Original Article

Comparison of Genotypic and Phenotypic Methods of Metallo-β- lactamase Detection in *Acinetobacter* spp.

Priyam Batra, Vijeta Bajpai, Aishwarya Govindaswamy, Surbhi Khurana, Muruganantham Ayyanar, Purva Mathur, Rajesh Malhotra[1]

Departments of Laboratory Medicine and [1]Orthopaedics, JPNA Trauma Centre, All India Institute of Medical Sciences, New Delhi, India

Abstract

Introduction: MBL containing genes have been reported in all GNBs including Acinetobacter spp since 1990s which are worrisome as they are transmitted by mobile genetic elements. Thus, early detection of MBL encoding organisms is necessary. The current study was designed to identify the most sensitive cost-effective test which could be used as a screening test for detection of cabapenamase producing Acinetobacter isolates. **Methodology:** All consecutive strains of Acinetobacter spp isolated from various clinical samples were included. All isolates found resistant to any of the carbapenems were tested for MBL production using MHT (on MacConkey Agar and Mueller Hinton Agar), Etest (using Imipenem/Meropenem-EDTA) and Combined Disc Test (using EDTA and 2 MPA as inhibitors and Ceftazidime/Imipenem/Meropenem as substrate discs). PCR was performed for representative strains for IMP, VIM, KPC, OXA and NDM-1 gene. **Results:** Total of 154 non-duplicate strains of Acinetobacter spp were isolated and identified, of which, 134 (88%) and 126 (82%) were resistant to meropenem and imipenem respectively. All 134 meropenem resistant strains were tested for MBL production and PCR was performed on 100 strains. 3(3%), 5(5%), 7(7%), 26(26%), and 51(51%) strains had IMP gene, VIM gene, KPC gene, OXA gene and NDM-1 gene. MHT on MAC had better performance than on MHA and dilution to 0.05 McFarland was not required. **Conclusion:** MHT on MAC had best sensitivity when compared with gold standard PCR and was also cost effective. With ROC curve, we found that 2MPA was not a good MBL inhibitor when compared with EDTA..

Keywords: Metallo-β-lactamase, modified Hodge test, receiver operating characteristic curve

Introduction

Multidrug-resistant *Acinetobacter spp.* are increasingly being reported to cause various outbreaks in the intensive care units.[1] Multidrug resistance has been found to be caused by various metallo-β-lactamase (MBL)-encoding genes which are detected by polymerase chain reaction (PCR). Various phenotypic methods are available such as combined disc test or E-test using various inhibitors or modified Hodge test (MHT) for the detection of MBL producers.[2] However, no single test has been found to be sensitive and cost-effective for MBL detection when compared to the gold standard genotypic tests. Thus, the current study was planned to determine the prevalence of carbapenem-resistant *Acinetobacter spp.* and to find the most sensitive and cost-effective methods of phenotypic detection of MBL.

Methodology

The study was conducted over a period of 3 years (2013–2015) at a 186-bedded Level 1 trauma center in India to include consecutive strains of *Acinetobacter spp.* isolated from various clinical samples. Identification and sensitivity (SN) testing were performed using Vitek 2 system (Biomerieux, France), and all carbapenem-resistant isolates were tested for MBL production. Phenotypic detection was done using the MHT[3] (on Mueller–Hinton agar [MHA] and MacConkey agar [MAC] using 0.5 and 0.05 McFarland density, respectively), double-disc synergy test[4,5] using ethylenediaminetetraacetic acid (EDTA) and 2 Mercaptopropionic Acid (2 MPA) as enzyme inhibitors, and MBL E-test.[6] All the methods were compared with the PCR as

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/jgid.jgid_38_19

Address for correspondence: Dr. Purva Mathur, Room No. 211, Second Floor, Department of Laboratory Medicine, JPNA Trauma Centre, All India Institute of Medical Sciences, New Delhi, India. E-mail: purvamathur@yahoo.co.in

How to cite this article: Batra P, Bajpai V, Govindaswamy A, Khurana S, Ayyanar M, Mathur P, *et al*. Comparison of genotypic and phenotypic methods of metallo-β- lactamase detection in *Acinetobacter spp*. J Global Infect Dis 2020;12:141-3.

