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Original Research Article

Comparison of combined disc synergy test and double disc synergy test for phenotypic detection of metallo-ß-lactamase among the clinical isolates of gram-negative bacilli

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ABSTRACT

Background: Metallo-β-lactamases (MBL) have a wide spectrum of activity and they confer a higher level of resistance to all β-lactams antibiotics including Carbapenem. The active site in MBLs contains either 1 or 2 Zn2+ ions for their catalytic mechanism. All MBLs share a common feature of being inhibited by EDTA. Metallo-β-lactamase-producing gram-negative bacteria are the most important nosocomial pathogens. The present study was conducted to detect Metallo-β-lactamase (MBL) production in gram-negative bacilli by Combined Disc Synergy Test (CDST) and Double Disc Synergy Test (DDST) with 0.1M EDTA as a chelator and to see their antibiotic susceptibility pattern of them.

Methods: The cross-sectional observational study was carried out in the Department of Microbiology, Chittagong Medical College, during the period of July 2015 to June 2016. Samples were collected from patients admitted to CMCH. Standard Microbiological procedures and biochemical tests were carried out for the isolation and identification of MBL. SPSS software is used for data analysis.

Results: When 66 screening positive MBL isolates were subjected to the phenotypic confirmatory test CDST detected 50 (25.4%) and DDST detected 48 (24.4%) as MBL producers. Among these isolates, we found *Acinetobacter spp.* 7 (100%), as the leading MBL producer followed by *Pseudomonas spp.* 16 (32.6%), *E. coli* 10 (20%) and *Klebsiella spp.* 15 (17.4%).

Conclusions: In a laboratory where multiplex PCR molecular set-up is not available CDST and DDST are convenient phenotypic methods and can be implemented in routine microbiological laboratories as well as in primary health care setup for daily application to monitor the production of MBLs.

Keywords: Microbiological, Antibiotics, Pathogen, Bacteria, MBL, Synergy

INTRODUCTION

The emergence of resistance to antimicrobial agents is becoming a major health problem worldwide, especially in hospital-acquired infections. One of the most important mechanisms of microbial resistance to β lactam antibiotics is hydrolyzed by β lactamase. Metallo-β-lactamase (MBL) has emerged as a powerful resistance

determinant in gram-negative bacteria. MBLs exhibit broad-spectrum activity and hydrolyses virtually all classes of β-lactams with the exception of Monobactams, e.g. Aztreonam.[1] These Metallo-b-lactamases are associated with resistance to Aminoglycosides and Fluoroquinolones further compromising the future therapeutic choices.[2] The first acquired MBL gene identified was blaIPM reported from Japan in Pseudomonas aeruginosa in 199.[3] Subsequently, other acquired MBLs; VIMs, NDMs, SPM-1, GIM-1, and SIM-1 have been identified.[4] On a global scale IPMs, VIMs, NDMs, and to some extent SPM-1 are the most prevalent MBLs. There are several risk factors in patients with Cabapenem-resistant MBL-positive isolates, like staying at the hospital for more than eight days, catheterization, intravenous infusion, previous antibiotic use, mechanical ventilation, and endotracheal intubation, graft application, surgical intervention, bronchoscopy, lacerated and contaminated wound.[5] The non-molecular methods used for detection of MBL were the Double Disc Synergy Test (DDST), Combined Disc Synergy Test (CDST), Micro dilution MIC test, Modified Hodge test, E test, and Carbapenem hydrolysis test. Molecular detection methods such as Isoelectric focusing, PCR, DNA probes, Cloning, and Sequencing.[6] The sensitivity and specificity of the disc diffusion tests, using a Ceftazidime disc and two MBL inhibitors [EDTA and mercaptopropionic acid] were comparable with those of PCR for detection of MBL production.[7] Later, the DDST method and CDST method for easy detection of MBL in routine laboratories.[8] These phenotypic methods are highly sensitive (100%) and specific (98%) in detecting Carbapenem-resistant MBL carrying isolates.[9] These methods were simple to perform and the materials used were cheap, nontoxic, and easily accessible making them highly applicable to routine clinical laboratories.[10]

