Determination of the prevalence of extended spectrum β-lactamase in clinical samples collected from Dehradun City Hospital

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Objective: To detect extended spectrum β-lactamase (ESBL) and determine its prevalence in various clinical samples collected from Dehradun City Hospital.

Methods: The samples were first cultured in MacConkey’s agar plates by streak plate method, then identified by Gram staining and biochemical tests. The isolated bacterial strains were then tested for antibiotic susceptibility by Kirby-Bauer method. The ESBL detection is then carried out by double disc diffusion method.

Results: Off the 56 samples cultured, 21 strains were identified which were six Escherichia coli (E. coli), six Klebsiella, four Proteus, four Pseudomonas aeruginosa (P. aeruginosa) and only one Acinetobacter. Eight out of 21 (38.1%) strains including three of E. coli, three of Klebsiella and two of P. aeruginosa, were found to be resistance to all five antibiotics (piperacillin, amikacin, ampicillin, gentamicin, and ciprofloxacin). Initial screening using four antibiotics (cefotaxime, ceftazidime, aztreonam and ceftriaxone) and the final confirmatory test using ceftazidime/clavulanic acid and ceftazidime alone showed that 19.05% of all strains isolated were ESBL producers. Individually, 16.67% E. coli, 16.67% Klebsiella pneumoniae, 25% P. aeruginosa and 100% Acinetobacter were found to be ESBL producers.

Conclusions: Antibiotic resistance by ESBL has become a major risk factor worldwide, therefore routine checkup and accordingly prescription are suggested.

1. Introduction

β-lactamase are enzymes which degrade the β-lactam antibiotics ranging from penicillin to carbapenems. This degradation in strain of Escherichia coli (E. coli) was first studied in 1940 by Abraham and Chain[1]. Based on substrate specificities, the β-lactamase family is divided into four functional groups including penicillinase, extended spectrum β-lactamase (ESBL), carbapenemase and AmpC type cephalosporinase[2]. These ESBLs can hydrolyze virtually all the penicillin and cephalosporin including extended spectrum cephalosporin such as cefotaxime or ceftazidime and comprise the largest and most prevalent group of enzyme[2]. Bacteria carrying ESBLs have been emerged as significant resistant to multiple antimiicrobial agent and can be challenging to treat as their therapeutic alternatives are few. There are various risk factors for the infection with the ESBL producing organisms such as length of hospital stay, the presence of vascular or urinary catheters, undergoing hemodialysis or emergency abdominal surgery, gut colonization, low birth weight and prior exposure to any antibiotic such as quinolones, trimethoprim-sulfamethoxazole, aminoglycoside and metronidazole[4]. The ESBLs is detected by initial screening for reduced susceptibility to different antibiotics like cefotaxime, ceftazidime, aztreonam and ceftriaxone) and the final confirmatory test using ceftazidime/clavulanic acid and ceftazidime alone showed that 19.05% of all strains isolated were ESBL producers. Individually, 16.67% E. coli, 16.67% Klebsiella pneumoniae, 25% P. aeruginosa and 100% Acinetobacter were found to be ESBL producers.

