Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, India

GAURAV DALELA

ABSTRACT

Background and Objectives: The resistance to broad spectrum β-lactams which is mediated by the extended spectrum beta lactamase (ESBL) enzyme is an increasing problem now-a-days. This resistance mechanism has been responsible for nosocomial outbreaks, serious therapeutic failure if it is not detected on time and the outbreak of multidrug resistant, gram negative pathogens that need expensive control measures. As no data was available on the prevalence of ESBL in this region, the current study was undertaken to determine the prevalence of the ESBL producing strains in our hospital based population of Jhalawar.

Aim: To know the prevalence of ESBL producing organisms at our tertiary care hospital at Jhalawar.

Material and Methods: A total of 219 consecutive, non-repetitive, gram negative isolates, which were resistant to one of the third generation cephalosporins (cefotaxime, ceftriaxone or ceftazidime) were selected as “Suspicious for ESBL production” as recommended by the Clinical and Laboratory Standards Institute (CLSI). These isolates were confirmed for ESBL production by the double disc synergy test (DDST) and the phenotypic confirmatory disc diffusion test (PCDDT) and they were further confirmed by the E-test ESBL strip randomly.

Result: Out of the 219 isolates which were tested, 135 (61.6%) were found to be ESBL producers by PCDDT and 126 (57.5%) were found to be ESBL producers by DDST. Twenty-eight randomly selected isolates were further confirmed by the E-test ESBL strip, which showed a highly significant correlation with PCDDT (p value <0.001). The isolates of Escherichia coli (73.5%) were the most common ESBL producers, followed by Proteus vulgaris (60%), K. pneumoniae (58.1%) and others. The maximum ESBL production was seen in urine (66.4%), followed by pus (57.3%) and others (54.2%). Imipenem (98.5%), piperacillin/tazobactum (72.6%) and amikacin (64.5%), in the decreasing order, were the most active and reliable agents for the treatment of the infections which were caused by the ESBL producing organisms.

Conclusion: There is a high prevalence of ESBL production in our hospital and so, it is essential to report the ESBL production along with the routine sensitivity reports, which will help the clinician in prescribing proper antibiotics. Also, control measures which include the judicious use of antibiotics, antibiotic cycling, the implementation of appropriate infection control measures and the formulation of an antibiotic policy must be done, to prevent the spread of these strains.

Key Words: Extended spectrum beta lactamase, DDST, PCDDT, E-test ESBL, Prevalence

INTRODUCTION

The ever-increasing bacterial resistance to antibiotics is one of the most challenging tasks of all the medical issues which are being faced by us today. A single mutation in bacteria which leads to a new resistance mechanism against various drugs is like undoing within moments, the great efforts in developing these drugs, of a great mind.

The persistent exposure of the bacterial strains to a multitude of β-lactams has induced a dynamic and continuous production and mutation of β-lactamases in the bacteria, expanding their activity even against the third and fourth generation cephalosporins such as ceftazidime, cefotaxime and cefepime and also against aztreonam. These new β-lactamases are called extended spectrum β-lactamases (ESBLs) [1].

ESBL enzymes are plasmid borne and they have evolved from point mutations which altered the configuration of the active site of the original and long known β-lactamases, which have been designated as TEM-1, TEM-2, and SHV-1 [1]. The resistance to newer β-lactams which are a result of these β-lactamases, has emerged quickly.

The first report of plasmid-encoded β-lactamases which are capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 [2].

ESBL production has become more and more common in a variety of enteric bacilli other than Klebsiella spp. or Escherichia coli (e.g. Enterobacter aerogenes, E. cloacae, Serratia marcescens, Morganella morganii, Providentia spp., Citrobacter freundii and C. koserii), as well as in non-enteric bacilli (e.g. Pseudomonas aeruginosa) [3]. ESBLs have also been reported in Acinetobacter...
Extended spectrum beta lactamase, DDST, PCDDT, E-test ESBL, Prevalence

The risk factors for the infection or colonization with the ESBL producing organisms include: the length of hospital or ICU stay, the presence of vascular or urinary catheters, undergoing haemodialysis or emergency abdominal surgery, gut colonization, low birth weight, prior exposure to any antibiotic (e.g., quinolones, trimethoprim-sulfamethoxazole, aminoglycoside and metronidazole), prior ceftazidime or aztreonam administration and prior stay in a long term care facility [2].