Received: 01 April 2019 **Revised:** 09 January 2020
Accepted: 20 March 2020 **Published:** 29 August 2020

Table 1: Primers for the detection of carbapenemase genes

Primer name	Sequence	Amplicon size
IMP-F	GGCAGTCGCCCTAAAACAAA	737
IMP-R	TAGTTACTTGGCTGTGATGG	
VIM-F	AAAGTTATGCCGCACTCACC	865
VIM-R	TGCAACTTCATGTTATGCCG	
OXA-1-F	CGCAAATGGCACCAGCTTCAAC	464
OXA-1-R	TCCTGCACCAGTTTTCCCATACAG	
KPC-F	ATGTCACTGTATCGCCGTC	382
KPC-R	AATCCCTCCGAGCGCGAGT	
NDM-1-F	GGTGCATGCCCGGTGAAATC	660
NDM-1-R	ATGCTGGCCTTGGGGAACG	

Table 2: Performance of MacConkey agar and Mueller-Hinton agar

Inoculum (McFarland)	Mean distortion in millimeter	
	MacConkey Agar	MHA
0.5	5.2	4.3
0.05	3.82	3.12

MHA: Mueller-Hinton agar

the gold standard.[5,7] PCR was performed to detect the presence of bla_{IMP}, bla_{VIM}, bla_{OXA}, bla_{KPC}, and bla_{NDM}, the primer details of which are summarized in Table 1 and a picture of the same is depicted in Figure 1.

Result

Of the 154 nonduplicate strains of *Acinetobacter spp.* isolated, 134 (88%) and 126 (82%) strains were resistant to meropenem and imipenem, respectively, but PCR of only 100 strains could be performed. PCR analysis of the 100 strains showed that 3 (3%), 5 (5%), 7 (7%), 26 (26%), and 51 (51%) strains had IMP gene, VIM gene, KPC gene, OXA gene, and NDM-1 gene, respectively. The mean distortion in MHT on MHA and MAC is summarized in Table 2. A picture of the distortions in MAC and MHA is depicted in Figure 2. To evaluate the combined disc test with EDTA and 2 MPA as the inhibitors and imipenem/meropenem/ceftazidime as the substrates, receiver operating characteristic (ROC) curve was plotted between the difference in the zone sizes obtained and the PCR results (keeping PCR results as the gold standard). The ROC curve was made using the STATA software (STATA Version 12.1, Stata Corp, Texas-77845, USA). ROC is a graph which shows the relationship between SN and specificity (SP) of any test for all cutoff values. Thus, the curve plots the true positives against the false positives. As the ROC graph is between SN and 1 − SP, the most accurate test would pass through the upper left corner of the graph, and the closer the graph to the 45° diagonal line, the least accurate the test would be. In addition, area under the ROC curve (AUC) measures the test accuracy. With the help of ROC curve, we found that 2 MPA was not a good MBL inhibitor when compared with EDTA (as seen by the AUC). The results are depicted in Figure 3.

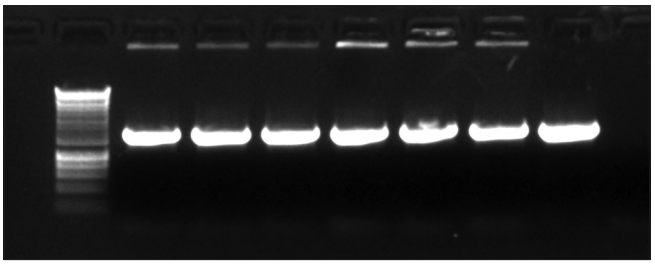

Figure 1: Gel doc picture showing samples 1–7 as positive and sample 8 as negative