The most common Carbapenemase among Enterobacteriaceae in the United States was the Ambler class A *Klebsiella pneumonia*. Among the most recent Carbapenemases to appear in the United States was the newly described New Delhi Metallo β Lactamase (NDM-1) first reported in 2009. NDM-1 was initially identified in K. pneumoniae. isolated from a Swedish patient who had been hospitalized in India.[11] In Europe, Veronese imipenemase (VIM) type MBLs and the so-called K. pneumoniae Carbapenemases (KPC) was the most frequently isolated Carbapenemases.[12] In Bangladesh, 22.86% NDM-1(gene) positive isolates by PCR have been detected from the Imipenem resistant organisms.[13] However, another study has been conducted in BIRDEM, Dhaka, in which 43% of Pseudomonas spp. isolates from various clinical samples were MBL producers by the MIC method.[14] A study in Bangladesh among 53 Pseudomonas spp. 67.9% were positive by DDST on the other hand, 69.8% were positive by CDST. In the case of 19 *Acinetobacter spp*. isolates, 51.7% were positive by DDST similarly 55.2% were positive by CDST.[15]

MBL-producing gram-negative bacilli are increasing worldwide including in Bangladesh. Multiplex PCR is the gold standard method for detecting MBL producers which is not feasible in routine microbiological laboratories. So simple screening test like CDST and DDST with 0.1M EDTA as a chelator is used in this study for the detection of MBL.

Objectives

General objective

Comparison of combined disc synergy test and double disc synergy test for phenotypic detection of metallo-b-lactamase among the clinical isolates of gram-negative bacilli.

Specific objectives

Isolation and identification of Metallo-ß-lactamase producing gram-negative bacilli. To compare the results of two different phenotypic methods.

METHODS

This cross-sectional study was conducted at the Department of Microbiology, Chittagong Medical College and Hospital, Chittagong. During the period July 2015 to June 2016. A total of 197 participants were selected for this study following the selection criteria. Samples were collected from patients admitted to the Surgery, Gynae, Intensive Care Unit (ICU). Urology Department and Burn unit of Chittagong Medical College. Patients were selected from those who were clinically isolated with gram-negative bacilli. The Metallo-β-lactamase screening test was done to screen the patients for gram-negative organism types and their distribution among patients. Kirby Baurer Disk Diffusion Method was also used among the participants. A combined disk synergy test and double disk synergy test were done among patients who were resistant to both IPM and CAZ. Informed written consent was obtained from the study participants, and the study was commenced after the approval of the protocol by the ethical review committee of Chittagong Medical College. Samples were collected from patients admitted to the Surgery, Gynae, Intensive Care Unit (ICU). Urology Department and Burn unit of Chittagong Medical College. Data was collected using a pre-prepared questionnaire, and statistical analysis of the collected data was done using SPSS software.

RESULTS

Results of MBL screening test among gram-negative isolates

Among 197 (100.0%) gram-negative bacilli 66 (33.5%) were both Imipenem and Ceftazidime resistant, 81 (41.1%) were Imipenem sensitive but Ceftazidime resistant and 50 (25.4%) were both Imipenem and Ceftazidime sensitive. There were no Imipenem resistant but Ceftazidime sensitive gram-negative bacilli found.

Table 1: Results of Metallo-β-lactamase (MBL) screening test among gram-negative isolates.

Name of gram-negative organism	Number	Kirby Baurer Disk Diffusion Method			
		Both IPM and CAZ Resistant	IMP Sensitive and CAZ Resistant	IMP Resistant and CAZ Sensitive	Both IPM and CAZ Sensitive
E. coli	50 (25.4)	14 (28.0)	20 (40.0)	0	16(32.0)
***Klebsiella* spp.**	86 (43.7)	24 (27.9)	39 (45.3)	0	23 (26.8)
***Pseudomonas* spp.**	49 (24.9)	19 (38.8)	19 (38.8)	0	11 (22.4)
***Proteus* spp.**	5 (2.5)	2 (40.0)	3 (60.0)	0	0 (0.0)
***Acinetobacter* spp.**	7 (3.5)	7 (100.0)	0 (0.0)	0	0 (0.0)
Total	197 (100.0)	66 (33.5)	81 (41.1)	0	50 (25.4)

Table 2: Results of CDST and DDST among the screening test positive cases (n=66).

	Positive for MBL	Negative for MBL
Combined disc synergy test (CDST)	50 (75.8)	16 (24.2)
Double disc synergy test (DDST)	48 (72.7)	18 (27.3)
Figures within parentheses indicate percentages		

Among a total of 50 (25.4%) *E. coli* isolates 14 (28.0%) were resistant to both Imipenem and Ceftazidime. Among the total 86 (43.7%) Klebsiella isolates 24 (27.9%) were resistant to both Imipenem and Ceftazidime. Among the total 49 (24.9%) Pseudomonas isolates 19 (38.8%) were resistant to both Imipenem and Ceftazidime. Among the total 5 (2.5%) Proteus isolates 2 (40.0%) were resistant to both Imipenem and Ceftazidime. Among the total 7 (3.5%) Acinetobacter isolates all are resistant to both Imipenem and Ceftazidime.

