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Original Research Paper

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DETECTION OF VARIOUS BETA-LACTAMASES IN MDR ISOLATES OF FAMILY ENTEROBACTERIACEAE

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ABSTRACT

Introduction: Infections due to Gram-negative bacilli are on rise world over. The rampant 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.

Material and methods: The present study was conducted over a period of one year on a total of 100 non repetitive MDR isolates of Gram-negative bacilli of family Enterobacteriaceae. All isolates were identified by standard microbiological techniques and antimicrobial susceptibility pattern was determined. American Type Collection (ATCC) strain viz. E. coli. ATCC 25922 was employed as a control strain. Isolates showing reduced susceptibility to third generation cephalosporins were tested for ESBL production as per CLSI guidelines. Isolates showing reduced susceptibility to cefoxitin were screened by AmpC disc test. Isolates showing reduced susceptibility to carbapenems were tested for MBL production by ceftazidime-EDTA disc test.

Results: The prevalence of ESBL, AmpC and MBL was 70%, 39% and 45% respectively. ESBL production along with MBL production was seen in 19% of the isolates. ESBL production along with AmpC production was seen in 12% of the isolates. MBL production along with AmpC production was observed in 10% of the isolates. Coproduction of all three β-lactamases was observed in 9% of the isolates.

Conclusion: The high prevalence of beta-lactamases emphasizes the need for an early detection by simple screening methods, which can help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistant strains.

KEYWORDS : MDRO, ESBL, AmpC, MBL

INTRODUCTION

Infections due to Gram-negative bacilli are on rise world over. The rampant 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]

Extended spectrum β-lactamases (ESBLs), have the ability to hydrolyze and cause resistance to various types of the newer β-lactam antibiotics, including cephalosporins like cefotaxime, ceftriaxone, ceftazidime, and monobactams (e.g., aztreonam) but not the cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., Imipenem, meropenem and ertapenem).[2] Plasmid-mediated AmpC β-lactamases have arisen through the transfer of chromosomal genes from the inducible AmpC β-lactamase onto plasmids. This transfer has resulted in plasmid mediated AmpC β-lactamases in isolates of Escherichia coli, Klebsiella pneumoniae, Salmonella spp., Citrobacter freundii, Enterobacter aerogenes and Proteus mirabilis.[3]

AmpC beta-lactamases are clinically significant, since they confer resistance to cephalosporins in the oxyimino group (cefotaxime, ceftriaxone, ceftazidime), 7 alpha methoxy cephalosporins (CX) and are not affected by available beta-lactamase inhibitors (clavulanate, sulbactam).[4]

Plasmid mediated AmpC beta-lactamases differ from chromosomal AmpC in being non-inducible and are typically associated with broad multi-drug resistance. Therapeutic options for infections caused by Gram-negative organisms expressing AmpC beta-lactamases are limited except for fourth generation cephalosporins such as cefepime and carbapenems. This emphasizes the need for detecting AmpC beta-lactamase harbouring isolates so as to avoid therapeutic failures and nosocomial outbreaks.[5]

Metallo β-lactamases (MBLs) have the ability to hydrolyze almost all drugs including carbapenems as well as aminoglycosides and fluoroquinolones and an ability to rapidly disseminate as they are plasmid mediated.[6]

The mechanism of hydrolysis is based on the interaction of zinc ions in the enzyme's active site and this is the part inhibited by EDTA.[7] The currently emerging MBL is the NDM (New Delhi metallo-beta-lactamase) detected in the chromosome of Acinetobacter baumanii, but detected in Enterobacteriaceae mainly in Klebsiella spp. and E. coli.[8]

The Clinical Laboratory Standards Institute (CLSI) has not yet published the guidelines for the detection of AmpC beta - lactamases and MBLs . Though a number of phenotypic methods have been proposed, the coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. Hence, it is necessary to know the accurate prevalence of the β-lactamase producing strains in the high risk areas, so as to formulate a policy of the empirical therapy in the ICUs where the infections which are caused by the resistant organisms are much higher.[9,10]

In view of need of cheap and easy methods for the diagnosis of various β-lactamases in basic microbiological laboratories ensuring an evidence-based medicine as prescribed by CDC, we planned a cross-sectional prospective analytical study to determine resistance mechanism by various β-lactamases in Gram-negative clinical isolates using various phenotypic methods.

