Prevalence of ESBLs among Enterobacteriaceae and their Antibiotic Resistance Pattern from Various Clinical Samples

Sanjo Gupta and Veena Maheshwari*

Department of Microbiology RKDFMCH & RC Jatkhedi Bhopal, India

*Corresponding author

A B S T R A C T

Beta-lactam antibiotics are the most commonly used antimicrobials against bacterial infections. In recent years, emergence of resistance to these antimicrobial agents due to production of β-lactamases has become a serious global health concern. It was reported as leading cause to ineffectiveness to antibiotic usage, increased morbidity and high cost of treatment. Extended spectrum beta-lactamases (ESBLs) detection amongst the Gram – negative bacilli is considered as an important marker of endemicity. Knowledge of the patterns of antimicrobial resistance and effective surveillance has significant implications for patient management and guiding clinicians to take appropriate interventions. A prospective study was conducted over a duration of 1 year (March 2014 to February 2015) in the Department of Microbiology, of a teaching tertiary care hospital. The prevalence of potential ESBLs producers was explored. Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines. A total of 250 samples from male and female patients visiting the outpatient department (OPD) and inpatient department (IPD) of our hospital were collected. Among the ESBL producers maximum number of isolates were identified as E. coli 45(44.1%) followed by Klebsiella spp. 30(29.4%), Citrobacter spp. 10(9.8%) Proteus spp. 12(11.7%), and Enterobacter spp. 5(4.9%) respectively. ESBL producing isolates were found to be multi-drug resistant when compared to non-ESBL producers. High prevalence of ESBLs producing Enterobacteriaceae in hospitals, with a tendency for multi-drug resistance, suggests that routine detection is mandatory as this may help in regulating hospital antibiotic policy.

Key words
Antimicrobial resistance, Enterobacteriaceae, Extended spectrum beta-lactamase (ESBL). Phenotypic confirmatory disc diffusion test.

Introduction

Beta-lactam antibiotics are the most commonly used antimicrobials against bacterial infections. In recent years, emergence of resistance to these antimicrobial agents due to production of β-lactamases has become a serious global health concern. It leads to antibiotic ineffectiveness, increased severity of illness and cost of treatment. These enzymes are numerous and they mutate continuously in response to overuse or misuse of beta-lactam antibiotics and have lead to the development of extended spectrum β-lactamases (ESBL). Extended spectrum beta-lactamase (ESBL) producing organisms are those that hydrolyze the oxyimino beta-lactams and monobactams, but have no effect on the cephamycins and carbapenems and are commonly inhibited by β- lactamase inhibitors such as clavulanic acid, sulbactam, and Tazobactam. ESBL are placed under Bush’s functional class 2be. They are plasmid-mediated enzymes and are derived from point mutation of TEM on SHV β-lactamases that are widely distributed among the Enterobacteriaceae. In
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.\(^2\)

Risk factors for infection with ESBL producing organisms are prolonged antibiotic usage, ICU stay, recent invasive procedure, pressure sores, anaemia and permanent urinary catheter.\(^4\)

The prevalence of these organisms varies geographically and in hospital settings. ESBLs have been reported worldwide in many different genera of Enterobacteriaceae and Pseudomonas aeruginosa\(^5\). However, majority of the ESBLs are found in Klebsiella spp. and Escherichia coli of the Enterobacteriaceae family.\(^6,7\) The serious increase in the prevalence of ESBL’s worldwide creates a need for effective and easy to perform screening methods for detection.\(^8\)

ESBL producing isolates remain undetected as they are difficult to detect by routine susceptibility testing methods and may show false susceptibility to antibiotics by Kirby-Bauer disc diffusion methods.\(^9\)

Several methods have been developed to detect the presence of ESBL including Double disc synergy test (DDST) and double-disc diffusion test (DDDT) using cefotaxime and ceftazidime, disc with or without clavulanic acid.\(^9\)

Although various studies have been conducted on the ESBL-producing strains of Enterobacteriaceae in different regions of India like Bhopal,\(^2\) Dibrugarh,\(^10\) Bangalore,\(^11\) Pondicherry,\(^12\) Bijapur,\(^13\) Hyderabad,\(^14\) Mumbai,\(^15\) no published data are available on the prevalence of ESBL production in the Moradabad region of Uttar Pradesh in Northern India. ESBL detection is of utmost importance to formulate infection control measures and to prevent their spread.