Generally, an isolate is suspected to be an ESBL producer when it shows in vitro susceptibility to the second generation cephalosporins (cefoxitin and cefotetan) and resistance to the third generation cephalosporins and to aztreonam. Moreover, one should suspect these strains when the treatment with these agents for gram negative infections fails despite the in vitro susceptibility reports. Once an ESBL producing strain is detected, the laboratory should report it as “resistant” to all penicillins, cephalosporins, and aztreonam, even if it tests as susceptible [1].

The best means of testing for ESBLs is an initial screening for reduced susceptibility to cefpodoxime, cefotaxime, ceftaxzone, ceftazidime, or aztreonam, and then performing the phenotypic confirmatory test by demonstrating a synergistic effect between an indicator cephalosporin and a β-lactamase inhibitor (usually clavulanic acid) [3]. The NCCLS/CLSI have recommended the combined disk method and the MIC method for ESBL confirmation [3].

As no data was available on the prevalence of ESBL in this region, the current study was undertaken to determine the prevalence of ESBL producing, gram negative bacilli from various clinical isolates in our hospital based population of Jhalawar.

MATERIAL AND METHODS

Bacterial isolates: A total of 219 consecutive, non-repetitive, gram negative isolates from various clinical samples such as urine (n=113), pus (n=82), ear swab (n=8), stool (n=7), vaginal swab (n=4), sputum (n=2), pleural fluid (n=2) and conjunctival swab (n=1) which were received in the clinical bacteriology laboratory, Jhalawar Medical College, Jhalawar, Rajasthan, from December 2010 to mid-August 2011, were included in the study.

Antimicrobial susceptibility testing: The isolates were tested by the disc diffusion method (modified Kirby-Bauer method) on Muller Hinton agar (Hi-Media) following the zone size criteria which was recommended by the CLSI [4]. The antibiotics (µg) which were included were amikacin (30), piperacillin (100), piperacillin/tazobactum (100/10), cefepime (30), cefotaxime (30), ceftaxzone (30), ceftazidime (30), amoxyclav (20/10), cotrimoxazole (25), ciprofloxacin (5), imipenam (10), doxycycline (30) and azithromycin (15).

Criteria for the selection of the ESBL producing strains: The isolates were tested for their susceptibility to the third generation cephalosporins (3GCs) e.g. ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) by using the standard disc diffusion method as recommended by the CLSI [4]. If a zone diameter of ≤ 22 mm for ceftazidime, ≤ 27 mm for cefotaxime and ≤ 25 mm for ceftriaxone were recorded, the strain was considered to be “suspicious for ESBL production” [4]. Only those isolates which were resistant to one of the 3 GCs were selected for the study and were processed for ESBL production.

The double disc synergy test (DDST): According to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines [5], isolates which were presumed to be ESBL producers on the basis of the screening test results, were picked up and emulsified in saline to a 0.5 McFarland’s turbidity standard. Discs of ceftazidime (30 µg), cefotaxime (30 µg) and amoxyclav (20 µg amoxicillin and 10 µg clavulanic acid) were placed at a distance of 20 mm from center to center in a straight line, with the amoxyclav disc in the middle on a plate of Mueller Hinton Agar (MHA) being inoculated with the test strain. The plates were incubated at 37°C aerobically overnight. Isolates which showed an enhancement of the zone of inhibition as greater than 5 mm on the amoxyclav side of the disc as compared to that which was seen on the side without amoxyclav, were confirmed as ESBL producers [5]. [Table/Fig-1]

The phenotypic confirmatory disc diffusion test (PCDDT): All the strains which were screened out for ESBL production were also subjected to confirmation by using the PCDDT, as recommended by the CLSI [4]. The ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg discs) were applied onto a plate of Mueller Hinton Agar (MHA) which was inoculated with the test strain. An increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be a...
Gaurav Dalela, Prevalence of ESBL Producers among Gram Negative Bacilli from Various Clinical Isolates

marker for ESBL production [4] [Table/Fig-2].

The ESBL E Test: The E-test ESBL strips (AB Biodisk, Sweden) [6] carry two gradients, ceftazidime (0.5-32 µg/ml) on the one end and ceftazidime plus clavulanic acid (0.064-4 µg/ml) in a different concentration gradient on the other end, along with a fixed concentration of clavulanic acid (4 µg/ml). A lawn culture of the test organism was plated on Mueller Hinton Agar (MHA) on which the E-test ESBL strip was placed on the centre of the plate. The plates were incubated aerobically at 37°C for 16-18 hours. The MIC was interpreted at the point of intersection of the inhibition eclipse with the E-test strip edge. The presence of ESBL was confirmed by the appearance of a phantom zone or by the deformation of the TZ eclipse or when the ceftazidime MIC was reduced by >3 log, dilutions (ratio TZ/TZL: >8) in the presence of clavulanic acid [Table/Fig-3] as per the manufacturer’s guidelines [6].