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 non repetitive 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 antibiotic susceptibility testing by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) criteria. Mueller-Hinton agar (MHA) was used for antibiotic sensitivity testing. American Type Collection (ATCC) strain viz. E. coli. ATCC 25922 was employed as a control strain.[11,12]

Isolates showing reduced susceptibility to third generation cephalosporins were tested for ESBL production as per CLSI guidelines. Isolates showing reduced susceptibility to cefoxitin were screened by AmpC disc test. Isolates showing reduced susceptibility to carbapenems were tested for MBL production by ceftazidime-EDTA disc test.

ESBL Detection

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 ± 20 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.[11]

AmpC disc test

A lawn culture of E. coli ATCC 25922 was prepared on MHA plate. Several colonies of the test organism were inoculated on sterile disc moistened with sterile saline (20µl). The inoculated disc was placed beside a cefoxitin disc (30µg) on agar plate. The plates were inoculated overnight at 35^{0}C ± 20 C. A positive test appeared as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc. A negative test showed an undistorted zone.[13]

MBL detection

Test organism was inoculated on MHA plate. Two 30µg ceftazidime discs were placed on the surface of agar plate and 10 µl of 0.5 M EDTA solution was added to one ceftazidime disc to obtain a desired concentration of 750µg. The inhibition zones of ceftazidime and ceftazidime EDTA discs were compared after 16-18 hours of incubation at 35^{0}C. A positive test was indicated by zone enhancement with EDTA impregnated ceftazidime discs. The zone size enhancement of ≥7mm for ceftazidime-EDTA as compared to ceftazidime disc alone respectively was taken as positive criteria for MBL production.[14,15]

RESULTS

Majority of MDR strains were E. coli (54%) followed by Klebsiella species (28%), Proteus species (7%), Citrobacter species (4%), Enterobacter species (4%) and Providencia species (3%).

Out of 100 MDR isolates, 70 (70%) were ESBL producers by CLSI method. Eighty nine (89%) isolates were cefoxitin resistant and 39(43.8%) were found to be AmpC producers. Out of these, 31 (31%) isolates showed flattening and 8 (8%) showed indentation.

Out of total 100 MDR isolates, 48(48%) were imipenem/meropenem resistant i.e. screen positive. Out of these 48 screen positive isolates, 45 (93.7%) were MBL producers by ceftazidime-EDTA combined disc method.

The prevalence of ESBL, AmpC and MBL was 70%, 39% and 45% respectively.

ESBL production was seen maximally in E. coli(52.85%) followed by Klebsiella spp. (30%) and Proteus spp.(7.14%). AmpC production was seen maximally in E. coli (61.5%), followed by Klebsiella spp.(20.5%) and Proteus spp(7.6%). MBL production was maximally seen in E. coli (55.5%), followed by Klebsiella spp.(26.6%) and Citrobacter spp. (6.6%).

ESBL production along with MBL production was seen in 19 (19%) isolates. ESBL production along with AmpC production was seen in 12 (12%) isolates. MBL production along with AmpC production was observed in 10 (10%) isolates. Coproduction of all three β-lactamases was observed in nine (9%) isolates.

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), AmpC β-lactamases, as well as carbapenemases, including metallo-β-lactamases (MBLs). Increasing prevalence and severity of infections caused by multidrug-resistant (MDR) Gram-negative pathogens, particularly MDR Enterobacteriaceae, and the meager pipeline of novel antibiotic therapies in development to treat Gram-negative infections have resulted in limited treatment options for an increasing number of patients.[28]

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 and in avoiding the development and the dissemination of these multidrug resistant strains.[9] The prevalence of ESBLs among clinical isolates varies greatly worldwide and in geographic areas, and is rapidly changing over time. It varies from country to country and institution to institution. In our study the prevalence of ESBL is 70%. Various studies have been conducted showing difference in the prevalence of ESBLs as depicted in Table 1.