Among a total of 50 (25.4%) *E. coli* isolates 20 (40.0%) were resistant to Ceftazidime and sensitive to Imipenem. Among the total 86 (43.7%) *Klebsiella spp.* isolates 39 (45.3%) were resistant to Ceftazidime and sensitive to Imipenem. Among the total 49 (24.9%) Pseudomonas spp isolates 19 (38.8%) were sensitive to Imipenem and resistant to Ceftazidime. Among the total 5 (2.5%) *Proteus spp.* isolates 3 (60.0%) were sensitive to Imipenem and resistant to Ceftazidime. No Imipenem sensitive and Ceftazidime resistant Acinetobacter spp. found.

Among a total of 50 (25.4%) *E. coli* isolates 16 (32.0%) were sensitive to both Imipenem and Ceftazidime. Among the total 86 (43.7%) Klebsiella spp. isolates 23 (26.8%) were sensitive to both Imipenem and Ceftazidime. Among the total 49 (24.9%) Pseudomonas spp. isolates 11 (22.4%) were sensitive to both Imipenem and Ceftazidime. Among the total 5 (2.5%) Proteus species, no Proteus spp. were sensitive to both Imipenem and Ceftazidime. Among the total 7 (3.5%) Acinetobacter spp. no Acinetobacter spp. were sensitive to Imipenem and Ceftazidime.

Results of CDST and DDST among the screening test positive cases (n=66)

Out of 197 gram-negative bacteria, 66 (33.50%) were resistant to both Imipenem and Ceftazidime by Kirby Bauer disc diffusion technique taken as screening positive for MBL. Among the 66 (100%) screening test positive cases 50 (75.8%) were positive for MBL producers by the Combined Disc Synergy Test and 48 (72.7%) were positive for MBL producers by the double disc synergy test.

Comparison between combined and double disc synergy tests on detection of MBL producing strains among screening test positive cases

The comparison between combined and double disc synergy tests on detection of MBL producing strains among gram-negative bacteria. Higher rate MBL producers were observed in Pseudomonas species. Out of 19 (38.8%) screening positive cases Pseudomonas spp. 16 (32.6%) strains were found positive by both Combined Disc Synergy Test and Double Disc Synergy Test. Out of 24 (27.9%) screening positive Klebsiella species, 15 (17.4%) strains were found positive by Combined Disc Synergy Test and 13 (15.1%) found positive by Double Disc Synergy Test. Out of 14(28.0%) screening test positive *E. coli* species, 10 (20.0%) strains were found positive by both combined disc synergy test and double disc synergy test. Out of 7 (100.0%) screening positive Acinetobacter species 7 (100.0%) strains were found positive by both Combined Disc Synergy Test and Double Disc Synergy Test. Out of 2 (40.0%) Proteus species, 2 (40.0%) strains were found positive by both combined disc synergy test and double disc synergy test.

Table 3: Comparison between combined and double disc synergy tests on detection of MBL producing strains among screening test positive cases.

Name of strains tested	Total No.	Screening positive (imipenem resistant)	Combined disc synergy test positive	Double disc synergy test positive
E. coli	50 (22.7)	14 (28.0)	10 (20.0)	10 (20.0)
***Klebsiella* spp.**	86 (43.7)	24 (27.9)	15 (17.4)	13 (15.1)
***Pseudomonas* spp.**	49 (24.9)	19 (38.8)	16 (32.6)	16 (32.6)
***Proteus* spp.**	5 (2.5)	2 (40.0)	2 (40.0)	2 (40.0)
***Acinetobacter* spp.**	7 (3.5)	7 (100.0)	7 (100.0)	7 (100.0)
	197 (100.0)	66 (33.5)	50 (25.4)	48 (24.4)

Table 4: Association between CDST and DDST (with χ2 test significance).

		Double disc synergy test			χ^2 test significance
		Positive	**Negative**	**Total**	
Combined Disc Synergy Test	Positive	46	4	50	χ^2= 34.720
	Negative	2	14	16	
Total		48	18	66	p<0.001. Highly Significant

No strains were found Double Disc Synergy Test positive but Combined Disc Synergy Test negative. Two strains of Klebsiella spp. were found Double Disc Synergy Test negative but Combined Disc Synergy Test positive.

Association between CDST and DDST (with χ2 test significance)

The difference in MBL detection between combined disc synergy test and the double disc synergy test was statistically highly significant (p<0.001).