Quality control: β-lactamase negative Escherichia coli ATCC 25922 was used as the negative control and ESBL-producing Klebsiella pneumoniae ATCC 700603 was used as the positive control throughout the study [4].

Statistical analysis: Statistical analysis was performed by the Chi-square test and a p value of less than 0.05 was considered as statistically significant.

RESULT

The present study was conducted in the Department of Microbiology, Jhalawar Medical College, Jhalawar, Rajasthan from December 2010 to mid August 2011, to know the prevalence of ESBL producing gram negative bacilli in various clinical isolates at our tertiary health care centre.

The antibiotic sensitivity pattern revealed that the maximum sensitivity was seen for imipenem (96.8%), followed by piperacillin/tazobactum (69.9%), amikacin (59.8%), azithromycin (45.7%), ciprofloxacin (42.5%), and cefepime (32.4%). A high resistance rate was seen for cotrimoxazole (83.5%), ceftriaxone (83.1%), ceftazidime (80.8%), doxycycline and amoxycilav (80.4%), piperacillin (77.6%), and cefotaxime (78.1%) [Table/Fig-4].

Out of the 219 gram negative bacilli, 135 (61.6%) were confirmed as ESBL producers. DDST detected only 126 ESBL producers and all the 135 were detected by PCDDT (p value <0.05) [Table/Fig-5].

Three strains of Klebsiella pneumoniae, 4 strains of Escherichia coli and 2 strains of Pseudomonas aeruginosa were not diagnosed as ESBL producers by DDST, which were detected as ESBL producers by PCDDT. The rest of the bacteria showed similar prevalence by both DDST and PCDDT, thus indicating a highly significant correlation (p value <0.001) [Table/Fig-5].

Escherichia coli was the most common ESBL producer which was found, followed by Proteus vulgaris and others, as shown by both PCDDT and DDST [Table/Fig-5].

Twenty-eight randomly selected isolates (14 Escherichia coli, 9 Klebsiella pneumoniae and 5 Pseudomonas aeruginosa) were further confirmed by the E-test ESBL strip (AB Biodisk, Sweden), which showed a highly significant correlation with PCDDT (p value <0.001).

The specimen wise distribution of the ESBL producers is shown in [Table/Fig-6]. The maximum ESBL production was seen in urine (66.4%), followed by pus (57.3%) and others (54.2%) (p value <0.05). Escherichia coli showed the maximum ESBL prevalence in pus (76.9%), followed by others (75%) and urine (72.2%). [Table/Fig-6]

The ESBL producers had more sensitive isolates for imipenem (98.5%), piperacillin/tazobactum (72.6%) and amikacin (64.5%) as compared to their non ESBL producing counterparts (p value >0.05). The non ESBL producers had different mechanisms for their resistance pattern. For other antibiotics, the resistance pattern was found

<table>
<thead>
<tr>
<th>Antibiotic (n = 219)</th>
<th>Sensitive (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>131 (69.8%)</td>
<td>4 (1.8%)</td>
<td>84 (38.4%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>93 (42.5%)</td>
<td>6 (2.7%)</td>
<td>120 (54.8%)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>71 (32.4%)</td>
<td>0 (0%)</td>
<td>148 (67.6%)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>40 (18.2%)</td>
<td>3 (1.4%)</td>
<td>176 (80.4%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>49 (22.4%)</td>
<td>0 (0%)</td>
<td>170 (77.6%)</td>
</tr>
<tr>
<td>Piperacillin/</td>
<td>153 (69.9%)</td>
<td>2 (0.9%)</td>
<td>64 (29.2%)</td>
</tr>
<tr>
<td>Tazobactum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>212 (96.8%)</td>
<td>3 (1.4%)</td>
<td>4 (1.8%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>100 (45.7%)</td>
<td>4 (1.8%)</td>
<td>115 (52.2%)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>35 (16%)</td>
<td>1 (0.5%)</td>
<td>183 (83.5%)</td>
</tr>
<tr>
<td>Amoxycilav</td>
<td>41 (18.7%)</td>
<td>2 (0.9%)</td>
<td>176 (80.4%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>46 (21%)</td>
<td>2 (0.9%)</td>
<td>171 (78.1%)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>35 (16%)</td>
<td>2 (0.9%)</td>
<td>182 (83.1%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>41 (18.7%)</td>
<td>1 (0.5%)</td>
<td>177 (80.8%)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>PCDDT*</th>
<th>DDST #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>25/43</td>
<td>22/43</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>86/117</td>
<td>82/117</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>13/35</td>
<td>11/35</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Acinetobacter lowffi</td>
<td>4/7</td>
<td>4/7</td>
</tr>
<tr>
<td>Citrobacter koserii</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>126</td>
</tr>
</tbody>
</table>

