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PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA SPP* AMONG URINARY ISOLATES

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Abstract: Extended spectrum beta lactamases are enzymes that mediate resistance to third generation cephalosporins and monobactams. ESBL producing organisms confer resistance not only to beta lactams but also to other antimicrobials like aminoglycosides and fluoroquinolones. So their detection helps the clinician in deciding the appropriate treatment strategies. **MATERIAL AND METHODS**: This study conducted over a period of 1 year detected ESBL production in urinary isolates by double disk diffusion test and the three dimensional test. Association of ESBL producers with various risk factors like history of hospitalization, antibiotic use and catheterization was studied. Antimicrobial susceptibility testing for imipenem was done using the Kirby Bauer's disc diffusion method. **RESULTS**: The prevalence of confirmed ESBL producers was 54.86% by the double disk diffusion test and 59.81% by the three dimensional test. There was significant association of ESBL producers with various risk factors like history of hospitalization, antibiotic use and catheterization. Sensitivity to imipenem in ESBL producers was found to be 94.93%. **CONCLUSIONS**: Both *Escherichia coli* and *Klebsiella spp* which are the main pathogens in urinary tract infections showed high rate of ESBL production. The double disc diffusion test and the three dimensional test were found to be comparable for confirmation of ESBL producers. ESBLs are emerging as an important resistance threat in hospitals and their detection has thus become a great concern.

Keywords: Extended spectrum beta lactamase, *Escherichia coli, Klebsiella* spp, Double disc diffusion test, Three dimensional test

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INTRODUCTION

In recent years there has been an increased incidence and prevalence of extended spectrum β-lactamases (ESBLs), enzymes that hydrolyze and cause resistance to oxyimino-cephalosporins and aztreonam.[1]

ESBLs represent a major group of β-lactamases currently being identified worldwide in large numbers and are now found in a significant percentage of *E.coli* and *K. pneumoniae* strains. They have also been found in *Pseudomonas aeruginosa* and other enterobacteriaceae strains like *Enterobacter, Citrobacter, Proteus*, *Morganella morganii*, *Serratia marcescens*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Capnocytophaga ochracea*.[2]

With the ability to produce highly effective β lactamase enzymes, these organisms are resistant to all β-lactam antibiotics except cephamycins (cefoxitin, cefotetan) and carbapenems. In addition, ESBL-producing organisms are frequently resistant to many other classes of antibiotics, including aminoglycosides and fluoroquinolones. This leaves an extremely limited range of antibiotics for the treatment of these resistant organisms and the use of drugs which are destroyed by the enzymes produced by these organisms will result in treatment failure.

The various detection methods which can be employed are the screening methods and confirmatory methods. The screening methods are antimicrobial susceptibility testing by Kirby Bauer's disc diffusion method and the minimum inhibitory concentration (MIC) method. The confirmatory tests are double disk method, MIC, automated method, three dimensional test (TDT) and E-test.[3]

The antibiotics recommended for screening of potential ESBL producers are cefpodoxime, ceftazidime, cefotaxime, ceftriaxone and aztreonam. The presence of ESBL activity in an isolate that is positive by the screening test should be then established by a confirmatory test.

The main objective of our study was to know the prevalence of ESBL producing *Escherichia coli* and *Klebsiella spp* isolated in urine samples received from the various intensive care units and wards of our hospital. We also studied the association of various risk factors with ESBL production and finally we studied the antibiotic sensitivity pattern of the ESBL producing organisms to imipenem.

MATERIAL AND METHODS

This study was conducted over a period of one year from April 2008 to March 2009 in the department of Microbiology, on patients admitted in various intensive care units and wards in Dayan and Medical College and Hospital, Ludhiana. All *Escherichia coli* and *Klebsiella spp.* isolated from the urine samples collected from these patients over a period of one year were

studied. The bacterial isolates were identified according to standard microbiological procedures.

Screening Tests for Probable ESBL Producers

Antibiotic susceptibility testing by Kirby-Bauer's disc diffusion method:

Antibiotic susceptibility testing was done according to the Kirby Bauer's disc diffusion method on Mueller Hinton agar. Antimicrobial disc containing 10 µg of imipenem (BD, Diagnostics) was used.

