Prevalence and Antimicrobial Resistance Pattern of Extended Spectrum Beta Lactamase Producing Gram Negative Bacterial Isolates Obtained From Various Clinical Samples of Indoor Patients Admitted in a Tertiary Care Hospital

Akanksha Chopra1, Kanwardeep Singh2, Shailpreet Sidhu3, Loveena Oberoi4, Arpandeep Kaur5

ABSTRACT

Introduction: Antimicrobial resistance is a cause of global concern as resistance is emerging enormously in hospital and community settings. The occurrence of resistance to cephalosporins due to production of Extended Spectrum Beta- Lactamases is known worldwide. Hence, this study was undertaken to detect the prevalence and antimicrobial resistance pattern of ESBL-producing gram-negative bacteria isolated from various clinical samples received from the indoor patients of a tertiary care hospital.

Material and Methods: Clinical specimens received from the patients admitted in Guru Nanak Dev Hospital, Amritsar from January 1, 2018 to June 30, 2018 were included in the study. The samples were processed based on standard microbiological techniques. ESBL screening and confirmation were done based upon CLSI guidelines. Antimicrobial resistance pattern of ESBL producing gram negative bacteria was determined.

Result: A total of 8147 samples were received out of which 1061(13.02%) gram negative bacteria were isolated. 227 (21.97%) of the gram negative isolates were positive on screening and 107 (10.08%) were confirmed to be ESBL producers phenotypically. Maximum antimicrobial resistance was observed to ciprofloxacin and amikacin. All the isolates were sensitive to sulbactam ceftriaxone and imipenem.

Conclusion: The present study highlights the prevalence of ESBL-producing gram negative bacterial isolates in a tertiary care hospital in Amritsar, Punjab. Measures such as the establishment of antimicrobial stewardship activities, monitoring surveillance and infection control programmes, emphasizing on effective hand hygiene practices together with coherent antibiotic policies should be enforced in the hospitals to arrest the spread of ESBLs.

Keywords: Prevalence and Antimicrobial Resistance Pattern, Spectrum Beta Lactamase, Gram Negative Bacterial Isolates

INTRODUCTION

Antimicrobial resistance is a cause of global concern as resistance is emerging enormously in hospital and community settings. Rapid detection of resistance is important for the early recognition of antimicrobial resistant organisms. New resistance mechanisms are emerging and spreading globally limiting our ability to treat common infectious diseases, resulting in prolonged hospital stay, and increased mortality. As suggested by the World Health Organization, microorganisms that develop antimicrobial resistance are referred to as “superbugs”. As a result, the antimicrobials have become ineffective and infections persist in the body, increasing the risk of spread to others. One of the most commonly used and effective group of antibiotics, cephalosporins, exhibit resistance due to production of Extended Spectrum Beta- Lactamases (ESBLs). The prevalence of ESBL producing gram negative bacteria has increased throughout the world and is a major cause of treatment failure in hospital settings. Thus, the prudent use of antimicrobials is as crucial as finding alternatives of carbapenems to treat the infections caused by ESBL-producing isolates and avoid exacerbation of the spreading of ESBLs.

When detecting ESBL-positive strains, microbiology laboratories should provide the clinician with reliable therapeutic options for successfully treating infected patients, since ESBL-distribution has been shown to differ among countries. Inadequate surveillance practices and inefficient regulatory system play a key role in the determination of antimicrobial resistance. The testing of the samples is generally undertaken only when patients fail to respond to common treatments. Hence, this study was undertaken to detect the prevalence of ESBL-producing gram-negative bacterial isolates obtained from various clinical samples received from the indoor patients of a tertiary care hospital and to study their antimicrobial susceptibility pattern.

MATERIAL AND METHODS

A prospective study was conducted in the department of

1-Junior Resident, Department of Microbiology, 2-Professor, Department of Microbiology, 3-Assistant Professor, Department of Microbiology, 4-Professor and Head Of Department, Department of Microbiology, 5-Senior Resident, Department of Microbiology, Government Medical College, Amritsar, India

Corresponding author: Dr. Kanwardeep Singh, Professor, Department of Microbiology, Government Medical College, Circular Road, Amritsar, India

How to cite this article: Akanksha Chopra, Kanwardeep Singh, Shailpreet Sidhu, Loveena Oberoi, Arpandeep Kaur. Prevalence and antimicrobial resistance pattern of extended spectrum beta lactamase producing gram negative bacterial isolates obtained from various clinical samples of indoor patients admitted in a tertiary care hospital. International Journal of Contemporary Medical Research 2019;6(3):C1-C4.