Hence the present study was undertaken to find the prevalence and resistance pattern of ESBL producing organisms and to help in implementing an effective antibiotic policy.

**Materials and Methods**

A prospective study was conducted over a duration of 1 year (March 2014 to February 2015) in the Department of Microbiology, of a teaching tertiary care hospital. The study protocol was approved by the Institutional Ethical Committee and informed consent was obtained from patients.

A total of 250 samples from different clinical specimens such as urine, tracheal aspirate, pus, blood and sputum were collected from patients of all age groups and either sex in sterile containers.

Samples were processed and isolates were identified by standard laboratory methods.\(^16,17\) Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines.\(^18\)

The following antibiotics were tested: Amikacin (30 mcg), gentamycin (10 mcg), ampicillin (10 mcg), ampicillin/sulbactam (10/10 mcg), ciprofloxacin (5 mcg), levofloxacin (5 mcg), chloramphenicol (30 mcg), co-trimoxazole (1.25/23.75 mcg), ceftriaxone (30 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), tigecycline (30 mcg) piperacillin-tazobactum (100/10 mcg), imipenem (10 mcg) and meropenem (10 mcg), ertapenem (10 mcg). Norfloxacin (10 mcg) and nitrofurantoin (300 mcg) were tested against isolates from urine samples only. Dehydrated media and antibiotic discs were procured from Hi-Media, Mumbai.
All isolates were subjected for ESBL screening test. Potential ESBL producer was then subjected for ESBL Phenotypic confirmatory test – Disc Diffusion method (PCDDT).

**Method for ESBL detection**

ESBL production was detected by using the phenotypic confirmatory test along with routine antibiotic susceptibility testing according to Clinical Laboratory Standard Institute (CLSI) guidelines. Cefotaxime and ceftazidime discs alone and in combination with clavulanic acid were used. A >5 mm increase in zone size was confirmed for ESBL production.

Although CLSI described phenotypic confirmatory test is applicable for *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*, an attempt was made to look for ESBL production among the other members of Enterobacteriaceae.

ESBL-producing Enterobacteriaceae isolates which were resistant to cefoxitin (zone diameter<18 mm) were not considered for the study. This is to exclude associated Amp-C type of β-lactamases.

In the study, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 (HiMedia Laboratories, Mumbai) were used as positive and negative controls throughout, for ESBL production.

**Results and Discussion**

A total of 250 samples were collected from patients who consisted of 60 (40%) males and 90 (60%) females’ patients visiting the outpatient departments (OPD) and inpatient departments (IPD). These included Urine, pus, swabs, Blood, sputum and tracheal aspirate. Most of the patients presented in the active age group of more than 21-30 years.

Prevalence of ESBL producers among male patients was 33(32.3%) and female patients was 69(67.6%) respectively. Among the 150 Gram negative isolates 102 (68%) were found to be ESBL producers and rest 48(32%) were non-ESBL producers (Table 1).

The age group more commonly affected was between 21 and 30 years (n=38) (Table 2).

Among the ESBL producers maximum number of isolates were identified as *E. coli* 45(44.1%) followed by *Klebsiella spp.* 30(29.4%), *Citrobacter spp.* 10(9.8%), *Proteus spp.* 12(11.7%), and *Enterobacter spp.* 5(4.9%) respectively.

And Amongst the Non ESBL producers highest percentage of isolates identified were *E. coli* 15(31.25%) followed by *Klebsiella spp.* 12(25%), *Citrobacter spp.* 10(20.8%), *Proteus spp.* 6 (12.5%) and *Enterobacter spp.* 5(10.4%) respectively.

Prevalence of ESBLs was maximum in ICU 34.2% and least in ENT 3.9% (Table 3).