Screening for ESBL producers was done by using 30 µg ceftazidime, cefotaxime and ceftriaxone discs (BD, Diagnostics).[3] Zones of inhibition were measured and compared with the performance standards for antimicrobial disk susceptibility test provided by clinical and laboratory standards institute (CLSI).

Zone Size

Ceftazidime	≤ 22 mm
Cefotaxime	≤ 27 mm
Ceftriaxone	≤ 25 mm

If the zone sizes of these antibiotics were less than the above standards given, then these strains were considered resistant to these drugs and thus were labeled as probable ESBL producers.

Quality Control: *Klebsiella pneumoniae* ATCC 700603

E.coli ATCC 25922

Confirmatory Tests for ESBL Producers

1. Double Disc Diffusion Test

Double disc diffusion test was done using Mueller Hinton agar plates. The discs applied were ceftazidime alone (30 µg) and a combination disc of ceftazidime (30 µg) + clavulanic acid (10 µg) and similarly cefotaxime (30 µg) alone and in combination with clavulanic acid (10 µg). The discs were placed in such a way that the centre to centre distance between the discs was a minimum of 30mm. The increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was observed. An increase in zone

diameter of >5 mm in either of the two combination discs was considered as an indication of ESBL production.

2. Three Dimensional Test[4]

Indirect method: Mueller Hinton agar plates were seeded with the inoculums of a standard sensitive strain (*E. coli* ATCC 25922) adjusted to McFarland 0.5 standard. A disc of ceftazidime, ceftriaxone, cefotaxime or aztreonam was placed in the center of the plate. A well of 4 mm diameter was punched at a distance of 2mm. The test strain (30 µl) in brain heart infusion broth preadjusted to 5.0 Mc Farland standard was seeded into the well. Plates were then incubated at 37°C for 24hours. Heart shaped distortion of the zone of inhibition around the beta lactam disc was indicative of ESBL activity.

RESULTS

A total of 6058 urine samples received in the department of Microbiology for culture from various intensive care units and wards were processed.

Out of 6058 urine samples received, growth of *Escherichia coli* and *Klebsiella* species was obtained in 627 (10.34%) samples. Out of the 627 *E. coli* and *Klebsiella* isolates, probable ESBL producers were 460 (73.37%) (Table1). The overall prevalence of ESBL producers was 54.86% by the double disc diffusion test and 59.81% by the three dimensional test (p-value - 0.0933, not significant). Prevalence of ESBL producers among *E coli* was 59.96% and among *Klebsiella* spp was 59% (Table 2).

History of hospitalization, antibiotic usage and catheterization were found to be significant risk factors for ESBL production (p-value < 0.05) (Table- 3, 4 and 5). Sensitivity to imipenem was found to be 95.93% in the ESBL producing organisms.

DISCUSSION

Because of the spread of resistance to other antibiotics, ESBL producing organisms have become a threat to clinical therapeutics. They are becoming emerging pathogens causing major outbreaks all over the world. Due to this increasing spread of ESBL producing organisms their detection has become an important issue to know their prevalence in a hospital and thereby provide the clinicians with alternate antibiotic policies.

In our study we found prevalence of ESBL producers to be 59.81% by the three dimensional test. There was no significant difference in the prevalence rate by the double disk diffusion test and the three dimensional test. In a study by Bhavnani *et al*,[5] prevalence of ESBL producing organisms was found to be 77%. In another study by Agrawal *et al*,[6] the prevalence of ESBL

producing *E coli* and *Klebsiella* isolates was found to be 22%. In our study the ESBL producing rate of *E coli* was 59.96% and in *Klebsiella* species was 59.00%. In a study by Kumar *et al*,[7] a high prevalence of ESBLs was found among *E coli* (63.7%) as compared to *Klebsiella pneumoniae* (14%), while in our study the prevalence rates between the two were comparable which were consistent with the study by Babypadmini and Appalaraju[8] who observed prevalence rate of ESBL producers to be 41% in *E coli* and 40% in *K pneumoniae* in urinary samples.