DOI: http://dx.doi.org/10.21276/ijcmr.2019.6.3.17
Microbiology, Government Medical College and hospital, Amritsar. Various Clinical specimens (blood, urine, sputum, pus, fluids, etc.) received from the patients admitted in various departments of Guru Nanak Dev Hospital, Amritsar from January 1, 2018 to June 30, 2018 were included in the study.

The samples were processed based on standard microbiological techniques. All the samples were cultured on Blood Agar and McConkey’s Agar and incubated for 24 hours aerobically at 37°C. Provisional identification of the organisms was made based on the colony characters, motility and gram staining. Final identification was made on the basis of biochemical reactions. Gram negative bacterial isolates were identified. Test organisms from stock culture were activated by inoculation into Nutrient Broth and incubated at 37°C for 15-20 minutes. The concentration of the bacterial suspension was adjusted to be equivalent to 0.5 McFarland unit. The test organism was seeded on the surface of freshly prepared Mueller Hinton agar using a sterile Dacron swab, as per the CLSI guidelines. 10 The plates were then allowed to stand at room temperature for 15 minutes prior to the application of antibiotic discs.

The Antibiotic Susceptibility pattern of the gram negative bacterial isolates were determined against various antibacterial agents. The antibiotics discs which were included were amoxicillin (10 µg), amoxicillin clavulanic acid (20/10 µg), piperacillin (100 µg), piperacillin/tazobactam (100/10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), imipenem (10 µg). Plates were incubated aerobically at 37°C for 18-24 hours, and the diameter of the zone of inhibition (if any) around the antimicrobial discs was measured in mm using a ruler.

The screening of ESBL producing organisms was done by standard disc diffusion procedure using antimicrobial discs of cefpodoxime (10 µg), cefazidime (30 µg), cefotaxime (30 µg) and ceftiraxone (30 µg). More than one of these agents was used for screening to improve the sensitivity of ESBL detection, as per CLSI guidelines. 10 Thereafter, the isolates which were suspected to be ESBL producers were tested for the confirmatory test of ESBL production, discs of cefotaxime (30 µg) and ceftazidime (30 µg) discs with and without clavulanate (10 µg) were used for phenotypic confirmation of the presence of ESBL. A difference of ≥ 5 mm between the zone diameters of either of the cephalosporin discs and their respective cephalosporin/clavulanate discs was taken for phenotypic confirmation of ESBL production. 9 To detect the isolates co-producing AmpC and ESBL, AmpC inhibitor cloxacillin was added to the culture media while performing the confirmatory test.

**RESULT**

A total of 8147 samples were received during a period of six months. Out of them 1061 (13.02%) gram negative bacteria were isolated. All of these bacterial isolates were screened for ESBL production and 227 (21.97%) of the gram negative isolates were found to be resistant to the third generation cephalosporins. Confirmatory test was performed on the isolates which were found to be positive on screening and 107 (10.08%) isolates were confirmed to be ESBL producers phenotypically. 23 (10.132%) isolates were found to be AmpC and ESBL co-producers (graph-1).

The highest prevalence of ESBL producing gram negative bacteria was observed in urine samples (53.2%) followed by body fluids (25%), sputum (18.6%), pus (16.11%), and blood (11.11%).

![Figure-1: Sample wise distribution of various ESBL producing gram negative bacilli](image)

**Table-2: Sample wise distribution of Extended Spectrum Beta Lactamase Producing Gram negative bacteria**

<table>
<thead>
<tr>
<th>Site</th>
<th>Urine</th>
<th>Pus</th>
<th>Blood</th>
<th>Respiratory Samples</th>
<th>Vaginal Swabs</th>
<th>Body Fluids</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
<td>No.</td>
<td>%age</td>
<td>No.</td>
<td>%age</td>
<td>No.</td>
<td>%age</td>
<td>No.</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>49</td>
<td>85.9</td>
<td>9</td>
<td>45</td>
<td>1</td>
<td>8.3</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>6</td>
<td>10.52</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>3.50</td>
<td>7</td>
<td>35</td>
<td>1</td>
<td>8.3</td>
<td>-</td>
</tr>
<tr>
<td><em>Citrobacter</em> species</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td><em>Proteus</em> species</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>57</td>
<td>20</td>
<td>12</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>107</td>
</tr>
</tbody>
</table>
pus (18.6%), blood cultures (11.21%), respiratory samples (8.41%) Vaginal swabs (4.67%) followed by Body fluids (3.73%) (table-1).

*Escherichia coli* (57.9%) showed the highest prevalence of ESBL production, followed by *Klebsiella pneumoniae* (25.2%), *Pseudomonas aeruginosa* (9.3%), *Citrobacter* species (4.6%) and *Proteus* species (2.8%) (figure-2, table-2). Antimicrobial susceptibility testing of the ESBL producing gram negative isolates was performed. All the strains were found to be sensitive to imipenem and sulbactam ceftriaxone. Maximum resistance was observed to ciprofloxacin and aminoglycosides (table-3).

