Original Research Paper

Volume-8 | Issue-3 | March-2018 | PRINT ISSN No 2249-555X

Microbiology

COMPARISON OF VARIOUS METHODS OF EXTENDED SPECTRUM BETA LACTAMASE DETECTION AMONG GRAM NEGATIVE ISOLATES OF FAMILY *ENTEROBACTERIACEAE.*

Shuchi Mehra* Junior Resident, Department of Microbiology, PGIMS Rohtak *Corresponding Author

Rama Sikka Professor, Department of Microbiology, PGIMS Rohtak

Uma Chaudhary Sr. Prof. & Head, Department of Microbiology,PGIMS Rohtak

ABSTRACT

Introduction: ESBL producing members of family *Enterobacteriaceae* have been responsible for numerous outbreaks of infection throughout the world. ESBL detection thus propagates the use of higher end antibiotics.

Material and methods: A total of 100 non repetitive MDR strains isolated from various clinical samples were included in our study. All isolates were identified by standard microbiological techniques and antimicrobial susceptibility pattern was determined. Detection of ESBL production was carried out by three methods: phenotypic confirmatory test (CLSI method), method using sulbactam as inhibitor and chromogenic medium.

Results: ESBL production was seen in 70 (70%) isolates by CLSI method, whereas 68 (68%) isolates showed ESBL production when sulbactam was used as the inhibitory agent. By using chromogenic medium, 77 (77%) out of 100 isolates showed ESBL production.

Conclusion: Chromogenic media are rapid and better screening method for ESBL detection.

KEYWORDS : MDR, ESBL, chromogenic medium

Introduction

Gram-negative bacteria cause serious and potential life-threatening infections particularly in hospitalized patients. The injudicious use of broad-spectrum antibiotics can lead to colonization with resistant strains with an increase in morbidity, mortality, and significant economic loss. Multidrug-resistant organisms (MDRO) by virtue of production of various β-lactamases confer resistance to many classes of antibiotics, particularly cephalosporins.[1]

ESBLs are capable of conferring bacterial resistance to the penicillins, early and extended-spectrum cephalosporins, and aztreonam (but not to cephamycins or carbapenems) by hydrolysis of these antibiotics. These are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam.[2] The ESBL producing organisms also express the AmpC β-lactamases and are co-transferred with the plasmids, thus mediating the fluoroquinolone and the aminoglycoside resistance.[3,4]

For detection of various types of ESBLs, molecular methods are the best but facilities are not available in most of the laboratories, especially in the developing countries. Therefore, various phenotypic methods are recommended for routine use to detect ESBL production. These methods used are tedious. Moreover, with the pharmaceutical pipeline running almost dry, rapid detection of such pan resistant organisms offers one of the best solutions to improve patient screening and hospital infection control practices.

The present study was undertaken to detect the prevalence of ESBLs in MDR isolates of family *Enterobacteriaceae* using various phenotypic tests.

Material and Methods: The present study was conducted in the Department of Microbiology, Pt. B.D. Sharma PGIMS Rohtak over a period of one year i.e from June 2016 to May 2017. Study was done on a total of 100 MDR isolates of Gram-negative bacilli of family *Enterobacteriaceae*, obtained from clinical samples (urine, blood, pus, sputum, stool, body fluids etc.) received in Microbiology laboratory from various inpatient and outpatient departments. All isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI 2016) guidelines. Antibiotic discs used in the study were procured from Hi- media laboratories, Mumbai, India. American Type Collection (ATCC) strain viz. *E. coli.* ATCC 25922 was employed as a control strain.[5,6]

The following antimicrobial discs were put up: ampicillin (10µg), gentamicin (10µg), amikacin (30µg), amoxicillin/ clavulanic acid (20µg/10µg), ampicillin/ sulbactam (10µg/10µg), ticaricillin/ clavulanic acid (75µg/10µg), cefuroxime (30µg), cefepime (30µg), ceftriaxone (30µg), cefotaxime (30µg), ciprofloxacin (5µg), levofloxacin (5µg), ertapenem (10µg), imipenem (10µg), meropenem (10µg), trimethoprim-sulfamethoxazole (1.25µg/23.75µg), aztreonam (30µg), ceftazidime (30µg), ofloxacin (5µg), norfloxacin (10µg), nitrofurantion (300µg), piperacillin/tazobactam (100 µg/10 µg), colistin(10µg) and polymyxin B(300 units).

ESBL Detection

Isolates showing reduced susceptibility to third generation cephalosporins were tested for ESBL production as per CLSI guidelines, method using sulbactam as inhibitory agent and method using chromogenic medium.