*Phenotypic confirmatory disc diffusion test; # Double disc synergy test.

[Table/Fig-3]: E-test ESBL strip showing clear cut ESBL positive organism showing ceftazidime (TZ) MIC is reduced by >3 log, dilutions (ratio TZ/TZL: >8) in the presence of clavulanic acid

[Table/Fig-4]: Antibiotic susceptibility pattern of gram negative bacilli in various clinical isolates

[Table/Fig-5]: ESBL producers among gram negative bacilli in various clinical specimens

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to be more in the ESBL producers as compared to their non-ESBL producing counterparts (p value <0.01) [Table/Fig-7, 8].

Only 2 ESBL producing strains, one of which was Proteus mirabilis, showed a sensitive pattern to ceftazidime and the second, Proteus vulgaris, showed intermediate sensitivity to ceftazidime. All of the other isolates showed resistance to ceftazidime, indicating that ceftazidime was a good drug for the detection of the ESBL activity having significant correlation (p value <0.01) [Table/Fig-7, 8].

DISCUSSION

The spread of ESBL producing bacteria has become strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. The therapeutic options for the infections which are caused by these organisms have also become increasingly limited [7].

A number of nosocomial outbreaks which were caused by ESBL producing organisms, have been reported in the United States [8-10] Although most of the outbreaks were limited to high risk patient care areas such as ICUs, oncology units etc., the first report of an outbreak in nursing homes appeared in the literature in the year 1999 [11]. Therefore, now-a-days, the threat of ESBL producing isolates is not limited to ICUs or tertiary care hospitals only.

The Clinical and Laboratory Standards Institute (CLSI) has issued recommendations for ESBL screening, for the confirmation of the isolates of Escherichia coli and Klebsiella spp., and for reporting the confirmed organisms. No CLSI recommendations existed for ESBL detection and for the reporting of other organisms [12] which were included in our study.

Previous studies from India have reported the prevalence of the

<table>
<thead>
<tr>
<th>Sample Organism</th>
<th>Urine ESBL producer/Isolate tested (%)</th>
<th>Pus ESBL producer/Isolate tested (%)</th>
<th>Others ESBL producer/Isolate tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>11/17 (64.7%)</td>
<td>10/20 (50%)</td>
<td>4/6 (66.7%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>57/79 (72.2%)</td>
<td>20/26 (76.9%)</td>
<td>9/12 (75%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2/7 (28.6%)</td>
<td>11/23 (47.8%)</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0/1 (0%)</td>
<td>2/3 (66.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>2/3 (66.7%)</td>
<td>1/2 (50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>2/3 (66.7%)</td>
<td>2/4 (50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>0/1 (0%)</td>
<td>0/2 (0%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>75/113 (66.4%)</td>
<td>47/82 (57.3%)</td>
<td>13/24 (54.2%)</td>
</tr>
</tbody>
</table>

