Detection of Extended Spectrum Beta-lactamase (ESBL) Producing Gram Negative Bacteria from Clinical Specimens of Sir Salimullah Medical College and Mitford Hospital

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Abstract:
Detection of Extended spectrum beta lactamase (ESBL) enzyme producing bacteria in hospital settings is vital as ESBL genes are transmissible. This study was carried out to determine the distribution of ESBL producing gram negative isolates at a tertiary care hospital in Dhaka city which deals with the patients hailing from relatively low socioeconomic status. One hundred and twenty four gram negative bacteria isolated from different clinical specimens from outpatient and inpatient departments of Sir Salimullah Medical College and Mitford Hospital (SSMC & MH) were tested for ESBL by E test ESBL method in the department of microbiology of Sir Salimullah medical college (SSMC) from March 2013 to August 2013. Out of 124 gram negative bacteria 69 (55.65%) were positive for ESBL. Among the ESBL producers, Esch. coli was the highest (46.38%) which was followed by Serratia spp (11.59%), Enterobacter spp (10.14%), Proteus spp, (8.70%), Acinetobacter spp (7.24%) and Klebsiella spp (5.79%). Out of 32 Esch. coli isolated from outpatient department, 10 (31.25%) were positive for ESBL. On the other hand out of 27 Esch. coli isolated from inpatient department, 22 (81.48%) were positive for ESBL. The difference was statistically significant (p<0.001). So the present study reveals that the distribution of ESBL producers is more among the hospitalized patients than the patients of the community.

Key word: Bangladesh, ESBL, Gram Negative Bacteria.

Introduction:
Gram negative bacteria intrinsically can produce both chromosomal and plasmid mediated beta lactamases enzymes due to selective pressure created by beta lactam substances produced by soil organisms. TEM-1 was the 1st plasmid mediated beta lactamase enzyme described in early 1960. Subsequently extended spectrum beta lactamases (ESBLs) are identified¹. ESBLs are 2be group enzymes of Bush-Jacoby-Medeiros classification and some of group 2d enzymes which has similar functional properties like group 2be enzymes². These enzymes are produced by members of Enterobacteriaceae such as Esch. coli, K. pneumoniae, Enterobacter spp., Citrobacter spp., Proteus spp, Morganella morganii, Serratia marcescens, Shigella dysenteriae, and other Gram negative bacteria like Pseudomonas aeruginosa and Acinetobacter baumanii³. ESBLs are usually TEM, SHV and CTX-M gene mediated beta lactamases⁴. Since 1995 rapidly proliferating CTX-M gene mediated ESBLs are taking upper hand over TEM or SHV gene mediated enzymes due to its greater ability to spread. Gram negative bacteria obtain CTX-M genes from environmental Kluyvera species⁴. CTX-M enzymes hydrolyze cefotaxime better than ceftazidime. Many hydrolyze cefepime as well⁵. Same organism can harbor many types of enzyme along with other new beta lactamase like AmpC-type beta lactamases, carbapenemases, which change antibiotic susceptibility pattern⁵. These ESBLs are efficiently capable of hydrolyzing penicillins, early cephalosporins such as cephalexin and cephalothin except cephamycins, the oxyimino group containing cephalosporins like cefotaxime, ceftazidime, and monobactam and are usually inhibited by beta-lactamase inhibitors such as clavulanicacid, sulbactam, and tazobactam⁵. In addition ESBLs genes are frequently intermingled with other antibiotic resistance genes such as tetracycline, aminoglycosides, trimethoprim, sulphonamide, chloramphenicol and quinolones making them multidrug resistance⁶. High prevalence of ESBL producers are documented from all over the country. Prevalence of ESBLs differs significantly geographically and depends on various factors⁶. Enterobacteriaceae are the most common group of gram-negative rods isolated in clinical laboratories,⁷ hence
detection of ESBL production by *Enterobacteriaceae* and other gram negative bacteria has paramount importance to ensure appropriate antibiotic treatment. With this view this study was designed to find out the distribution of ESBL producing bacteria isolated from different clinical specimens of Sir Salimullah Medical College and Mitford Hospital in Dhaka city of Bangladesh.