CLSI method:

ESBL production was detected as per CLSI guidelines. The test organism was inoculated on MHA plate. A 30µg disc of ceftazidime and a 30µg disc of cefotaxime and another 30/10 µg disc of ceftazidime/ clavulanic acid and 30/10 µg disc of cefotaxime/ clavulanic acid were placed on surface of agar plate. The plates were then incubated at 35^0 C ± 2^0 C for 16-18 hours. An increase in zone diameter of ≥5mm for antimicrobial agent tested in combination with clavulanic acid verses its zone when tested alone was considered positive for ESBL production.[5]

Method using sulbactam as an inhibitory agent:

The test organism was inoculated on MHA plate as per CLSI guidelines. One 75µg disc of cefoperazone and 30 µg disc of ceftriaxone were placed on the surface of MHA plate and another 75/30µg disc of cefoperazone/sulbactam and 30/15µg ceftriaxone/ sulbactam were placed on the same agar plate at a distance of approximately 15mm from cefoperazone and ceftriaxone respectively. A ≥ 5mm increase in zone diameter for antimicrobial agent tested in combination with sulbactam versus its zone when tested alone was considered positive for ESBL production.[7]

Method using chromogenic medium:

HiCrome ESBL Agar (procured from Hi-media laboratories, Mumbai, India) was inoculated with liquid suspension equivalent to 0.5 McFarland turbidity. ESBL producing *E.coli* produced either pink or purple colonies. ESBL producing members of the KESC (*Klebsiella* spp*, Enterobacter* spp*, Serratia* spp *and Haffnia* spp.) group produced bluish green colonies; *Proteus* spp, *Morganella* spp. and *Providencia* spp. did not utilize any chromogen resulting in colourless to light brown colonies.

Statistical analysis

Two or more sets of variables were compared by using SPSS software. If the p-value was <0.05, it was considered to be statistically significant.

Results

Among the 100 MDR isolates studied, ESBL production was seen in 70 (70%) isolates by CLSI method, when clavulanic acid was used as

inhibitor. ESBL production was seen highest in *E. coli* (52.85%) followed by *Klebsiella* spp. (30%) and *Proteus* spp.(7.14%). Prevalence of ESBL production was studied by other phenotypic methods also. Table 1 shows evaluation of these methods in comparison to the CLSI method (gold standard).

Table 1: Comparison of various methods of ESBL detection in 100 MDR isolates

Name of Method	ESBL producers		ESBL non producers	
	No.	Percentage (%)	No.	Percentage (%)
CLSI method	70	70	30	30
Method using sulbactam as inhibitory agent	68	68	32	32
Method using chromogenic medium	77	77	23	23

On comparing various phenotypic methods for ESBL production, it was found that 68 (68%) isolates showed ESBL production when sulbactam was used as the inhibitory agent whereas 77 (77%) isolates showed ESBL production by using chromogenic medium.

Statistical analysis of different methods is shown in table 2.

Table 2: Statistical analysis of different methods of ESBL detection in 100 MDR isolates

Method	Sensitivity (%)	Specificity (%)	NPV(%)	PPV(%)
Chromogenic medium	100	85	100	90.9
Method using sulbactam as inhibitory agent	97.1	100	93.7	100

NPV-Negative predictive value; PPV-Positive predictive value

On analysis of various methods of ESBL detection, it was found that the sensitivity of chromogenic medium was 100% while that of method using sulbactam was 97.1%. The specificity of chromogenic medium for ESBL detection was 85% while that of method using sulbactam was 100%. The positive predictive value of method using chromogenic medium was 90.9% whereas that of method using sulbactam was 100%. The negative predictive value of method using chromogenic medium was 100% whereas that of method using sulbactam was 93.7%.

Discussion

The rate of antimicrobial drug resistance and particularly of multiple drug resistance are increasing among *Enterobacteriaceae*, thus limiting the armamentarium of potentially active antimicrobial agents. Of particular importance are pathogens of this family that produce β-lactamases with a broad profile of substrate activity such as extended-spectrum β-lactamases (ESBLs). The high prevalence of these organisms in the ICUs emphasizes the need for an early detection of the β-lactamase producing organisms by simple screening methods, which can help in providing an appropriate antimicrobial therapy.[3]

The prevalence of bacteria producing ESBLs varies from 20-71% in India and 8-45% worldwide.[8,9] In the present study 70% of MDR isolates were ESBL producers. *E. coli* was the predominant ESBL producer. Similar findings were reported in a study by Gupta et al. and Siddiqui et al.[10,11] We observed that a majority of the isolates in our study were susceptible to imipenem (77.2%). Similar findings were reported by Siddiqui et al and Ravikant et al.[11,12] However this drug should be used as reserve one, avoiding its irrational use thus preventing the emergence of resistance.