DISCUSSION

In this study, we found 66 (33.5%) suspected Metallo-β-lactamases producers from 197 gram-negative isolates based on Imipenem resistance (screening for MBL). Among the 66-screening positive, MBL-producing organisms in the present study were found to be 50 (75.8%) by CDST and 48 (72.7%) by DDST. In India, out of 126 gram-negative bacilli, 80 (63.49%) showed resistance to Imipenem. Among the screening positive MBL-producing organisms, 80% were detected by CDST and 76.3% by DDST which correlates with this study.[16] In another study, Wankhede et al showed 1546 gram-negative bacilli 300 (19.1%) showed resistance to imipenem. Among the screening positive isolates 59 (19.67%) were MBL producers by both CDST and DDST.[17] Among the total 197 gram-negative bacilli, we found 50 (25.4%) MBL producers by CDST and 48 (24.4%) by DDST. In India, among 350 gram-negative bacilli, 23.7% were MBL producers by both CDST and DDST which correlates with this study.[18] Another study showed among the 126 gram-negative bacilli 50.79% were MBL producers by CDST and 48.4% by DDST.[19] Among the gram-negative isolates, we found *Acinetobacter spp.* 7 (100%), as the leading MBL producer followed by Pseudomonas spp. 16 (32.6%), *E. coli* 10 (20%) and Klebsiella spp. 15 (17.4%). *Proteus spp.* is not under consideration because of total 5 (2.5%) number is low. In a recent study, Saini et al found *Acinetobacter spp.* 100% MBL producer while *Klebsiella spp.* 76.47%, *Pseudomonas spp* 66.66% and *E. coli* spp. 64%. According to another study, 100% of Pseudomonas species, 84% of *Acinetobacter species*, and 62.5% of *Klebsiella* species were found to be MBL positive strains.[19] In Bangladesh out of 208 gram-negative bacilli (69.8%) of *Pseudomonas spp*. and (51.7%) of Acinetobacter spp. were MBL producers.[15] In India, the prevalence of MBL production was found to be highest in *Pseudomonas spp.* (37.3%) followed by *Klebsiella spp* (31.3%) *Acinetobacter spp.* (16.4%) and *E. coli* (15%).[20] The variation in MBL positivity might be due to the number of isolates studied, variation in institution to institution, geographic location, and also the country to country.[21] The prevalence of MBL production is high in the referral centers and the intensive care units where the patients are referred from peripheral centers and where the antibiotic use is profuse.

In this study, CDST showed better detection of MBL than DDST which is similar to the observation of other workers. Though the global increase in the types of MBL, the Clinical Laboratory Standard Institute (CLSI) does not have any performance standards documented so far, various screening methods have been employed for screening clinical isolates for MBL production. In this study, both CDST and DDST are reliable, easy to perform, and cheap. Interpretation of the CDST is more objective than that of DDST results because the DDST depends upon the expertise in discriminating true synergism from the intersection of the inhibition zone. So

one major disadvantage of DDST was the subjective interpretation of results. In India showed that CLSI has recommended a modified Hodge test for detection of carbapenemases activity in *Enterobacteriaceae*.[22] Other methods such as PCR and E test have been used to identify MBL producers. However, these tests may not be cost-effective for routine testing in clinical laboratories.[23] PCR has become more difficult with the increasing number of MBLs and our institute does not have any molecular setup, we were not able to confirm these findings by the genotypic method which was the limitation of this study.

Limitations of the study

Only Imipenem-resistant isolates were included in this study. Imipenem susceptible strains were not screened for MBL. This organism may also carry the MBL gene. Due to time constrain the genotype of these Carbapenem-resistant MBL producing gram-negative bacilli by multiplex PCR was not determined. The species of these MBL positive *Enterobacteriacae, Pseudomonas spp. Acinetobacter spp.* could not be identified.

CONCLUSION

The high rate of MBL-producing gram-negative bacteria in this study emphasizes the need for active surveillance in the microbiology laboratories for the detection of these resistant strains. There is a need for a simple and accurate test for MBL detection to prevent the spreading of infection with nosocomial strain in hospital settings. So, both tests can be used as alternative methods. Microbiology laboratories must be prepared for screening of MBL-producing isolates by a low-cost, convenient and sensitive procedure. In absence of molecular detection techniques, the Combined Disc Synergy Test provides a sensible choice for phenotypic detection of MBL production and can be implemented in the clinical laboratory on a daily basis.