A total of 150 non repetitive clinical isolates of Enterobacteriaceae were identified, among which 60 (40%) isolates were *Escherichia coli*, followed by 42(28%) isolates of *Klebsiella spp.*, 20 (13.3) of *Citrobacter spp.* 18(12%) of *Proteus spp.*, and10 (6.6%) of *Enterobacter spp.*, respectively. The distribution of organisms isolated from the various clinical specimens is as shown in table 4.

Infections by ESBL producing organisms have emerged as a major problem and the failure of therapy with broad spectrum antibiotics are creating serious problems.

Although some studies report male sex to be a risk factor for ESBL production our study corroborates with the study conducted by Nibedita Das et al., where there was no
significant association between ESBL production and male sex\textsuperscript{10}. The present study revealed a slight female preponderance for ESBL production among the study subjects. This was similar to the findings of an earlier study which were reported by Kiratisin et al., \textsuperscript{24} which revealed a female preponderance.

In our present study, we isolated 102(68\%) ESBL producers and 48(32\%) were non-ESBL producers. Their findings were quite different to several studies worldwide reflect the general trend in prevalence of ESBL producing bacteria. A study reported by Nibedita das et al.,\textsuperscript{10} from South India on ESBL production in uropathogens showed 81.9\% ESBL producers.

Out of the 150 Enterobacteriaceae isolates, a majority were \textit{E. coli} (40\%), followed by \textit{Klebsiella pneumoniae} (28\%), \textit{Citrobacter} spp (13.3\%), \textit{Proteus} spp (12\%), \textit{Enterobacter} spp (6.6\%). This finding was on par with those of many studies\textsuperscript{25}. Mathur et al., \textsuperscript{26} from New Delhi, have also reported \textit{E. coli} and \textit{Klebsiella pneumoniae} as the most common Enterobacteriaceae which were prevalent in their clinical samples and this was well comparable to the reports from our study. Babypadministr et al., \textsuperscript{27} from Chennai too reported the prevalence of 49\% \textit{E. coli} and 8\% \textit{Klebsiella} spp.

Antibiotic resistance has been noted as a serious problem, even at our medical college hospital. The third generation cephalosporins have been used in a majority of patients and resistance even to these antibiotics has been reported. As there was no data which was available on the prevalence of ESBL production in this region, the current study was undertaken to know the prevalence of ESBL producing Enterobacteriaceae at our tertiary health care centre.

\textbf{Table.1} Distribution of ESBL strains among male and female patients

| Sex     | ESBL N=102(\%) | NON-ESBL N=48 | TOTAL   |
|---------|----------------|---------------|---------|
| MALE    | 33 (32.35\%)   | 27 (56.25\%)  | 60      |
| FEMALE  | 69 (67.64\%)   | 21 (43.75\%)  | 90      |
| TOTAL   | 102            | 48            | 150     |

\textbf{Table.2} Age-wise distribution of ESBL producing Enterobacteriaceae in different age groups

| Age interval | Males | Females |
|--------------|-------|---------|
| 0-10         | 0     | 1       |
| 11-20        | 3     | 5       |
| 21-30        | 10    | 28      |
| 31-40        | 8     | 12      |
| 41-50        | 5     | 8       |
| 51-60        | 4     | 9       |
| 61-70        | 2     | 4       |
| 71-80        | 1     | 2       |
Table 3: Department wise distribution of ESBL producers

| Wards         | No. of isolates | ESBL producer | Percentage (%) |
|---------------|-----------------|---------------|----------------|
| Medicine      | 25              | 17            | 16.6           |
| Surgery       | 25              | 15            | 14.7           |
| Orthopedics   | 20              | 14            | 13.7           |
| OBG           | 15              | 11            | 10.7           |
| ENT           | 9               | 4             | 3.9            |
| Medical ICU   | 17              | 13            | 12.7           |
| Surgical ICU  | 20              | 15            | 14.7           |
| Pediatric     | 10              | 6             | 5.8            |
| ICCU          | 9               | 7             | 6.8            |
| **Total**     | **150**         | **102**       | **68**         |