[Table/Fig-6]: Specimen and organism wise distribution of ESBL producers

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
<th>Sensitive (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>64.5</td>
<td>2.2</td>
<td>33.3</td>
<td>52.4</td>
<td>1.2</td>
<td>46.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>36.3</td>
<td>4.4</td>
<td>59.3</td>
<td>52.4</td>
<td>0</td>
<td>47.6</td>
</tr>
<tr>
<td>Cefepime</td>
<td>24.4</td>
<td>0</td>
<td>75.6</td>
<td>45.2</td>
<td>0</td>
<td>54.8</td>
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<tr>
<td>Doxycycline</td>
<td>14.1</td>
<td>0.7</td>
<td>85.2</td>
<td>25</td>
<td>2.4</td>
<td>72.6</td>
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<tr>
<td>Piperacillin</td>
<td>10.4</td>
<td>0</td>
<td>89.6</td>
<td>41.7</td>
<td>0</td>
<td>58.3</td>
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<tr>
<td>Piperacillin/Tazobactum</td>
<td>72.6</td>
<td>1.5</td>
<td>25.9</td>
<td>65.5</td>
<td>0</td>
<td>35.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>98.5</td>
<td>0</td>
<td>1.5</td>
<td>94</td>
<td>3.6</td>
<td>2.4</td>
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<td>Azithromycin</td>
<td>38.5</td>
<td>3</td>
<td>58.5</td>
<td>57.1</td>
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<td>42.9</td>
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<tr>
<td>Cotrimoxazole</td>
<td>13.3</td>
<td>0</td>
<td>86.7</td>
<td>20.2</td>
<td>1.2</td>
<td>78.6</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>15.6</td>
<td>1.5</td>
<td>82.9</td>
<td>23.8</td>
<td>0</td>
<td>76.2</td>
</tr>
<tr>
<td>Ceftotaxime</td>
<td>11.1</td>
<td>1.5</td>
<td>87.4</td>
<td>36.9</td>
<td>0</td>
<td>63.1</td>
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<tr>
<td>Ceftriaxone</td>
<td>20.7</td>
<td>0.7</td>
<td>78.6</td>
<td>8.3</td>
<td>1.2</td>
<td>90.5</td>
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<tr>
<td>Ceftazidime</td>
<td>0.7</td>
<td>0.7</td>
<td>98.5</td>
<td>47.6</td>
<td>0</td>
<td>52.4</td>
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</table>

[Table/Fig-7]: Comparative study of antibiotic susceptibility pattern in both ESBL producers and non ESBL producers


<table>
<thead>
<tr>
<th>Author</th>
<th>Place</th>
<th>Year</th>
<th>Prevalence %</th>
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<tr>
<td>Subha A et al</td>
<td>Chennai</td>
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<td>Jerestin Hansotia et al</td>
<td>Nagpur</td>
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<td>Bhattacharjee et al</td>
<td>Varanasi</td>
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<td>Meerut</td>
<td>2005</td>
<td>36</td>
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<td>S Babypadmini et al</td>
<td>Coimbatore</td>
<td>2004</td>
<td>40.3</td>
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<tr>
<td>C Rodrigues et al</td>
<td>Mumbai</td>
<td>2005</td>
<td>53</td>
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<tr>
<td>Present Study</td>
<td>Jalalpur</td>
<td>2011</td>
<td>61.6</td>
</tr>
<tr>
<td>S Singhal et al</td>
<td>Gurgaon</td>
<td>2005</td>
<td>64</td>
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<tr>
<td>Purva Mathur et al</td>
<td>New Delhi</td>
<td>2002</td>
<td>68</td>
</tr>
</tbody>
</table>

[Table/Fig-9]: Comparative studies in different regions of India
ESBL producers to be 6.6 to 68%. In south India, Subha et al. reported 6.6% ESBL producers among Klebsiella pneumonia from children, whereas Babypadmini et al. [14] reported 40.3% ESBL producers in their study cohort. The ESBL production which was reported among gram negative bacteria by Mathur et al. [15] was 68%. S Singhal et al. [16] detected ESBL positivity in 64% isolates and C. Rodrigues et al. [17] reported 53% ESBL production and 65.8%, 61.7% and 27.9% ESBL positivity among E. coli, K. pneumoniae and Pseudomonas aeruginosa respectively. These findings correlated well with those of our study. The occurrence of ESBL producers among gram negative bacilli in the current study was 135/219 (61.6%), while 73.5% E. coli, 60% Proteus vulgaris, 58.1% Klebsiella pneumoniae, 57.1% Acinetobacter lowffi, 50% Proteus mirabilis, 50% Citrobacter koserii and 37.1% Pseudomonas aeruginosa were found to elaborate ESBLs. [Table/Fig-9].

In the present study, we also observed that 73.5% E. coli and 58.1% Klebsiella pneumoniae isolates were ESBL producers. Although K. pneumoniae was more often reported as an ESBL producer in other studies, we observed that the ESBL production was more common in the E. coli isolates as compared to that in the K. pneumoniae isolates [14, 18, 19].

In Pseudomonas aeruginosa, the ESBL production was less (37.1%) as compared to that in other gram negative bacilli, because its resistance mechanism was mediated by the production of metallobetalactamase, lack of drug penetration due to mutations in the porins or due to the loss of certain outer membrane proteins and the efflux pump [16, 20, 21].

