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

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ABSTRACT

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), carbapenamase 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[3]. Bacteria carrying ESBLs have been emerged as significant resistant to multiple antimicrobial 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, ceftriaxone or cefpodoxime depending upon the bacterial isolates selected for study[5].

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]. In

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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 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, H_2S 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)

Table1

Identification of microorganisms isolated from different samples.

Sample number	Gram's staining	Oxidase	Catalase	Indole	Methyl red	Triple sugar iodine	Citrate	Urease	Sugar fermentation			
									Glucose	Lactose	Maltose	Sucrose
DPMC1	-	-	+	+	+	A/A	-	-	+	+	+	-
DPMC2	-	-	+	+	+	A/A	-	-	+	+	+	-
DPMC3	-	-	+	+	+	A/A	-	-	+	+	+	-
DPMC4	-	-	+	-	+	K/NC	-	-	-	-	-	-
DPMC5	-	-	+	-	+	A/A	+	+	+	+	+	+
DPMC6	-	-	+	-	+	A/A	+	+	+	+	+	+
DPMC7	-	-	+	+	+		-	+	+	-	+	+
DPMC8	-	-	+	+	+	A/A	-	-	+	+	+	-
DPMC9	-	-	+	+	+	A/A	-	-	+	+	+	-
DPMC10	-	-	+	-	+	A/A	+	+	+	+	+	+
DPMC11	-	-	+	+	+		-	+	+	-	+	+
DPMC12	-	-	+	-	+	A/A	+	+	+	+	+	+
DPMC13	-	-	+	-	+	A/A	+	+	+	+	+	+
DPMC14	-	-	+	-	+	A/A	+	+	+	+	+	+
DPMC15	-	+	+	-	-	A/NC	-	-	-	-	-	-
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.

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 2

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*	Stool sample
DPMC6	*Klebsiella*	Stool 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*	Stool sample
DPMC13	*Klebsiella*	Stool 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

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	Gentamycin	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	8	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 $\leqslant$ 22 mm, 30 μg of aztreonam $\leqslant$ 27 mm, 30 μg of cefotaxime $\leqslant$ 27 mm and 30 μg of ceftriaxone $\leqslant$ 25 mm).

Table 4 shows initial screening of ESBL and Figure 1 shows its observations.

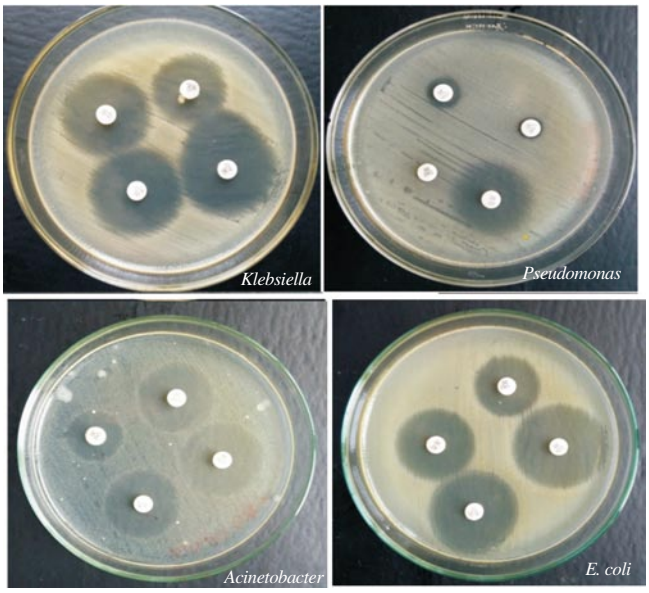

Figure 1. Initial screening of ESBLs.

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	11
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.

Table 5
Detection of ESBLs producers using double disc diffusion test.

Sample number	Zone of inhibition (mm)		Difference	Interpretations
	Ceftazidime/ clavulanic acid	Ceftazidime		
DPMC1	20	26	6	ESBL producer
DPMC2	33	30	3	Not ESBL producer
DPMC3	34	30	4	Not ESBL producer
DPMC4	24	15	9	ESBL producer
DPMC5	25	23	2	Not ESBL producer
DPMC6	29	29	0	Not ESBL producer
DPMC7	28	24	4	Not ESBL producer
DPMC8	30	28	2	Not ESBL producer
DPMC9	36	33	3	Not ESBL producer
DPMC10	29	25	2	Not ESBL producer
DPMC11	28	26	2	Not ESBL producer
DPMC12	20	20	0	Not ESBL producer
DPMC13	28	23	5	ESBL producer
DPMC14	32	29	3	Not ESBL producer
DPMC15	29	27	2	Not ESBL producer
DPMC16	28	27	1	Not ESBL producer
DPMC17	33	30	3	Not ESBL producer
DPMC18	34	30	4	Not ESBL producer
DPMC19	26	19	7	ESBL producer
DPMC20	20	17	3	Not ESBL producer
DPMC21	29	25	4	Not ESBL producer

4. Discussions

This study demonstrates the prevalence of ESBLs in clinical samples collected from Dehradun City Hospital. ESBLs 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. pneumoniae*, 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. 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.

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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