The association of ESBL production with history of hospitalization was statistically significant in our study (p-value 0.0479). This result was consistent with a study by Alici *et al*[9] in Ankara who also observed a statistically significant difference in distribution of ESBL producing isolates between hospitalized and non-hospitalized patients. Mangeney *et al*[10] found a significant association between length of hospital stay and ESBL production. Regarding the prior history of antibiotic use, in our study we observed a slightly significant difference in ESBL production in patients with prior history of antibiotic use as compared to the non ESBL producers (p-value-0.0057). Rafay *et al*[11] observed that excess use of third generation cephalosporins led to increased rate of ESBLs. Colodner *et al*[12] also found association of ESBLs with previous use of second generation cephalosporins, third generation cephalosporins, quinolones and penicillin.

It has been observed in various studies that history of urinary catheterization is also associated with increased rate of production of ESBLs. Goyal *et al*[13] in their study on ESBLs in *E coli* and *Klebsiella pneumoniae* observed that the presence of urinary catheters were associated with ESBL infection. Atifah *et al*[14] also found a similar association with presence of urinary catheters. The observation in our study was consistent with these studies as we found a slightly significant association between presence of catheterization and prevalence of ESBLs (p-value 0.0499).

We observed 95.93% of ESBL producers were sensitive to imipenem. Schwaber *et al*[15] in their study in Israel found that none of the ESBL producing isolates were resistant to imipenem. Liao *et al*[16] in a study in Taiwan observed that almost none of the isolates were resistant to carbapenems. Manchanda *et al*[17]studied the susceptibility profiles of ESBL producing *Klebsiella pneumoniae* and found 100% sensitivity to imipenem.

ESBLs are emerging as an important diagnostic and therapeutic challenge to the microbiologists and the clinicians. So, it has become very essential for the microbiologists to become the pioneers for the detection of these resistance threats. Inappropriate selection of antimicrobials for the treatment of these infections may result in treatment failures leading to prolonged hospital stay and also increase in cost of treatment to the patient. So, it becomes equally

important for the microbiologists to inform the clinicians at the earliest about the treatment policies for these infections.

Thus, from our study we have become aware of the fact that ESBL infections are becoming a potential challenge to the hospitals and therefore, it becomes important for us to reach to the root of the problem so as to put an end to it at the right time.

Table 1: Distribution of isolates according to ESBL production (n=627)

ESBL Status	No. (%)
Probable ESBL producers	460 (73.37)
Confirmed ESBL Producers by Double Disc Diffusion Test	344 (54.86)
Confirmed ESBL Producers by Three Dimensional Test	375 (59.81)
Non ESBL Producers	252 (40.19)
p-value	0.0933

Table 2: Prevalence of ESBL producers among *E coli* and *Klebsiella* species isolates

Isolates (n=627)	ESBL Producers	Non ESBL Producers
	No. (%)	No. (%)
E.coli (n=527)	316 (59.96)	211 (40.04)
Klebsiella (n=100)	59 (59.00)	41 (41.00)

Table 3: ESBL production in relation to prior history of hospitalization

Prior History of Hospitalization	ESBL Producers (n=375)	Non ESBL Producers (n=252)
(n=627)	No. (%)	No. (%)
Present (n = 224)	146 (65.18)	78 (34.82)
Absent (n = 403)	229 (56.82)	174 (43.18)
p-value	0.0479	

Table 4: ESBL production in relation to prior history of antibiotic usage

Prior history of Antibiotic usage	ESBL Producers (n=375)	Non ESBL Producers (n=252)
(n=627)	No. (%)	No. (%)
Present (n = 221)	146 (66.06)	75 (33.94)
Absent (n = 406)	229 (56.40)	177 (43.60)
p-value	0.0415	

Table 5: ESBL production in relation to history of catheterization

History of Catheterization	ESBL Producers (n=375)	Non ESBL Producers (n=252)
(n= 627)	No. (%)	No. (%)
Present (n=223)	151 (67.71)	72 (32.29)
Absent (n=404)	224 (56.68)	170 (43.31)
p-value	0.0499	

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