Different methods were used in the present study for detection of ESBL production. The confirmatory method recommended by CLSI had detected ESBL production in 70% of isolates. With method using sulbactam as inhibitory agent, 68 (68%) out of 100 MDR isolates were detected to be ESBL producers. The sensitivity and specificity of this method when compared with CLSI method was 97.1% and 100% respectively. Although clavulanic acid is more potent than sulbactam, its ability to induce AmpC production may interfere with ESBL production.[13] AmpC producing organisms act as hidden reservoirs for ESBLs. Such isolates, when tested by clavulanic acid inhibition test, produce high levels of AmpC enzymes thus masking the effect arising due to inhibition of ESBLs. False negative results are seen in such cases. Induction of AmpC β-lactamases is less likely with sulbactam and tazobactam which are thus considered as preferable inhibitors for ESBL detection tests.[14]

In our study, out of 100 MDR isolates, 77 were ESBL producers by method using chromogenic medium. Sensitivity of this method was found to be 100% while specificity was 85%. In a study done by Kaluzna et al, using the CHROMagar ESBL and ChromID ESBL for detection of ESBL-positive strains of *E. coli*, maximum number of positive results were obtained. The results of these studies and the results of our own suggest that chromogenic media for the detection of ESBL-positive strains are characterized by a relatively high sensitivity, but lower specificity, which creates the risk of false-positive results.[15]

Conclusion

The high prevalence of ESBLs emphasizes the need for their early detection by simple screening methods, which can help in providing an appropriate antimicrobial therapy. In our study, chromogenic media were found to have high sensitivity, whereas method using sulbactum has high specificity, when compared with CLSI method. Routine screening for ESBL-producing bacteria using chromogenic culture medium can be adopted in Clinical Microbiology Laboratories. Though expensive, it significantly reduces the need for other phenotypic confirmatory test or molecular tests which are time consuming and require technical expertise.

References

1. Livermore DM. Bacterial resistance:origins, epidemiology, and impact. Clin Infect Dis. 2003;36: S11-23.
2. Spellberg B, Blaser M, Guidos RJ. Combating antimicrobial resistance: policy recommendations to save lives. Clin Infect Dis. 2011;52:397-428.
3. Oberoi L, Singh N, Sharma P, Aggarwal A. ESBL, MBL and AmpC beta-lactamases producing superbugs-Havoc in the Intensive Care Units of Punjab. India J Clin Diagn Res. 2013;7(1):70-3.
4. Pitout JD, Reisbig MD, Venter EC, Church DL, Hanson ND. Modification of the double disc test for the detection of the Enterobacteriaceae which produced the extended spectrum and the AmpC β lactamases. J Clin microbiol. 2003;41:3933-5.
5. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility test. Twenty first international supplement. CLSI document. M100-S21. Wayne PA;2011.
6. Magiorakos AP, Srinivasan A, Carey RB, Struelens MJ, Weber JT, Monnet DL et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standards definition for acquired resistance.Clin Microbiol Infect. 2012;18(3): 268-81.
7. Jacoby GA, Han P. Detection of extended-spectrum beta-lactamases in clinical isolates of K.pneumoniae and E.coli. J Clin Microbiol. 1996;34(4):908-11.
8. Metri BC, Jyothi P, Peerapur BV. The prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India. J Clin Diag Res. 2011;5(3):470-5.
9. Winokur PL, Canton R, Casellas JM, Legakis N. Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, America, and the Western Pacific Region. Clin Infect Dis. 2001;32 Suppl 2:S94-103.
10. Gupta V, Singla N, Chander J. Detection of ESBLs using third & fourth generation cephalosporins in double disc synergy test. Indian J Med Res. 2007;126:486-7.
11. Siddiqui N, Bhakre J, Ajit Damle, Bajaj J. Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing Gram Negative Bacilli from various clinical isolates. IOSR Journal of Dental and Medical Sciences. 2014;13(9): 8-11.
12. Ravikant, Kumar P, Ranotkar S. Prevalence and identification of extended spectrum beta-lactamases (ESBL) in Escherichia coli isolated from a tertiary care hospital in NorthEast India. Indian J Exp Biol. 2016;54:108-14.
13. Haider M, Rizvi M, Fatima N, Shukla I, Malik A. Necessity of detection of extended spectrum betalactamase, AmpC and metallo-beta-lactamases in Gram negative bacteria isolated from clinical specimens. Muller J Med Sci Res. 2014;5:23-8.
14. Rajini E, Sherwal BL, Anuradha. Detection of extended-spectrum β-lactamases in AmpC β-lactamases producing nosocomial gram-negative clinical isolates from a tertiary care hospital in Delhi. Ind J Pract. 2008;4(6):1-2.
15. Kałuzna E, Zalas-Wiecek P, Gospodarek E. Comparison of detection methods for extended-spectrum beta-lactamases in Escherichia coli strains. Postepy Hig Med Dosw . 2014;68:808-13.