The epidemiology of ESBLs is quite complicated. Various studies have been conducted worldwide which show the different extent of ESBLs prevalence in different regions. A study in Nepal reported that 31.57% E. coli were confirmed as ESBL producers and these isolates further exhibited co-resistance to several antibiotics[6].
Tanzania, the ESBL prevalence was 64% in **Klebsiella pneumoniae** (*K. pneumoniae*) but 24% in *E. coli* and in Mali, 63% of the adults and 100% of the children were found to carry ESBL-producing Enterobacteriaceae (PE)[7,8]. About 26.5% of *E. coli* and 43% of *K. pneumoniae* were ESBL-positive in a study conducted in Iran. They indicated the high prevalence of ESBL-PE family especially in inpatients[9]. In Kuwait, the levels of ESBLs were found lower in community isolates of *K. pneumoniae* (17%) and *E. coli* (12%) than in the corresponding hospital isolates (28% and 26%, respectively)[10]. Researchers in Lebanon found that recently 24.8% carried ESBL-PE[11]. Recently, ESBL production was observed in 48% of *E. coli*, 44% of *K. pneumoniae* and 50% of *Pseudomonas aeruginosa* (*P. aeruginosa*) isolates in a tertiary hospital in Patiala, Punjab[12]. In other recent studies in India, prevalence of ESBL was found 46% for outpatients and 50% in inpatients, and 80% of clinical samples were found to be ESBL producers[13,14]. Similarly, Enterobacteriaceae, a prime producer of ESBLs was found containing New Delhi metallo-β-lactamase in India (6.9%) and in Pakistan (18.5%)[15]. Qureshi et al. reported that 72% of *E. coli* and 65.8% of *K. pneumoniae* were ESBL producers in Lahore[16]. In a most recent study, Rath et al. reported 12.11% ESBL-positive among ICU and NICU isolates and 22.47% ESBL-positive from nosocomial isolates[17]. According to a study, 53% of *E. coli* isolates were ESBL producers in Dehradun, India[18]. These all reports collectively indicate the increased risk of antibiotic resistance by ESBLs worldwide.

However, as very few data were 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 hospital based the population of Dehradun.

2. Materials and methods

2.1. Collection of clinical samples

In this study, 56 clinical samples were collected from outpatients of Doon Hospital Dehradun suspected for nosocomial infection like bronchitis, abscesses and gastritis during the period of May 2015. Samples collected were blood, respiratory pus, stool and gastric aspirate. Further processing and experimental work was carried out in the Department of Microbiology, Doon (PG) Paramedical College and Hospital Dehradun.

2.2. Isolation of pure culture

The bacterial samples, collected in sterile vials, were inoculated on MacConkey’s agar plates by streak plate method[19]. These plates were incubated at 37 °C in incubator for 24 h. The pink colored and pale yellow colored colonies appeared were further sub-cultured repeatedly on nutrient agar medium and incubated at 37 °C for 24 h. Pure cultures were further processed for the identification of bacteria.

2.3. Identification of bacteria

Identification of bacteria was carried out by Gram staining methods followed by various biochemical tests, namely, catalase test, sugar fermentation test, urease test, H₂S production test, citrate utilization test, methyl-red and Voges-Proskauer tests, and indole production test.

2.4. Antibiotic sensitivity test

Antimicrobial sensitivity test of all isolates was performed on diagnostic sensitivity test plates by the Kirby Bauer method following National Committee of Clinical Laboratory Standards guidelines[20]. Fresh cultures of tested isolates were inoculated into 5 mL normal saline. Then, suspension of bacterial culture was spread over the surface of Mueller-Hinton agar plates using sterile cotton swabs. Commercially available antibiotics discs, namely, piperacillin (10 mg/disc), gentamycin (10 mg/disc), amikacin (30 mg/disc), ampicillin (10 mg/disc) and ciprofloxacin (5 mg/disc) from Hi-Media, Mumbai were placed on plates using clean and sterile forceps and plates were incubated for 24 h at 37 °C. After 24 h of incubation, growth inhibition zone diameters were measured.

2.5. Analysis of ESBLs producer strains

2.5.1. Initial screening

Four antibiotics (cefotaxime, ceftazidime, aztreonam and ceftriaxone) were tested against 21 bacterial isolates[21].

2.5.2. Phenotypic confirmatory test

The isolates showing positive test were further tested with ceftazidime (30 μg) and in combination with clavulanic acid (30 μg/10 μg). The difference of zone of inhibition between ceftazidime/clavulanic acid and ceftazidime alone was determined[21].