**Methods:**
Clinical specimens were collected from the patients attending the microbiology laboratory of Sir Salimullah Medical College (SSMC) from outpatient and inpatient department, Dhaka, during the period of March 2013 to August 2013. Total 124 gram negative bacteria were isolated and identified from different biological samples such as urine, pus, wound swab, stool, blood and high vaginal swab (HVS). Samples were collected following standard procedures. MacConkey’s agar and blood agar media were used for the primary isolation of the bacteria. Identification of particular gram negative bacteria was done by gram staining, observing colony morphology, oxidase test, inoculation into Triple sugar iron (TSI), Motility indole urea (MIU) and Simmons citrate agar media. ESBL producing bacteria was detected by Screening test, Double disc synergy test (DDST) and E test ESBL method. E test ESBL method was taken as gold standard. *Klebsiella pneumoniae* ATCC 700603 was used as reference strain for ESBL positive control. The strain of *Esch.coli*, which was sensitive to ceftazidime, ceftriaxone, cefotaxime and aztreonam was used as negative control.

Screening test 8,9; Standard inoculum of bacterial suspension matched to 0.5 McFarland was made and Muller Hinton agar (MHA) plate was inoculated properly with bacterial suspension. Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs (Oxoid, England) were placed onto MHA plate and incubated overnight at 37°C. When inhibition zone of any isolate to Ceftazidime < 22 mm or Aztreonam ≤ 27 mm, or Cefotaxime ≤ 27 mm or Ceftriaxone ≤ 25 mm alone or in combination was found then the isolate was taken as screening test positive.

Double disc synergy test (DDST)8,9; The MHA plate was inoculated with bacterial suspension matched to 0.5 McFarland. Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs were placed 15 mm distance centre to centre from amoxiclav disc (20mg amoxicillin and 10mg of clavulanic acid) which was placed at middle. Any extension of inhibition zone of antimicrobial discs (one or more) towards amoxiclav disc confirmed the presence of ESBL.

E test ESBL Method8,9; Triple ESBL detection (Ezy MICTM) strip was used. The upper half of this phenotypic ESBL detection strip had ceftazidime, cefotaxime and cefepime (mixture) plus beta lactamase inhibitor mixture (clavulanic acid and tazobactum) with highest concentration tapering downwards. Whereas lower half was similarly coated with ceftazidime, cefotaxime and cefepime (mixture) in a concentration gradient in reverse direction. Standard inoculum of bacterial suspension matched to 0.5 McFarland was made and Muller Hinton agar plate was inoculated properly with matched bacterial suspension. With the help of applicator one ESBL detection strip was placed on one MHA plate. Plates were transferred in the incubator at 37°C for 24 hours. ESBL producing strain was confirmed either when the ratio of the value obtained for combination of antibiotic mixture plus beta lactamase inhibitor mixture and antibiotic mixture alone was greater than or equal to eight or presence of phantom zone that is no inhibitory zone was formed at antibiotic mixture side but inhibitory zone was found at combination of antibiotic mixture plus beta lactamase inhibitor mixture side.

