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Original Research Article

In Vitro Susceptibility to Piperacillin-Tazobactam and Imipenem among Gram-Negative Bacilli Isolated From Various Clinical Samples in a Tertiary Care Hospital

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ABSTRACT

Background: An alarming rise in the rates of the antibiotic resistance has now become a serious and an increasingly common public health concern, with severe implications, especially in the intensive care units. A variety of β-lactamases which include ESBLs, AmpCß-lactamases and metallo-ß-lactamases, have emerged as the most worrisome mechanism of resistance among the gram negative bacteria, which poses a therapeutic challenge to the health care settings.
Aims: The present study was conducted to detect ESBLs in Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa isolated from various clinical samples in a tertiary care hospital.
Material and Methods: A total of 190 consecutive, non-repetitive, gram negative isolates, which were resistant to 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).
Result: out of 190 ESBL producer, 110 isolates of Klebsiella pneumoniae, 50 isolates of E. coli and 30 isolates of pseudomonas aeruginosa were isolated. Klebsiella pneumoniae was the most common ESBL producer. Hospitalized patients (63.16%) showed more ESBL production in comparison to outpatients (26.31%). The antibiotic sensitivity pattern revealed that the maximum sensitivity was seen for piperacillin-tazobactam (98%), followed by imipenem (96.8%), amikacin (95.8%).
Conclusion: There is a high prevalence of ESBL production in our hospital 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, 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. coli, K. pneumoniae, P. aeruginosa.

INTRODUCTION

Increasing rates of bacterial resistance among common pathogens are threatening the effectiveness of even the most potent antibiotics. While the spread of multidrug resistant Gram-positive organisms, such as methicillin-resistant Staphylococcus aureus, routinely capture headlines, Gram-negative pathogens attract less attention, although their emergence and spread are associated with serious public health concerns. [1,2] Many clinical laboratories, do not screen for extended-spectrum β-lactamase (ESBL) producing enterobacteriaceae, although they are increasingly found in the community and associated with treatment failure. [3] It is time to intensify attention to Gram-negative resistance. Due to extensive use of β-lactam antibiotics over the last several decades in the clinical practice, various β-lactamases have emerged. Extended spectrum β-lactamases (ESBLs) are the enzymes produced by Gram-negative bacilli that have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (third generation cephalosporins and aztreonam) and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. [4] ESBLs are usually plasmid-mediated β-lactamases, most commonly found in Klebsiella pneumoniae, Escherichia coli and other Gram-negative bacilli. [5] Since 1983 the number of ESBL variants has been constantly growing; at present more than 300 different ESBL variants are known. [6]

The problems that are associated with ESBLs include multidrug resistance, difficulty in detection and treatment and increased mortality. Awareness and the detection of these enzymes are necessary for optimal patient care. The judicious use of antimicrobial agents and improved infection control methods must become health care priorities. Klebsiella pneumoniae and Escherichia coli remain the major ESBL-producing organisms isolated worldwide which are recommended to be routinely tested for and reported by the Clinical and Laboratory Standards Institute. [7,8] Prevalence of ESBLs varies from one institute to other.

The objective of the present study was to determine the prevalence and antibiotic sensitivity pattern of ESBL producing Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa that were isolated from various samples from both inpatients and outpatients who attended a tertiary care hospital.

MATERIALS AND METHODS

Bacterial isolates: A total of 190 consecutive, non-repetitive, Gram negative isolates from various clinical samples such as sputum (n=70), pus (n=70) and others(n= 50) including ear swab, vaginal swab, pleural fluid, conjunctival swab which were received in the clinical bacteriology laboratory from January 2015 to mid-April 2015 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. [9] The antibiotics (µg) which were included were amikacin (30), piperacillin (100), piperacillin-tazobactam (100/10), cefepime (30), cefotaxime (30), ceftriaxone (30), ceftazidime (30), amoxyclav (20/10), cotrimoxazole (25), ciprofloxacin (5),

imipenem (10), doxycycline (30) ceftazidime-clavulanic acid (30/10) 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 (30µg) and ceftriaxone (30µg) by using the standard disc diffusion method as recommended by the CLSI. [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". [9] Only those isolates which were resistant to one of the third generation cephalosporins 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, 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. [10] Discs of ceftazidime (30µg), cefotaxime (30µg) and amoxyclav (20µg amoxicillin and 10µg 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^{o}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 [Figure-1]. [10]