3. Results

3.1. Identification of isolates

Six strains (DPMC1, DPMC2, DPMC3, DPMC8, DPMC9 and DPMC21) were identified as *E. coli*. Six strains (DPMC5, DPMC6, DPMC10, DPMC12, DPMC13 and DPMC14) were identified as *K. pneumoniae*. Four strains (DPMC15, DPMC18, DPMC19 and DPMC20)
were *P. aeruginosa*. Four strains (DPMC7, DPMC11, DPMC16 and DPMC17) were identified as *Proteus* and only one strain, DPMC4 was identified as *Acinetobacter*. Table 1 shows the identification of microorganisms isolated from different samples using Gram staining and biochemical tests and Table 2 shows the list of bacteria and their corresponding samples.

### Table 1
Identification of microorganisms isolated from different samples.

| Sample number | Organism       | Source          |
|---------------|----------------|-----------------|
| DPMC1         | *E. coli*      | Blood sample    |
| DPMC2         | *E. coli*      | Blood sample    |
| DPMC3         | *E. coli*      | Stool sample    |
| DPMC4         | *Acinetobacter*| Stool sample    |
| DPMC5         | *Klebsiella*   | Blood sample    |
| DPMC6         | *Klebsiella*   | Blood sample    |
| DPMC7         | *Proteus*      | Pus sample      |
| DPMC8         | *E. coli*      | Gastric aspirate sample |
| DPMC9         | *E. coli*      | Gastric aspirate sample |
| DPMC10        | *Klebsiella*   | Blood sample    |
| DPMC11        | *Proteus*      | Gastric aspirate sample |
| DPMC12        | *Klebsiella*   | Blood sample    |
| DPMC13        | *Klebsiella*   | Blood sample    |
| DPMC14        | *Klebsiella*   | Stool sample    |
| DPMC15        | *P. aeruginosa*| Stool sample    |
| DPMC16        | *Proteus*      | Pus sample      |
| DPMC17        | *Proteus*      | Pus sample      |
| DPMC18        | *P. aeruginosa*| Gastric aspirate sample |
| DPMC19        | *P. aeruginosa*| Gastric aspirate sample |
| DPMC20        | *P. aeruginosa*| Gastric aspirate sample |
| DPMC21        | *E. coli*      | Gastric aspirate sample |

### Table 2
Microorganisms isolated from different samples.

| Sample number | Gram’s staining | Oxidase | Catalase | Indole | Methyl red | Triple sugar | Iodine | Citrate | Urease | Glucose | Lactose | Maltose | Sucrose |
|---------------|----------------|---------|----------|--------|------------|--------------|--------|---------|--------|---------|---------|---------|---------|
| DPMC1         | -              | +       | +        | +      | +          | A/A         | -      | +       | +      | +       | +       | +       |         |
| DPMC2         | -              | +       | +        | +      | A/A        | A/A         | -      | +       | +      | +       | +       | -       | -       |
| DPMC3         | -              | +       | +        | A/A    | -          | -            | -      | +       | +      | +       | +       | -       | -       |
| DPMC4         | -              | +       | -        | +      | A/A        | K/NC         | -      | -       | -      | -       | -       | -       | -       |
| DPMC5         | -              | +       | -        | +      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC6         | -              | +       | -        | +      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC7         | -              | +       | +        | -      | +          | -            | +      | +       | +      | -       | +       | -       | -       |
| DPMC8         | -              | +       | +        | -      | A/A        | -            | -      | -       | -      | -       | -       | -       | -       |
| DPMC9         | -              | +       | +        | -      | +          | -            | A/NC   | -       | -      | -       | -       | -       | -       |
| DPMC10        | -              | +       | +        | -      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC11        | -              | +       | +        | -      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC12        | -              | +       | -        | +      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC13        | -              | +       | -        | +      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC14        | -              | +       | -        | +      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC15        | -              | +       | -        | +      | A/A        | +            | +      | +       | +      | +       | +       | -       | -       |
| DPMC16        | -              | +       | +        | -      | +          | -            | +      | +       | +      | -       | +       | -       | +       |
| DPMC17        | -              | +       | +        | -      | +          | -            | +      | +       | +      | -       | +       | -       | +       |
| DPMC18        | -              | +       | +        | -      | -          | A/NC         | -      | -       | -      | +       | -       | +       | -       |
| DPMC19        | -              | +       | +        | -      | -          | A/NC         | -      | -       | -      | -       | -       | -       | -       |
| DPMC20        | -              | +       | -        | -      | A/NC       | -            | -      | -       | -      | +       | -       | +       | -       |
| DPMC21        | -              | +       | +        | -      | +          | A/A          | -      | +       | +      | +       | +       | +       | +       |

A: Acid; K: Alkaline; NC: No change; A/A: Acidic slant/acidic butt; K/NC: Alkaline slant/no change in butt; A/NC: Alkaline slant/no change in butt.