**Result:**
Total isolated gram negative bacteria was 124 and the most frequently isolated bacteria was *Esch.coli*. (Figure 1). Out of 59 *Esch.coli*, 32 (54.24%) was confirmed as ESBL producers by E test ESBL method (Table 1). Significantly highest (81.48%) percentage of ESBL producing *Esch.coli* had been identified from inpatient department (IPD) and only 31.25% ESBL producing *Esch.coli* was detected from outpatient department (OPD) (Table 1). Urine was the most common specimen. Sixty three percent of total *Esch.coli* was isolated from urine samples (Figure 2). Out of 15 *Esch.coli* isolated from urine sample of inpatient department, 13 (86.67%) were ESBL producers and 8 (36.36%) were ESBL producers among 22 *Esch.coli* isolated from urine of outpatient department (Figure 3). Difference of ESBL production by *Esch.coli* between inpatient and outpatient department was statistically significant. Next to *Esch.coli*, *Enterobacter* spp numbered second, *Proteus* spp. numbered third, *Pseudomonas* spp and *Serratia* spp numbered fourth position among 124 isolations (Table 2). ESBL producing *Enterobacter* spp, were 7 (43.75%) out of 12, ESBL *Proteus* spp, were 6 (54.55%) among 11 and ESBL producing *Pseudomonas* spp was 1 (12.50%) out of 8, ESBL producing *Serratia* spp were 08 (100%) , ESBL producing *Klebsiella* spp, were 04 (57.14%) among 7 and ESBL producing *Acinetobacter* spp were 5 (83.33%) out of 6 (Table-2). In all strains number of inpatient ESBL producing isolates were higher than outpatient department (Figure-3). 32 (54.24%) ESBL *Esch.coli* out of...
total 59 *Esch. coli*, and isolated 43.75% of *Enterobacter* spp, 54.55% of *Proteus* spp., and 57.14% of *Klebsiella* spp were ESBL producers. (Table 2),

![Pie chart showing the percentage of isolated bacteria from different clinical specimens]

Figure-1: The Percentage of isolated bacteria from different clinical specimens

**Table 1: ESBL producing Esch. coli detected by different methods**

<table>
<thead>
<tr>
<th>Tests</th>
<th><em>Esch. coli</em> from Inpatient department subjected to ESBL detection test</th>
<th><em>Esch. coli</em> from outpatient department subjected to ESBL detection test</th>
<th>Total Isolated <em>Esch. coli</em> (%)</th>
<th>Total ESBL detected By E test (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening test</td>
<td>Positive (%) 27 0 27 13 19</td>
<td>Negative (%) 0 19 0 32 59</td>
<td>Total IPD (%) 13 19</td>
<td>Total OPD (%) 59 32 19 19 32</td>
</tr>
<tr>
<td>DDST</td>
<td>Positive (%) 13 14 0 5 7</td>
<td>Negative (%) 0 7 0 22 7</td>
<td>Total IPD (%) 14 7 0 5 19</td>
<td>Total OPD (%) 0 14 0 7 22</td>
</tr>
<tr>
<td>E test</td>
<td>Positive (%) 22 05 10 5 22</td>
<td>Negative (%) 0 22 0 10 22</td>
<td>Total IPD (%) 22 5 0 10 22</td>
<td>Total OPD (%) 5 22 0 10 22</td>
</tr>
</tbody>
</table>

$p<0.001$

![Bar chart showing the number of ESBL positive and negative *Esch. coli* in urine isolated from inpatient and outpatient department (p<0.001)]

Figure 3: ESBL positive and negative *Esch. coli* in urine isolated from inpatient and outpatient department (p<0.001)

![Bar chart showing the number of ESBL positive and negative isolates other than *Esch. coli*.]

Figure-4: The number of ESBL positive and ESBL negative isolates other than *Esch. coli*.
Detection of ESBL Producing Gram Negative Bacteria