Table 1: Prevalence of beta-lactamases in different studies

ESBL producers	
Present study	70%
Siddiqui et al[18]	61%
Mathur et al[19]	68 %
Nurul et al[20]	58.6 %.
Nevine et al[21]	65.8 %.
Ali et al[22]	45 %
AmpC producers	
Present study	39%
Mohamudha et al[23]	47%
Rawat et al[24]	20.8%
Oberoi et al[9]	5.4%
Singhal et al[13]	8%
Haider et al[25]	19.52%
MBLproducers	
Present study	45%
Franklin et al[26]	62.68%
Jain et al[27]	16%
Haider et al[25]	17.93%

This wide variation could be explained by the high amount of geographical variation shown by ESBL producers.

The prevalence of AmpC producing isolates in this study was 39%. Various studies have been conducted showing difference in the prevalence of AmpC as depicted in Table 1 . The high prevalence of the AmpC producers in our study could be due to the differences in the geographical distribution, which may have produced variations in the prevalence of the β-lactamases and also due to the extensive use of third-generation cephalosporins that has resulted in the increased prevalence of plasmid-mediated AmpC among these organisms.

Considering the antimicrobial susceptibility profile, AmpC

producing isolates in our study were most susceptible to imipenem. Only six out of 39(15.3%) strains were resistant to imipenem. The results were concordant with the study conducted by Handa et al, which also showed least resistance with imipenem (5.56%) among all AmpC producing isolates.[5] Thus it can be concluded that imipenem is still the most effective antibiotics among AmpC beta- lactamase producing bacteria.

The prevalence of MBL producers in our study was 45%. Various studies have been conducted showing difference in the prevalence of MBLs as depicted in Table 1 .

The high prevalence of MBL in our study may be due to the excessive use of broad-spectrum antibiotics in our institute.

Coproduction of various β-lactamases

In the present study, the coproduction of ESBL and MBL enzymes was detected in 19 (19%) out of 100 MDR isolates. the coproduction of ESBL and AmpC enzymes was detected in 12 (12%) out of 100 MDR isolates. MBL and AmpC enzymes were coproduced by 10 (10%) out of 100 MDR isolates.

In the present study, the coproduction of ESBL, AmpC β-lactamases and MBL was observed in 9(9%) out of 100 MDR isolates. Various studies have been conducted showing the coproduction of different beta-lactamases as depicted in Table 2, 3 4 and 5 . The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. The AmpC producing organisms can act as a hidden reservoir for the ESBLs. Also, the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBLs and it may result in a fatal and an inappropriate antimicrobial therapy. [6]

The detection of the coproduction of various β-lactamases singly or in combinations is essential for enhanced infection control and effective antimicrobial therapy. Although ESBL detection and reporting is recommended routinely by CLSI, it lacks guidelines for the AmpC or combination of various β-lactamases. [17] In spite of many phenotypic tests, a reference laboratory for beta-lactamase isoelectric focusing and gene localization by genotype characterization are considered as the gold standard. This will help us to know the actual prevalence of these enzymes and characterize them for epidemiological purpose.[5]

Table 2: Results of various studies showing coproduction of ESBL and MBL

Present study	19%
Kolhapure et al[16]	4.81%
Anusuya et al[28]	16%
Rawat et al[24]	0%

Table 3: Results of various studies showing coproduction of ESBL and AmpC

Present study	12%
Kolhapure et al[16]	9.77%
Anusuya et al[28]	24%
Rawat et al[24]	25%
Nagdeo etal[17]	27.14%

Table 4: Results of various studies showing coproduction of MBL and AmpC

Present study	10%
Kolhapure et al[16]	6.23%
Rawat et al[24]	0%
Nagdeo etal[17]	11.40%

Table 5: Results of various studies showing coproduction of ESBL, MBL and AmpC

Present study	9%
Kolhapure et al[16]	5.09%
Oberoi et al9	19.04%
Nagdeo etal17	4.21%