Table 4: Distribution of organisms isolated by specimen (N=150)

| Organisms             | Urine | Pus | Swab | Sputum | Blood | Tracheal aspirate | Total |
|-----------------------|-------|-----|------|--------|-------|-------------------|-------|
| *Escherichia coli*    | 42    | 10  | 9    | 5      | 4     | 2                 | 60    |
| *Klebsiella spp.*     | 17    | 9   | 8    | 5      | 4     | 2                 | 42    |
| *Citrobacter spp.*    | 9     | 2   | 3    | 4      | 2     | 0                 | 20    |
| *Proteus spp.*        | 7     | 3   | 2    | 2      | 3     | 1                 | 18    |
| *Enterobacter spp.*   | 3     | 2   | 1    | 2      | 1     | 1                 | 10    |
| **Total**             | **78**| **26**| **23**| **18**| **10**| **6**             | **150**|

Fig. 1: Graphical representation of the antibiotic resistance profile of various extended spectrum beta-lactamase (ESBL) and non-ESBL clinical isolates.
As of now, no countrywide study has been conducted for the detection of the prevalence of ESBL production in India. Individual studies which were done in different parts of the country showed a varying prevalence, based on various risk factors and local reasons.

In the present study, the highest number of ESBL producers were obtained in the isolates from the ICU (N=35, 34.31%), followed by Medicine (N=17, 16.6%) and Surgery (N=15, 14.7%). This was comparable with a study which was carried out at AIIMS, New Delhi, India. This could be due to the prolonged hospital stay, inappropriate therapy, total antibiotic use, indwelling catheters, endotracheal or nasogastric tubes, gastrostomies or tracheostomies and the severity of the illness.

The antibiogram of the ESBL producing isolates showed a high degree of resistance towards routinely prescribed antibiotics when compared to non-ESBL producing isolates. These findings are similar to those reported by Sasirekha et al., Ndugulile et al., and Mehrgan et al., Since ESBLs are plasmid-mediated enzymes, which can be transferred from one bacterium to another and as these transferable plasmids also code for resistance determinants to antimicrobial agents other than beta-lactams. This finding is further explained by such fact.

In our study, Resistance shown to piperacillin-tazobactum (55.8% and 27% resistance among ESBL and non-ESBL) was low, reflects their less use for treatment of community-acquired infections. Not only ESBL producing isolates (resistance towards cefotaxime, ceftazidime, ceftriaxone and cefoparazone sulbactum in the range of 89-94%) but also ESBL nonproducers (cefotaxime: 66.7%; ceftazidime: 70.7%; ceftriaxone 68.2%; Cefoparazone sulbactum: 60.1%) possessed high degrees of resistance towards 3rd and 4th generation cephalosporins and mono-bactams. Such observation can be attributed to overuse of antibiotics in both community and hospital set-up, uncontrolled practices in prescription writing and incomplete dose consumed by patients.

Drugs such as imipenem which is used as last resort in the health-care settings was found resistant (12.7% and 15.8% among ESBL producers and non-producers, respectively). In addition, drugs such as meropenem exhibited high degree of resistance (reported as 34.3% and 45% by ESBL and non-ESBL producers respectively) which is quite alarming. Resistance against carbapenem can be attributed to multifactorial causes. The mechanism through which it occurs is either bacterial production of beta-lactamases, that hydrolyze the antibacterial agent or through changes in porin channels in the bacterial cell wall that decrease the permeability of the drug into the organism. In addition, upregulation of efflux pumps result into reduced susceptibility of organisms toward meropenem.

Most studies showed 100% sensitivity toward imipenem. Incidences of meropenem resistance higher than that of imipenem among nosocomial pathogens was observed by Gupta et al. The resistance exhibited in our case is due to existence of carbapenemase producing isolates in our setting. This may be because patients in Intensive Care Unit are directly being treated with carbapenems that has led to development of such multidrug-resistant isolates in our health-care setting.

The detailed insight of antibiotic resistance pattern has been illustrated in figure 1.

The present study highlights that there is a high prevalence of ESBL producing bacteria among Enterobacteriaceae mostly E. coli and Klebsiella spp. In view of these findings, we
suggest that routine screening of ESBL should be performed on all isolates which are showing decreased susceptibility to third generation cephalosporins. The strict compliance to antibiotic stewardship and enforcement of infection control practices should also be strengthened in all our tertiary health centers.

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