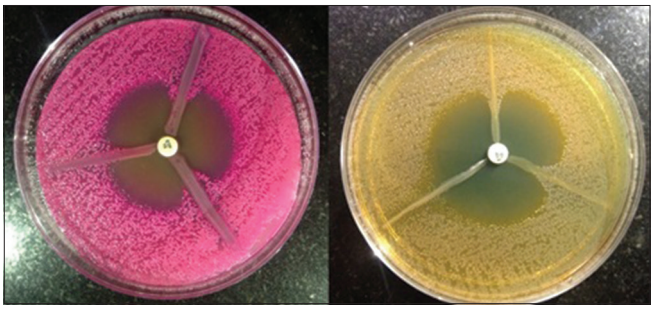

Figure 2: Modified Hodge test on MacConkey agar and Mueller–Hinton agar

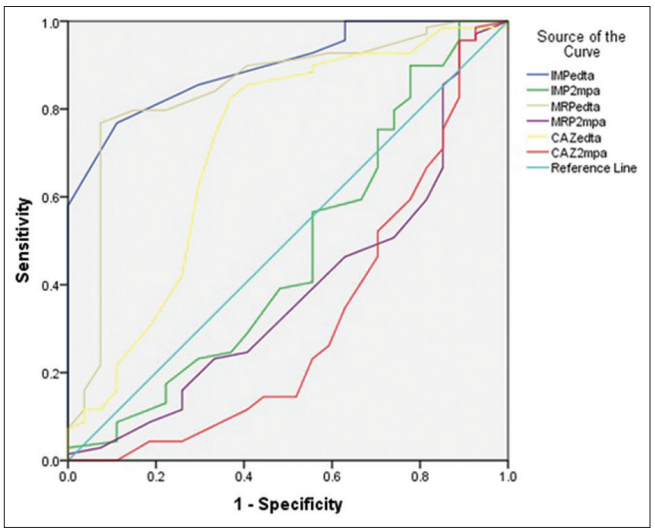

Figure 3: Receiver operating characteristic curve of the various methods of MBL detection includes IMP-EDTA, IMP-2 MPA, MRP-EDTA, MRP-2 MPA, CAZ-EDTA, and CAZ-2 MPA. IMP: Imipenem, EDTA: Ethylenediaminetetraacetic acid, MRP: Meropenem, CAZ: Ceftazidime, MPA: 2 Mercaptopropionic Acid, MBL: Metallo-β-lactamase

Conclusion

In our study, we found that MHT on MAC had better SN when compared with the gold standard PCR than MHT on MHA. In addition, there is no need of dilution to 0.05 McFarland to perform MHT on MAC plates. With the help of ROC curve, we found

that 2 MPA was not a good MBL inhibitor when compared with EDTA (as seen by the AUC). Upon comparison of the AUC of ceftazidime/imipenem/meropenem-EDTA AUC, it was seen that imipenem-EDTA performed the best with a cutoff of 4-mm increase in zone diameter. The SP (83.3%) and SN (90.3%) of imipenem EDTA E-test were better than those of the combined disc test.

Financial support and sponsorship

This study was funded by a grant from the Indian Council of Medical Research (ICMR) (project code: I-800, grant number: 5/3/3/26/2011-ECD-I). We acknowledge the financial support of ICMR for the performance of this study.

Conflicts of interest

There are no conflicts of interest.

References

1. Bergogne-Bérézin E, Towner KJ. Acinetobacter spp. As nosocomial pathogens: Microbiological, clinical, and epidemiological features. Clin Microbiol Rev 1996;9:148-65.
2. 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 compounds. J Clin Microbiol 2000;38:40-3.
3. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 26th ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2016. p. M100-S26.
4. Hemlata V. Detection of metallo β-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients Indian J Med Res 2005;122:148-52.
5. Queenan AM, Bush K. Carbapenemases: The versatile β-lactamases. Clin Microbiol Rev 2007;440-58.
6. Lee K, Yong D, Yum JH, Lim YS, Bolmström A, Qwärnström A, *et al*. Evaluation of Etest MBL for detection of blaIMP-1 and blaVIM-2 allele-positive clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2005;43:942-4.
7. Malvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA. New Delhi Metallo-β-Lactamase in *Klebsiella pneumoniae* and *Escherichia coli*. Emerg Infect Dis 2011;17:103-6.