REFERENCES

1. Livermore DM. Bacterial resistance:origins, epidemiology, and impact. Clin Infect Dis. 2003;36: S11-23.
2. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001;14:933-51.
3. Thompson KS. Controversies about Extended-Spectrum and AmpC Beta-lactamases. Emerg Infect Dis. 2001;7:333-6.
4. Abraham EP, Chain E. An enzyme from bacteria able to destroy penicillin. Nature 1940;146;146:837.
5. Handa D, Pandey A, Asthana AK, Rawat A, Handa S, Thakuria B. Evaluation of phenotypic tests for the detection of AmpC beta-lactamase in clinical isolates of Escherichia coli. Indian J Pathol Microbiol. 2013;56:135-8.
6. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo beta-lactamases: the quiet before the storm? Clin Microbiol Rev. 2005;18:306-25.
7. Queenan AM, Bush K. Carbapenemases:the versatile β-lactamases. Clin Microbiol Rev. 2007;20:440–58.
8. Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of blaNDM-1 positive Enterobacteriaceae. Antimicrob Agents Chemother. 2011;55:5403-7.
9. 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.
10. 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.
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility test. Twenty first international supplement. CLSI document. M100-S21. Wayne PA;2011.
12. 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.
13. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, et al. Evaluation of methods for AmpC β-lactamases in Gram-negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol. 2005;23:120-24.
14. Falagas ME, Bliziotis IA. Pandrug-resistant Gram-negative bacteria: the dawn of the post-antbiotic era? Int J Antimicrob Agents. 2007;29:630-6.
15. Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB. The metallo β lactamase producing clinical isolates from the patients of a tertiary care hospital. Journal of Laboratory Physicians.2011;3(2):93-7.
16. Kolhapure RM, Kumar A, Rajkumar HR. Co-expression of ESBL, AmpC and MBL in Gram-negative bacilli. Int J Res Med Sci. 2015;3(10):2698-703.
17. Nagdeo NV, Kaore NM, Thombare VR. Phenotypic methods for detection of various beta-lactamases in Gram negative clinical isolates: Need of the hour. Chron Young Sci. 2012;3:292-8.
18. 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.
19. Mathur P, Kapil A, Das B, Dhawan B. The prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. Ind J Med Res. 2002;115:153-7.
20. Nurul MA, Loo HKC, Subramaniam G, Wong EH, Selvi P, Ho S et al. Faecal prevalence of extended-spectrum β- lactamase (ESBL)-producing coliforms in a geriatric population and among haematology patients. Malaysian J Pathol. 2005;27(2):75-81.
21. Nevine S. Fam, M M. El-Damarawy. CTX-M-15 Extended-Spectrum Beta-Lactamases Detected from Intensive Care Unit of an Egyptian Medical Research Institute. Research Journal of Medicine and Medical Sciences. 2008;3(1):84-91.
22. Ali A M, Rafi S, Qureshi AH. Frequency of Extended Spectrum Beta Lactamase Producing Gram Negative Bacilli among Clinical Isolates at Clinical Laboratories of Army Medical College, Rawalpindi. J Ayub Med Coll Abbottabad 2004;16(1):35-7
23. Mohamudha PR, Harish BN, Parija SC. AmpC Beta lactamases among gram negative clinical isolates from a tertiary hospital, South India. Brazilian J Microbiol. 2010;41:596-602.
24. Rawat V, Singhai M, Verma PK. Extended-spectrum-beta-lactamase, AmpC, and Carbapenamase issues. J Clin Microbiol 2010;48:1019-25.
25. 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.
26. Franklin C, Liolios L, and Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol. 2006;44:3139–44.
27. Jain P, Gandhi V, Patel K, Modi G, Parmar R, Soni S et al. Phenotypic Detection of Metallo-β-lactamase Producing Enterobacteriaceae. International Journal of Microbiology Research. 2012;9:326-9.
28. Devi A, Ramachander R. Detection of ESBL, Amp C and Metallo Beta Lactamases in Clinical Isolates of Enterobacteriacae from a Tertiary Care Hospital Emergence of Carbapenamase and Other Beta Lactamases among Enterobacteriacae from a Tertiary Care Hospital. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2015;6(5):1377-83.