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. [9] The ceftazidime (30µg) discs alone and in combination with clavulanic acid (ceftazidime -clavulanic acid, 30/10µg discs) were placed at a distance of 20 mm apart on the agar. Similarly piperacillin (100µg) and piperacillin-tazobactam (100µg/ 10µg) were placed 30 mm apart. An increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to the piperacillin/ceftazidime disc alone was considered to be a marker for ESBL production [Figure-2]. [9]

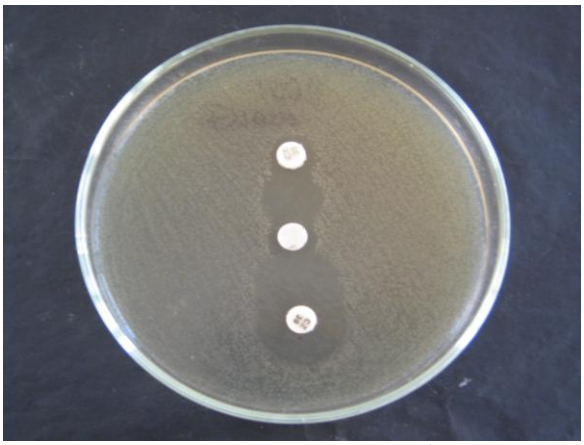

Figure- 1: Organism showing enhanced zone of inhibition between ceftazidime and cefotaxime and amoxicillin/clavulanic acid containing disc indicating ESBL production

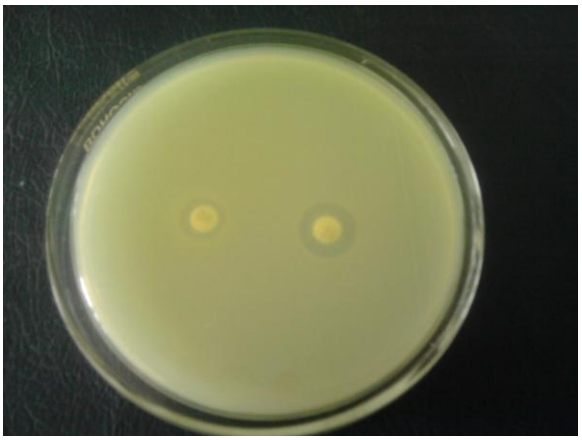

Figure 2: Phenotypic Confirmatory Disc Diffusion Test (PCDDT) Proposed by CLSI: ESBL production confirmed by an increase in zone diameter of ≥5mm for ceftazidime (CA) and ceftazidime – clavulanic acid (CAC).

Figure 3: Phenotypic Confirmatory Disc Diffusion Test (PCDDT) Proposed by CLSI: ESBL production confirmed by an increase in zone diameter of ≥5mm for piperacillin (P) and piperacillin-tazobactam (PIT).

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. [9]

RESULTS

We studied ESBL producing bacteria isolated from pus, sputum, and other specimens. Out of 190 ESBL producers, 110 isolates of Klebsiella pneumoniae, 50 isolates of E. coli and 30 isolates of pseudomonas aeruginosa. Klebsiella pneumoniae was the most common ESBL producer followed by Escherichia coli and Pseudomonas aeruginosa [Table -1].

Table-1 Distribution pattern of ESBL producing isolates from various sites

Specimen N= 190	ESBLs		
	Klebsiellapneumonia N=110	Escherichia coli N=50	Pseudomonas aeruginosa N=30
Sputum70	40(57.14%)	10(14.28%)	20(28.57%)
Pus70	40(57.14%)	20(28.57%)	10(14.28%)
Others50	30(60%)	20(40%)	-
Total190	110(57.90%)	50(26.31%)	30(15.79%)

The specimen wise distribution of the ESBL producers is shown in [Table -2]. ESBL production was seen in sputum (36.84%), and pus (36.84%) and others (26.31%).

Hospitalized patients (63.16%) showed more ESBL production in comparison to outpatients (26.31%).[Table-2]

Table 2: Distribution of ESBL producers in outpatients and Inpatients

ESBL producers		
	Inpatient Total-130 (63.16%)	Outpatient Total-60 (26.31%)
Klebsiella pneumoniae	85/110 (77.27%)	25/110 (22.73%)
Escherichia coli	25/50 (50%)	25/50 (50%)
Pseudomonas aeruginosa	20/30 (66.66%)	10/30 (33.33%)

Among the antimicrobial agents tested, piperacillin-tazabactam, imipenem and amikacin were overall the most consistently active antibiotics in vitro as the maximum sensitivity was seen for piperacillin-tazobactam (98%), followed by imipenem (96.8%), amikacin (95.8%) while sensitivity to ceftazidime-clavulanic acid (59.2%), azithromycin (45.7%), ciprofloxacin (42.5%), and cefepime (32.4%). A high resistance rate was seen for cotrimoxazole (83.5%), doxycycline (18.2%), amoxyclav (80.4%), piperacillin (77.6%), and cefotaxime (78.1%). [Table -3]

DISCUSSION

The β-lactamases are a large family of enzymes representing the major

mechanism of resistance of bacteria against β-lactam antibiotic. More than 340 β-lactamase enzymes have been detected until 2004. [11,12] ESBL production by gram negative bacteria has become a major problem in clinical practice in last few years due to extensive use of the β-lactam antibiotic. Cotransfer of resistance against aminoglycosides, trimethoprim, sulfonamides, tetracycline, chloramphenicol and quinolones is common on ESBL plasmids.

Table 3: Antimicrobial susceptibility pattern of ESBL producer isolates (n=190)

Antibiotic (n =190)	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	95.8%	0.2%	4%
Ciprofloxacin	42.5%	2.7%	54.8%
Cefepime	32.4%	0%	67.6%
Doxycycline	18.2%	1.4%	80.4%
Piperacillin	22.4%	0%	77.6%
Piperacillin-Tazobactam	98%	0%	2%
Imipenem	96.8%	1.4%	1.8%
Azithromycin	45.7%	1.8%	52.5%
Cotrimoxazole	16%	0.5%	83.5%
Amoxyclav	18.7%	0.9%	80.4%
Cefotaxime	21%	0.9%	78.1%
Ceftriaxone	16%	0.9%	83.1%
Ceftazidime	18.7%	0.5%	80.8%
Ceftazidime-clavulanic acid	59.2%	0.8%	40%

A number of nosocomial outbreaks which were caused by ESBL producing organisms, have been reported in the United States. [13-15] 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. [16] The threat of ESBL producing isolates is not limited to ICUs or tertiary care hospitals only. In the present study, 63.16% ESBLs were reported from patients admitted into hospital. A study conducted in Aligarh tertiary care hospital has reported 30.18% ESBL Klebsiella pneumoniae from clinical samples. [17]

In the present study, we observed that 57.90% Klebsiella pneumoniae and 26.31% E. coli isolates were ESBL producers. Other studies from India had reported a high prevalence of ESBL production ranging from 41.0 to 63.6 per cent in E. coli and 40 to 83.3 per cent in K. pneumoniae. [18,19]

In our study the ESBL production in Pseudomonas aeruginosa was less (15.79%) as compared to that in other Gram negative bacilli, because its resistance mechanism was mediated by the production of metallo-beta-lactamase, lack of drug penetration due to mutations in the porins or due to the loss of certain outer membrane proteins and the efflux pump. [20-22]

Looking at the overall trend of ESBL Klebsiella pneumoniae is on the rise and variable. This could partly be irrational use of cephalosporins at some institutions. The actual magnitude of problem posed by ESBL producers is not known as routine susceptibility testing fails to detect all ESBL producers. The two techniques used in the present study to confirm ESBL production are, namely, DDST and PCDDT. There is no instance of a DDST-positive and PCDDT-negative ESBL producers. This implies that PCDDT is more sensitive in detecting ESBL production than DDST. Observing the present and other similar studies it is confirmed that PCDDT is more sensitive than DDST for detection of ESBLs. [23-25]

In our study, we observed that a majority of the isolates were susceptible to piperacillin-tazobactam (98%) and imipenem (96.8%). Similarly, in a study from Coimbatore, all the members of Enterobacteriaceae were found to be susceptible to imipenem and piperacillin/ tazobactam. [26] In both the studies, amikacin also showed good activity against Gram negative bacteria as compared to other antibiotics. Therefore, piperacillin-

tazobactam is the most active drug for the treatment of infections which are caused by ESBL producers, followed by imipenem and amikacin. Thus, in our hospital, tazobactam appears to be much more effective ESBL inhibitor, and piperacillin-tazobactam is becoming drug of choice for infection suspected to be caused by ESBL-producing bacteria. Piperacillin-tazobactam is cost effective than carbapenem. So, here in our Hospital it has become a drug of choice.

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. [27] 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.

CONCLUSION

Piperacillin-tazobactam remains good choices for the treatment of infections suspected to be due to ESBL producing K. pneumoniae, E. coli and Pseudomonas aeruginosa.

Conflict of Interests: no conflict of interests.

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