### DISCUSSION

Monitoring of the prevalence of extended spectrum beta lactamase enzymes may contribute to defining the degree of the problem in a specific geographical area, and establishing a proper treatment protocol. A regional strategy on antimicrobial resistance has been proposed by the World Health Organization with the goal to preserve the effectiveness of antimicrobial agents in the treatment and prevention of microbial infections and therefore decreasing the morbidity and mortality.7

The prevalence of Extended spectrum beta lactamase producing gram negative bacteria is alarmingly increasing and will continue to rise if adequate and timely measures are not undertaken. The ability of the spread of the resistance mediating genes via horizontal drug resistance transfer further aggravates the need of an effective action plan to combat this drug resistance.

In our study, 10.132% of gram negative bacterial isolates were phenotypically confirmed to be ESBL producers, whereas prevalence ranging from 4% to 84% has been reported in various parts of India.11,12

In the present study, urine samples were the most common source of ESBL producing gram negative bacilli. This is in concordance to the study performed by Shanthi M et al and Abhikash KP et al.13,14

Among various gram negative bacterial isolates highest ESBL production was observed in *Escherichia coli* which was similarly found in other studies.15 As per Umadevi et al., ESBL production is less in *Pseudomonas spp* compared to *Enterobacteriaceae* and is in accordance with present study.16

Our study indicates that all the ESBL producing isolates were sensitive to imipenem. Various other authors have also reported imipenem sensitivity amongst ESBL producing gram negative bacterial isolates.17 Among β-Lactam/β-Lactamase inhibitor drugs highest sensitivity has been observed with ceftriaxone sulbactum followed by piperacillin tazobactum while high resistance was observed to ciprofloxacin and

<table>
<thead>
<tr>
<th>Organism</th>
<th><em>Escherichia coli</em> (n=62)</th>
<th><em>Klebsiella pneumonia</em> (n=27)</th>
<th><em>Pseudomonas aeruginosa</em> (n=10)</th>
<th><em>Citrobacter</em> species (n=5)</th>
<th><em>Proteus</em> species (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial</td>
<td>No.</td>
<td>%age</td>
<td>No.</td>
<td>%age</td>
<td>No.</td>
</tr>
<tr>
<td>Amikacin</td>
<td>32</td>
<td>51.61</td>
<td>16</td>
<td>59.25</td>
<td>5</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>18</td>
<td>290</td>
<td>8</td>
<td>29.6</td>
<td>4</td>
</tr>
<tr>
<td>Piperacillin Tazobactum</td>
<td>3</td>
<td>4.8</td>
<td>2</td>
<td>7.40</td>
<td>1</td>
</tr>
<tr>
<td>Subbactam Ceftriaxone</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>36</td>
<td>58</td>
<td>18</td>
<td>66.66</td>
<td>5</td>
</tr>
<tr>
<td>Amoxicillin clavulanate</td>
<td>15</td>
<td>24.1</td>
<td>11</td>
<td>40.7</td>
<td>-</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>28</td>
<td>45.1</td>
<td>12</td>
<td>44.44</td>
<td>3</td>
</tr>
<tr>
<td>Imipenam</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Table-3: Antimicrobial resistance pattern of ESBL producing gram negative bacteria

**Figure-2:** Sample wise distribution of Extended Spectrum Beta Lactamase Producing Gram negative bacteria
amikacin. All the ESBL producing gram negative bacterial isolates exhibited Multiple Drug Resistance (MDR). These findings were similarly found by agreement with various other studies.13,15

CONCLUSION

The present study highlights the prevalence of ESBL-producing gram negative bacterial isolates in a tertiary care hospital in Amritsar, Punjab. All the multi drug resistant strains isolated retained their sensitivity against imipenem which conserves its ability as a reserve antimicrobial agent. To retain the use of antimicrobial agents, routine ESBL testing along with conventional antibiogram is highly recommended. This will aid in the proper treatment of the patient and also prevent development of multi drug resistant bacteria in hospital and community settings. Measures such as the establishment of antimicrobial stewardship activities, monitoring surveillance and infection control programmes, emphasizing on effective hand hygiene practices together with coherent antibiotic policies emphasising on first line empirical therapy should be enforced in the hospitals and clinics to arrest the spread of ESBLs.

REFERENCES


Source of Support: Nil; Conflict of Interest: None
Submitted: 19-01-2019; Accepted: 05-03-2019; Published: 26-03-2019