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Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Walsh TR. Emerging Carbapenemases: a global perspective. International Journal of Antimicrobial Agents. 2011;508-14.
2. Peleg AY, Franklin C, Bell MJ, Spelman D. Dissemination of the Metallo-β-Lactamase Gene blaIMP-4 among Gram-Negative Pathogens in a Clinical Setting in Australia. Clinical Infectious Diseases. 2005;41:1549-56.
3. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy. 1991;35(1):147-51.
4. Cornaglia G, H Giamarellou, Rossolini GM. Metallo-β-lactamases last frontier for β-lactams? International Business Review. 2011;11(5):381-93.
5. Kumar SH, Baveja SM & Gore MA. Prevalance and risk factors of metallo beta lactamases producing Pseudomonas aeruginosa and Acinetobacter species in Burns and Surgical wards in a tertiary care hospital. Journal of Labratory Physicians. 2012;4(1):39-42.
6. Anwar S. Phenotypic detection of Metallo-β-Lactamases among the Clinical isolates of Pseudomonas spp. and Acinetobacter spp., M.Phil Thesis, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. 2010.
7. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, Goto M. Convenient Test for Screening Metallo-β-Lactamase Producing Gram-Negative Bacteria by Using Thiol Compound. Journal of Clinical Microbiology. 2000;38(1):40-3.
8. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA Disk Method for Differentiation of Metallo-β -lactamases- producing Clinical Isolates of Pseudomonas spp. and Acinetobacter spp. Journal of Clinical Microbiology. 2002;40(10):3798-801.
9. Franklin C, Liolios L & Peleg AY. Phenotypic Detection of Carbapenem-Suspectable Metallo-Beta-Lactamase Producing Gram-negative Bacilli in the Clinical Laboratory. Journal of Clinical Microbiology. 2006;44(9):3139-44.
10. Bashir D, Thoker MA, Fomda BA, Bashir G, Zahoor A, Toboli AS. Detection of Metallo-beta-lactamase (MBL) producing Pseudomonas aeruginosa at a Teritary Care Hospital in Kashmir. American Journal of Microbiology Research. 2011;5(2):164-72.
11. Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ et al. New Delhi Metallo-β-Lactamase Producing Enterobacteriaceae United States. Emerging Infectious Diseases. 2013;19(6):870-80.
12. Struelens MJ, Monnet DL, Magiorakos AP, O'Conner FS. New Delhi metallo-beta-lactamase 1 producing Enterobacteriaceae: emergence and response in Europe, European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden. 2010;1-10.
13. Shamuzzaman SM. NDM1 producing new superbug bacteria: a threat to control infection. Bangladesh J Med Microbiol. 2011;5(1):1-2.
14. Nasrin T, Jilani SA, Barai L, Haq JA. Metallo-β-lactamase Producing Pseudomonas species in a Teriary care Hospital of Dhaka City. Bangladesh J Med Microbial. 2011;4(1):43-5.
15. Anwar S, Miah MRA, Saleh AA, Sattar H, Ahmed S. Simple Screening Test for The Detection of Metallo-β-lactamase (MBL) Production In Clinical Isolates Pseudomonas and Acinetobacter. Ibrahim Med Coll J. 2010;4(1):26-30.

16. Agarwal R, Sumana MN, Kishore A, Kulkarni M. Simple Method for Detection of Metallo β Lactamase among Gram-negative Isolates. Online journal of Health and Allied Science. 2015;14(3):1-3.
17. Wankhede SV, Iyer VS, Bharadwaj RS. The Study of MBL Producers in Gram-negative Isolates from ICUs and Wards. Indian Journal of Basic and Applied Medical Research. 2011;1(1):38-46.
18. Saini M, Mishra A, Gupta S. Prevalence of Carbapenem Resistance in Gram-negative Bacilli Isolates and Their Antimicrobial Susceptibility Pattern. Int J Med Res Prof. 2016;2(3):28-32.
19. Agarwal R, Sumana MN, Kishore A, Kulkarni M. Simple Method for Detection of Metallo β Lactamase among Gram-negative Isolates. Online journal of Health and Allied Science. 2015;14(3):1-3.
20. Patel DD, Rathod SD, Panchal PD, Chauhan B, Pethani JD, Shah PD. Comparison of Two Phenotypic methods, Prevalence and Antimicrobial Suspectibility profile of Metallo-β-Lactamase(MBL) Producing Gram-negative bacilli. International Journal of Scientific Research. 2013;2(7):3124.
21. Bradford PA. Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology and Detection of This important Resistance Threat. Clinical Microbiology Reviews. 2001;14(4):933-51.
22. Noyal MJC, Menezes G, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases inclinical isolates of non-fermentative gram-negative bacteria. Indian j Med Res. 2009;707-12.
23. Tellis R, Muralidharan S, Peter AI. Evaluation of three phenotypic methods for the detection of metallo-beta-lactamase production in non-fermenting gram-negative bacilli. International Journal of Biomedical And Advance research. 2013;4(5):299-305.

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