### 3.2. Antimicrobial susceptibility of bacterial isolates

Five common antibiotics (piperacillin, gentamicin, amikacin, ampicillin and ciprofloxacin) were tested against all 21 bacterial isolates. Among all isolates, 8 (38.1%) strains (DPMC1, DPMC2, DPMC5, DPMC6, DPMC12, DPMC15, DPMC19 and DPMC21) were found resistant to all the five antibiotics tested. Table 3 shows the antimicrobial susceptibility of bacterial isolates.

### Table 3
Antimicrobial susceptibility of bacterial isolates (mm).

| Sample number | Zone of inhibition against following drugs (8 mm well diameter) |
|---------------|---------------------------------------------------------------|
|               | Piperacillin | Gentamicin | Amikacin | Ampicillin | Ciprofloxacin |
| DPMC1         | 8           | 11         | 9        | 8          | 10           |
| DPMC2         | 11          | 9          | 8        | 12         | 18           |
| DPMC3         | 24          | 22         | 20       | 19         | 18           |
| DPMC4         | 17          | 23         | 26       | 20         | 19           |
| DPMC5         | 8           | 8          | 12       | 11         | 12           |
| DPMC6         | 11          | 10         | 9        | 8          | 10           |
| DPMC7         | 19          | 18         | 29       | 25         | 12           |
| DPMC8         | 18          | 16         | 31       | 17         | 18           |
| DPMC9         | 18          | 18         | 17       | 16         | 21           |
| DPMC10        | 26          | 24         | 40       | 22         | 14           |
| DPMC11        | 34          | 26         | 33       | 15         | 15           |
| DPMC12        | 8           | 8          | 9        | 11         | 10           |
| DPMC13        | 15          | 26         | 21       | 22         | 19           |
| DPMC14        | 17          | 23         | 26       | 20         | 19           |
| DPMC15        | 8           | 11         | 9        | 8          | 10           |
| DPMC16        | 28          | 18         | 17       | 16         | 16           |
| DPMC17        | 21          | 18         | 31       | 22         | 18           |
| DPMC18        | 23          | 22         | 20       | 19         | 18           |
| DPMC19        | 8           | 8          | 9        | 12         | 12           |
| DPMC20        | 8           | 8          | 12       | 11         | 12           |
| DPMC21        | 11          | 10         | 9        | 8          | 10           |

### 3.3. Initial screening of ESBLs

Four antibiotics, namely, ceftazidime, aztreonam, cefotaxime and ceftriaxone were tested against 21 bacterial isolates. Among all isolates, 4 (19.04%) isolates which are strain DPMC1, DPMC4, DPMC13 and DPMC19 were found resistant to all the four antibiotics tested. The inhibitory zone diameter was less (30 μg of ceftazidime ≤ 22 mm, 30 μg of aztreonam ≤ 27 mm, 30 μg of cefotaxime ≤ 27 mm and 30 μg of ceftriaxone ≤ 25 mm).
Table 4 shows initial screening of ESBL and Figure 1 shows its observations.

