone (µg/ml) | | Ceftazidime (µg/ml) | |
|---|---|---|---|---|---|
| E.coli | Klebsiella | E.coli | Klebsiella | E.coli | Klebsiella |
| 512 | 256 | > 512 | 256 | 64 | 128 |

**Discussion**

Infection due to ESBL producing Enterobacteriaceae is a well known problem. Extensive use of newer generation Cephalosporins has been the strong factor for the evolution of newer beta-lactamases such as ESBLs. ESBLs are encoded by transferable plasmids which code resistant determinants to other antimicrobial agents. They are also responsible for dissemination of resistance to other gram negative bacilli in the Hospital and in the Community.

In our study, 18.95 % of the total Enterobacteriaceae strains are found to produce ESBLs. Our study is in accordance with study of Christopher[9] in which 18 % of the isolates were positive for ESBL. Whereas Vercauteren[6] in his study shows lesser percentage (5.8 %) of ESBL producers and the study of Subha[7] indicates higher incidence (66.6 %), when compared with our study. The highest level of ESBL production is exhibited by E. coli (26.42 %) followed by Klebsiella species (16.6 %) in our study. In this study the MIC values range from 256 to > 512 for Ceftriaxone and Cefotaxime and 64 to 128 for Ceftazidime. The MIC values obtained in our study are similar to the study of B.Sasirekha[10]

In this study resistance to 3GC was found to coexist with resistance to other antibiotics. Since 82 (90.1 %) ESBL producing isolates showed Multi Drug Resistance, the therapeutic strategies to control infection due to Enterobacteriaceae have to be formulated. Our study highlights the emergence of ESBL producing Enterobacteriaceae strains endowed with extremely wide spectrum of antibiotic resistance including resistance to Sulphonamides, Quinolones and Aminoglycosides thus limiting the therapeutic options. Only 2.3 % of ESBL producers were resistant to Imepenem as indicated in our study. However, Imepenem could only be used as the last therapeutic option for ESBL producers to prevent the emergence of carbapenem resistant strains. Though the presence of ESBL producers was confirmed by MIC in our study, there are still limitations that better results could have been given by further exploration with molecular techniques.

The detection of organisms producing ESBLs remains a contentious issue. The Centers for Disease Control and Prevention and the College of American Pathologists conducted proficiency testing studies and have raised concerns about the current capacity of many laboratories to detect organisms producing ESBLs[11,12,13]. If molecular detection assays, are available at the time of an outbreak and performed by a reference laboratory, early recognition and the possible mechanisms by which resistance is spread can be identified in a timely manner. Extra efforts such as molecular procedures to identify resistance mechanisms can promote optimal patient care by early detection of antimicrobial-resistant organisms and implementation of appropriate infection control procedures.

**Conclusion**

The presence of ESBLs would create significant therapeutic problems. Enhanced infection control coupled with antibiotic stewardship programme therefore plays an important role in limiting the spread of ESBL producing organisms.

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