The PCDDT test was compared with DDST and it was found to be an inexpensive alternative for the DDST, for the detection of ESBL producers. The DDST lacks sensitivity because of the problem of optimal disc space and the correct storage of the clavulanic acid containing discs. Assuming that a laboratory is currently testing the sensitivity for ceftazidime by using the disc diffusion test and it required only one disc to be added to the sensitivity plate by PCDDT and would screen all gram negative bacteria in the diagnostic laboratory for ESBL production. This method is technically simple and inexpensive [22].

The Clinical and Laboratory Standards Institute (CLSI) therefore, also recommended the use of PCDDT for the phenotypic confirmation of the ESBL producers among E. coli and K. pneumoniae [4].

In our study, we observed that a majority of the isolates were susceptible to imipenem (96.8%) and piperacillin/tazobactum (89.9%). Similarly, in a study from Coimbatore, all the members of Enterobacteriaceae were found to be susceptible to imipenem and piperacillin/tazobactum [23]. In both the studies, amikacin also showed good activity against gram negative bacteria as compared to other antibiotics. Therefore, imipenem is the most active drug for the treatment of infections which are caused by ESBL producers, followed by piperacillin/tazobactum and amikacin.

We need to keep in mind that carbapenem must be kept in reserve for non-life-threatening infections where other susceptible antibiotics can be used [17]. The heavy use of carbapenem, in fact, may favour the selection of Stenotrophomonas maltophilia (a species which is naturally resistant to these drugs) [24].

Cefpodoxime and cefazidime have been proposed as the indicators of ESBL production as compared to cefotaxime and ceftriaxone [25]. Only 2 ESBL producer strains, one of which was Proteus mirabilis, showed a sensitive pattern to ceftazidime and the second, Proteus vulgaris showed intermediate sensitivity to ceftazidime. All of the other isolates showed resistance to ceftazidime. So, ceftazidime is a better indicator for the detection of ESBL production as compared to cefotaxime and ceftriaxone, as was found in our study.

Many clinical laboratories are not fully aware of the importance of the ESBL producers and of methods to detect them. Laboratories may also lack the resources which are needed to curb the spread of these resistance mechanisms. This lack of understanding or resources is responsible for a continuing failure to respond appropriately to prevent the rapid, worldwide dissemination of the pathogens which possess these β-lactamases. The consequence of this has been avoidable therapeutic failures (sometimes fatal) in patients who received inappropriate antibiotics and outbreaks of infections which were caused by multidrug-resistant, gram negative pathogens that required expensive control efforts [12]. Hence, their detection must be quick, for formulating an antibiotic policy and containment measures to solve the issue of antibiotic resistance.

Therefore, the regular detection of ESBLs by conventional methods should be carried out in every laboratory where molecular methods cannot be performed, as genotyping is used only for the detection and confirmation of ESBLs and as it is not informative for selecting the right treatment. However, the techniques which are required for the task of identifying the exact ESBL subtype (e.g. DNA probing, polymerase chain reaction, restriction fragment length polymorphism and isoelectric focusing) are available only at research facilities [26].

**CONCLUSION**

A committee must be formed at all hospitals, which should provide guidelines for the judicious use of antibiotics and should formulate policies which will help in minimizing the emergence of resistant bacteria among the patients. There is a possibility that the restricted use of antibiotics can lead to the withdrawal of selective pressure and that the resistant bacteria will no longer have a survival advantage against these antibiotics.

To conclude, as we know, clinical laboratories are the first to encounter bacteria with new forms of antibiotic resistance and so they need appropriate tools to recognize these bacteria, including trained staff with sufficient time and equipment to follow up the important observations. Also, it is essential to report ESBL production along with the routine susceptibility testing, which will help the clinician in prescribing proper antibiotics. In the end, it has been felt that there is a need to formulate strategies to detect and prevent the emergence of ESBL producing strains for the effective treatment of infections which are caused by them.

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AUTHOR(S): 
1. Dr. Gaurav Dalela

PARTICULARS OF CONTRIBUTORS: 
1. MBBS, Assistant Professor, Department of Microbiology, 
Jhalawar Medical College, Jhalawar, Rajasthan (PIN – 326001), India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE 
CORRESPONDING AUTHOR: 
Dr. Gaurav Dalela, MBBS, MD, 
Assistant Professor, Deptt of Microbiology 
Jhalawar Medical College Jhalawar, Rajasthan (PIN – 326001), India. 
Phone: 09314607245 
E-mail: gauravdalela@yahoo.com

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