Figure 1. Initial screening of ESBLs.

|   |   |   |   |   |
|---|---|---|---|---|
|   |   |   |   |   |

Table 4
Initial screening of ESBLs (mm).

| Sample number | Zone of inhibition against following drugs (8 mm zone diameter) |
|---------------|---------------------------------------------------------------|
|               | Ceftazidime | Aztreonam | Cefotaxime | Ceftriaxone |
| DPMC1         | 18          | 10        | 14         | 17          |
| DPMC2         | 25          | 30        | 29         | 27          |
| DPMC3         | 26          | 27        | 28         | 25          |
| DPMC4         | 15          | 9         | 11         | 20          |
| DPMC5         | 30          | 29        | 25         | 30          |
| DPMC6         | 31          | 25        | 27         | 26          |
| DPMC7         | 31          | 27        | 29         | 31          |
| DPMC8         | 13          | 23        | 28         | 13          |
| DPMC9         | 24          | 27        | 30         | 25          |
| DPMC10        | 22          | 18        | 28         | 22          |
| DPMC11        | 23          | 28        | 31         | 28          |
| DPMC12        | 26          | 33        | 29         | 25          |
| DPMC13        | 19          | 10        | 15         | 19          |
| DPMC14        | 22          | 28        | 27         | 26          |
| DPMC15        | 24          | 32        | 28         | 25          |
| DPMC16        | 29          | 27        | 31         | 26          |
| DPMC17        | 23          | 30        | 28         | 27          |
| DPMC18        | 27          | 28        | 30         | 28          |
| DPMC19        | 13          | 19        | 19         | 13          |
| DPMC20        | 30          | 33        | 29         | 28          |
| DPMC21        | 25          | 28        | 30         | 26          |

3.4. Analysis of ESBLs producer strains

Analysis of ESBLs producer strains was carried out using phenotypic confirmatory test. These four isolates were further tested with ceftazidime (30 μg) and in combination with clavulanic acid (30 μg: 10 μg). A β-lactamase inhibitor interfered with the activity of ESBLs. As a result, 19.05% of total isolates, (K. pneumonia and Pseudomonas E. coli and Acinetobacter) were considered as ESBLs producer. About 16.67% E. coli (1:6), 16.67% K. pneumoniae (1:6), 25% P. aeruginosa (1:4) and 100% Acinetobacter (1:1) were found to be ESBL producers. Proteus was found to be susceptible to all four antibiotics and not confirmed to be ESBL producers. Table 5 shows the detection of ESBLs producers using double disc diffusion test.

4. Discussions

This study demonstrates the prevalence of ESBLs in clinical samples collected from Dehradun City Hospital. ESBL detection is not routinely carried out in many microbiology units of service laboratories. This could be attributed to the lack of resources and facility to conduct ESBL identification. This study is carried out to show the prevalence of ESBL in Dehradun area justifying the need of routine test.

The study includes the 21 isolates from 56 samples (blood, respiratory pus, stool and gastric aspirate). These isolates include six strains of E. coli, six strains of K. pneumonia, four strains of P. aeruginosa, four strains of Proteus and only one strain of Acinetobacter. These isolates were tested for their antibiotic susceptibility against five common antibiotics (penicillin, amikacin, ampicillin, gentamicin and ciprofloxacin). Eight (38.1%) of 21 isolates were found to be resistant to all five antibiotics. A total of 21 isolates of different bacteria were then initially screened for ESBLs using four antibiotics, namely, cefotaxime, ceftazidime, aztreonam and ceftriaxone. About 19.04% of isolates showed positive test for ESBLs. These were DPMC1 (E. coli), DPMC4 (Acinetobacter), DPMC13 (Klebsiella) and DPMC19 (P. aeruginosa). The confirmatory test for ESBLs was carried out using double disc diffusion method. The confirmatory test showed the similar result as that of screening test confirming that 19.04% of total isolates were ESBL producers.

The results of this study are, in significant extent, correspondence
with some previous reporting the prevalence of ESBL in E. coli, Klebsiella, P. aeruginosa and Acinetobacter [7,9,10,12,18,22,23]. Many studies also showed the prevalence of ESBLs in Proteus [24]. In our study, Proteus doesn’t show the presence of ESBLs, which may be attributed to the less number of Proteus isolates included in study and indicates the need of further study. Sometimes, correct identification of Proteus is misleading [25].

Conflict of interest statement

We declare that we have no conflict of interest.

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