Table 2: ESBL producing different bacteria detected by E test ESBL method

<table>
<thead>
<tr>
<th>Name of bacteria (n)</th>
<th>Inpatient department isolates</th>
<th>Outpatient department isolates</th>
<th>ESBL producers out of total ESBL (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESBL Positive</td>
<td>ESBL Negative</td>
<td>Total</td>
<td>ESBL Positive</td>
</tr>
<tr>
<td>Esch. coli (59)</td>
<td>02</td>
<td>05</td>
<td>01</td>
<td>27</td>
</tr>
<tr>
<td>Enterobacter spp. (10)</td>
<td>07</td>
<td>07</td>
<td>01</td>
<td>14</td>
</tr>
<tr>
<td>Proteus spp. (11)</td>
<td>05</td>
<td>03</td>
<td>08</td>
<td>01</td>
</tr>
<tr>
<td>Pseudomonas spp(08)</td>
<td>01</td>
<td>05</td>
<td>06</td>
<td>00</td>
</tr>
<tr>
<td>Serratia spp(08)</td>
<td>07</td>
<td>00</td>
<td>01</td>
<td>07</td>
</tr>
<tr>
<td>Klebsiella spp(08)</td>
<td>03</td>
<td>02</td>
<td>05</td>
<td>01</td>
</tr>
<tr>
<td>Acinetobacter spp. (06)</td>
<td>05</td>
<td>01</td>
<td>06</td>
<td>-</td>
</tr>
<tr>
<td>Salmorrella spp (05)</td>
<td>03</td>
<td>00</td>
<td>03</td>
<td>00</td>
</tr>
<tr>
<td>Citrobacter spp (03)</td>
<td>01</td>
<td>00</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>Morganella spp (01)</td>
<td>01</td>
<td>00</td>
<td>01</td>
<td>-</td>
</tr>
<tr>
<td>Total Bacteria (124)</td>
<td>55</td>
<td>23</td>
<td>78</td>
<td>14</td>
</tr>
</tbody>
</table>

Discussion:
Over the years beta lactams antibiotics are prescribed for both community acquired and hospital acquired infections. The continued use of these antibiotics produces selective pressure for pathogenic and commensal bacteria to produce and maintain beta lactam antibiotic destroying mechanisms. Discovery of different types of beta lactamase enzymes are the best example of this long continued pressure. Now a day's multiple broad-spectrum beta lactamases produced by multidrug-resistant K. pneumoniae, Acinetobacter spp., P. aeruginosa, and Enterobacter spp. have disseminated throughout gram-negative pathogens10.

In this research work ESBL producing isolates were identified from clinical specimens of outpatient (OPD) and inpatient department (IPD). ESBL strains obtained from outpatient department figured out community involvement. In contrast, ESBL infections of inpatient department denoted nosocomial or community participation. This study reported 32 (54.24%) ESBL Esch. coli out of total 59 Esch. coli, in which 81.48% was from inpatient department and 31.25% from the outpatient department. Significant presence of higher percentage of ESBL producing Esch. coli in inpatient department in present study indicates certain degree of nosocomial spread of infections. Significantly higher number of ESBL producing Esch. coli was detected from urine of inpatient department which is consistent with findings of several studies6,11,12. Isolated 43.75% of Enterobacter spp, 54.55% of Proteus spp., and 57.14% of Klebsiella spp were ESBL producers. Vinodhini et al., found low percentage of ESBL Enterobacter spp. and Proteus spp and Sridor et al., (2008) showed higher percentage of ESBL Proteus spp13, 14.

ESBL Enterobacter spp, ESBL Proteus spp and ESBL Klebsiella spp isolation numbers were high in IPD than OPD though statistically not significant. Other gram negative members could not be compared for significance test as number of isolated bacteria was very small. Identification of ESBL Acinetobacter spp was very important because this is one of the multdrug resistant pathogens10 and now a day it is being isolated from various biological specimens. This study also documented ESBL Pseudomonas spp from IPD samples as reported by other study14. Isolations of ESBL Acinetobacter (7.24%) and ESBL Pseudomonas strains (1.45%) were alarming because they are environmental bacteria, difficult to control8. All isolated Serratia spp were identified from IPD blood samples sent for blood culture and all of them were ESBL producers. Serratia infections are clearly related to hospitalization9. Comparable findings were documented by other studies15,16,17.

The present study reveals significant number of ESBL producing gram negative bacteria which demands routine practice of ESBL testing in microbiology laboratory of SSMC for reporting.

References:


