

DETECTION OF EXTENDED SPECTRUM β -LACTAMASES (ESBLs) IN CLINICAL ISOLATES

Bhavanarushi Sreekanth, Gogi Suresh Dattaraya

Department of Microbiology, DM Wayanad Institute of Medical Sciences (DMWIMS), Meppadi, Wayanad Dist, Kerala, India

Correspondence to: Bhavanarushi Sreekanth (drskan02@yahoo.com)

DOI: 10.5455/ijmsph.2014.010720142

Received Date: 02.06.2014

Accepted Date: 01.07.2014

ABSTRACT

Background: Treatment of extended Spectrum β -lactamase (ESBL) producing gram negative bacilli is an increasing problem in hospitalized patients. Increasing resistance to third generation cephalosporins among gram negative bacilli has important therapeutic and clinical implications. ESBL producers significantly affect the course and outcome of infection and are associated with increased morbidity and mortality. The high proportion of ESBL producers and the outbreak of multi drug resistant gram negative bacilli are quite alarming and need expensive control measures. Appropriate laboratory detection is important to avoid inappropriate antimicrobial therapy.

Aims & Objectives: The present study was undertaken to investigate the high incidence of extended Spectrum β -lactamases (ESBLs) producers and their antibiotic susceptibility pattern.

Materials and Methods: A total of 228 GNB isolates, were studied for ESBL production. The isolates were screened for ESBL production by the double disc synergy test (DDST) as recommended by the Clinical Laboratory Standards Institute (CLSI). Antimicrobial susceptibility testing was done along with screening for ESBL production for commonly used antibiotics. Isolates which showed positive results were confirmed by phenotypic confirmatory disc diffusion test (PCDDT).

Results: Out of 228 GNB isolates studied, 102 (44.73%) isolates were positive for ESBL production by presumptive screening test, of which 84 (36.84%) isolates were confirmed by PCDDT. The isolates of *Escherichia coli* (71.6%) were the most common ESBL producers, followed by *K. pneumoniae* (22.6%) and others. Imipenem (94.45%) was the most active and reliable agent for the treatment of the infections which were caused by the ESBL producing organisms.

Conclusion: ESBL detection and antibiotic sensitivity testing should be undertaken routinely to avoid misuse of antibiotics and also prevent spread of these strains.

Key Words: Extended Spectrum Beta Lactamases (ESBLs); Gram Negative Bacilli; 3rd Generation Cephalosporins

Introduction

The introduction of the third-generation Cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β -lactamase-mediated bacterial resistance to antibiotics. The third generation Cephalosporins had the major advantage of lessened nephrotoxic effects compared to Aminoglycosides and Polymyxin.^[1] Increasing resistance to third generation cephalosporin amongst gram negative bacilli is predominantly due to the production of extended-spectrum β -lactamases (ESBLs).^[2] ESBLs are β -lactamases conferring resistance to 3rd generation Cephalosporins and aztreonam but not to Cephameycins and Carbapenems.^[3] ESBL enzymes are plasmid borne and they have evolved from point mutations which altered the configuration of the active site of the original and long known β -lactamases, which have been designated as TEM-1, TEM-2, and SHV-1.^[4] The resistance to newer β -lactams which are a result of these β -lactamases, has emerged quickly. The first report of plasmid-encoded β -lactamases which are capable of hydrolyzing the extended-spectrum Cephalosporins was published in 1983.^[4] Their numbers have increased

significantly since 1989, and represent one of the largest groups of novel enzymes.^[3] At present, there are more than 300 different ESBL variants, and these have been clustered into nine different structural and section evolutionary families based on amino acid sequence. TEM and sulfhydryl variable SHV are the major types. However, CTX-M type is more common in some countries.^[5] The aim of the present study was to isolate and identify ESBL producing bacteria in different clinical samples and to determine the antimicrobial susceptibility pattern of ESBL and non-ESBL producers.

Materials and Methods

The present study was carried out over a period of four months from October 2013 to January 2014. A total of 640 specimens were collected. Various samples included in the study were urine, pus and sputum. The specimens were cultured on blood agar and MacConkey agar and the isolates were identified on the basis of colony morphology and biochemical reactions.^[3]

Antimicrobial Susceptibility Testing: The susceptibility of gram negative bacilli to antimicrobial

agents was performed on Muller Hinton agar by modified Kirby Bauer disc diffusion method following the criteria put forward by the CLSI.⁴ The antibiotics (μg) which were included were ampicillin (10), gentamicin (10), amikacin (30), piperacillin/ tazobactam (100/10), trimethoprim/ sulfamethoxazole (1.25/23.75), ciprofloxacin (5), and imipenem (10).

Selection Criteria for ESBL: The isolates were tested with 30 μg each of third generation Cephalosporins viz. ceftazidime, cefotaxime, and ceftriaxone – by using the standard disc diffusion method as recommended by the CLSI.⁷ Isolates found resistant or with decreased susceptibility (intermediate) to any of the third generation Cephalosporins were selected for presence of ESBL.

ESBL Detection: (i) Double Disc Method: The isolates presumed to be ESBL producers were inoculated on the Muller Hinton agar plate. Ceftazidime 30 μg , cefotaxime 30 μg and an amoxicillin / clavulanic acid 20+ 10 μg were placed at a distance of 25-30 mm apart, centre to centre. Following overnight incubation at 37°C, ESBL production is inferred when the zone of inhibition around ceftazidime was expanded by clavulanate. (ii) Phenotypic Confirmatory Test: This test was performed on Muller Hinton agar by disc diffusion test as recommended by CLSI. Pairs of disc containing ceftazidime (30 μg) alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 μg discs) were placed on opposite sides of the same inoculated plate. The organism was regarded as an ESBL producer if the zone of inhibition around the combination disc is at least 5mm larger than that of ceftazidime alone.⁸

Results

Out of 640 samples processed, 228 samples yielded various Gram negative bacilli. Of the 228 isolates, 102 isolates were found to be presumptive ESBL producers. A total of 84(36.84%) were positive for ESBL production. Of these, 61 (72.6%) isolates were *E. coli*, 19 (22.6%) isolates were *K. pneumoniae*, 2 (2.38%) isolates were *P. mirabilis* and 1 (1.1%) isolate was *C. freundii* and *enterobacter cloacae* each. None of the isolates of *Acinetobacter* or *Pseudomonas* spp was positive for ESBL production [Table 1]. Analysis of antimicrobial-resistance patterns showed that resistance is more frequent in ESBL-producing strains than in those which did not produce the enzyme [Table 2].

ESBL isolates were found to be more resistant to cotrimoxazole (64.28), ciprofloxacin (69.0%), gentamicin (39.28%) imipenem (5.55%), piperacillin/tazobactam (14.28%) and amikacin (15.47%) compared to their Non ESBL producing counterparts ($p > 0.05$). The highest rate of resistance in ESBL negative isolates was seen against ampicillin (71.42%) which was significantly ($p < 0.01$) lower than ESBL producing isolates. Imipenem was found to be the most effective antibiotic against ESBL producers (94.45% of isolates were sensitive), while in non-ESBL producing isolates, resistance was 4.86%. However, in this case, the difference was not significant ($p > 0.05$)

Table-1: Distribution of ESBL producers among Gram negative bacilli in clinical isolates

Organisms	Total	ESBL positive
<i>Escherichia coli</i>	122	61 (72.6%)
<i>Klebsiella pneumonia</i>	43	19 (22.6%)
<i>Proteus spp.</i>	11	02 (2.38)
<i>Citrobacter spp.</i>	8	01 (1.1%)
<i>Acinetobacter spp.</i>	4	0
<i>Enterobacter cloacae</i>	7	01 (1.1%)
<i>Pseudomonas aeruginosa</i>	33	0

Table-2: Antibiotic susceptibility pattern of ESBL & Non-ESBL producing organisms

Antibiotics	Resistant (%)	
	ESBL (n=84)	Non-ESBL (n=144)
Ampicillin	96.42	71.42
Piperacillin + Tozabactam	14.28	28.57
Amikacin	15.47	4.76
Cotrimaxazole	64.28	55.95
Ciprofloxacin	69	57.1
Gentamycin	39.28	28.57
Imipenem	5.55	4.86

Discussion

ESBLs have become a widespread serious problem, setting unique challenges to clinical microbiologists, physicians and infection control practitioners.⁹ In the present study, ESBL production was found to be 84 out of 228 (36.84%). The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different institutes. Previous studies from India have reported the prevalence of the ESBL producers to be 6% to 87%.¹⁰ These findings correlated well with those of our study. One reason for such variability may be less number of samples studied. In the present study, we also observed that 72.6% *E. coli* and 22.6% *Klebsiella pneumoniae* isolates were ESBL producers. Although *K. pneumoniae* was more often reported as an ESBL producer in other studies⁴, we observed that the ESBL production was more common in the *E. coli* isolates as compared to that in the *K. pneumoniae* isolates. Ananthkrishnan et al¹¹ and Kumar et al¹² also reported high prevalence of ESBL

among *Escherichia coli* isolates as compared to that in *Klebsiella pneumoniae* isolates. ESBL production was nil by *Pseudomonas species* and *Acinetobacter species* by the method we used – whether they were actually non-producers, or whether some of them did produce β -lactamases that were not inhibited by clavulanate, needs to be investigated. Jain A et al^[13] reported similar findings. The present study showed higher antimicrobial resistance among ESBL producers than among non-ESBL producers [Table 2]. Almost all the ESBL-positive isolates were found to be resistant to Ampicillin (96.42%) ($p < 0.01$) and sensitive to Imipenem (94.5%), ($p > 0.05$) compared to their counterparts, which again advocates the usage of Carbapenems antibiotics as the therapeutic alternative to β -lactam antibiotics as indicated in many previous studies.

Conclusion

Routine detection of ESBL-producing microorganisms should be done by each laboratory by the standard detection methods, so as to enable clinician to select appropriate drug regimen, to control and to prevent spread of these infections.

References

1. Paterson DL, Bonomo RA. Extended-Spectrum β -lactamases. A Clinical Update. Clin Microbiol Rev 2005;18:657–86.
2. Andrews J. Detection of extended-spectrum β -lactamases (ESBLs) in *E. coli* and *Klebsiella* species. 2003. <http://www.bsac.org.uk/uploads/Ecoliklebsiella.pdf>
3. Kulkarni R, Vaishali D, Dnyaneshwari G, Aravind B. A study of Extended spectrum betalactamase (ESBL) producers in clinical isolates. Med J of west India 2013;41:18-22.
4. Gaurav D. Prevalence of Extended spectrum β -lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, India. J Clin Diagn Res 2012;6:182-7.
5. Sharma M, Pathak S, Srivastava P. Prevalence and antibiogram of Extended Spectrum β -Lactamase (ESBL) producing Gram negative bacilli and furthemolecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* spp. Antibiogram and Molecular Characterization of ESBL Producers. J Clin Diagn Res 2013;7:2173-7.
6. Tsering DC, Das S, Adhiakari L, Pal R, Singh TS. Extended spectrum β -lactamase detection in gram negative bacilli of nosocomial origin. J Glob infect Dis 2009;1:87-92.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement ed. CLSI document M100-S20. Wayne, PA:CLSI; 2010.
8. Jonathan N. Screening for extended-spectrum Beta-Lactamase-producing pathogenic enterobacteria in district general hospitals. J Clin Microbiol 2005;43:1488–90.
9. Afridi FI, Farooqui BJ, Hussain A. Frequency of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae Among Urinary Pathogen Isolates. J Col Physicans Surg Pak 2011;21:741-2.
10. Agrawal P, Gosh AN, Satish K, Basu B, Kapila K. Prevalence of extended-spectrum β -lactamases among *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital. Indian J Pathol Microbiol 2008;51:139-42.
11. Anathakrishnan AN, Kanungo R, Kumar A, Badrinath S. Detection of Extended Spectrum β -lactamases producers among surgical wound infections and burn patients in JIPMER. Indian J Med Microbiol 2000;18:160-5.
12. Kumar MS, Lakshmi V, Rajgoplan R. Occurrence of Extended spectrum β -lactamases among Enterobacteriaceae species isolated at tertiary care Institute. Indian J Med Microbiol 2006;24:208-2.
13. Amita J, Indranil R, Mahendra KG, Kumar M, Agarwal SK. Prevalence of extended-spectrum β -lactamase producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. J Med Microbiol 2003;52:421-5.

Cite this article as: Sreekanth B, Dattaraya GS. Detection of extended spectrum β -lactamases (ESBLs) in clinical isolates. Int J Med Sci Public Health 2014;3:1132-1134.

Source of Support: Nil

Conflict of interest: None declared