International journal of basic and applied research
www.pragatipublication.com
ISSN 2249-3352 (P) 2278-0505 (E)
Cosmos Impact Factor-5.960

Prevalence of Metallobatalactamase (MBL) genes in *Acinetobacter baumannii* isolates in a tertiary care hospital of Punjab, India

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Received: 10 July Revised: 18 July Accepted: 26 July

Abstract

Background: *A. baumannii* is a non-fermenting Gram negative bacteria that plays important role in causing nosocomial infections. The world-wide emergence of multi-drug resistant *A.baumannii* strains is a growing concern. As carbapenems are the potent antimicrobial weapons against multidrug resistant *A. baumannii*, it has even developed resistance against this group of drugs by producing metallobetalactamases.VIM and IMP enzymes are most common MBL's found in carbapenem resistant bacteria.**Method:** A total of 116 clinical isolates of *A. baumannii* were recovered from various sites of infection . All carbapenem resistant strains were tested for MBL production by disc potentiation test. Polymerase chain reaction was performed for detection of blaVIM genes in phenotypically positive isolates.**Results**: A total of 52 isolates were found to be phenotypically positive for MBL production.Out of them, 32 isolates(62%) showed presence of blaVIM genes whereas in 20 isolates (38%) , no bla VIM gene was detected. **Conclusion**: The study demonstrates that MBL production detected by phenotypic tests must be confirmed by genotypic methods. Continuous surveillance and monitoring of *A baumannii* is important because of high prevalence of antibiotic resistance genes.

Keywords: *A. baumannii*, metallobetalactamases, blaVIM gene, Polymerase chain reaction.

Introduction

Acinetobacter baunmannii (*A. baumannii*) is a global pathogen and has been isolated from hospitals throughout the world.[1] It is the common cause of hospital acquired infections especially in intensive care units.[2] *A. baumannii* possesses mechanisms of resistance to all existing antibiotic classes as well as great propensity to for developing mechanisms of drug resistance rapidly. Over the past few years, metallobetalactamase producing isolates have emerged worldwide and are associated with outbreaks in healthcare settings.[3] Infections caused by MBL producing isolates are difficult to treat and are thus associated with high degree of morbidity, mortality and rising healthcare costs.Till date,

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six types of MBL's have been identified in *A. baumannii*. These are- IMP- like, VIM- like, Seoul imipenamase(SIM-1), New Delhi metallobetalactamases(NDM-1 and NDM-2).[4,5]. In particular, *bla* $_{VIM-2}$ has emerged as a dominant MBL variant worldwide.[6]

Though several methods are advocated in many studies, CLSI guidelines don't recommend a standardized method for detection of MBL producing isolates.Therfore, determination of MBL genes by molecular techniques in MBL producing isolates and their antibiogram can supply useful data about theie epidemiology and risk factors associate with these infections.It is important for surveillance and epidemiological purposes to be aware of antibiotic resistance genes in important healthcare associated pathogens such as *A. baumannii*.

The present study was conducted with an aim to determine the prevalence of *bla* $_{VIM}$ gene responsible for MBL production in *A. baumannii* iaolates in this hospital.

Material and Methods

The studuy was condcted in Department of Microbiology, Adesh Institute of Medical Sciences and Research and Centre for Interdisciplinary Biomedical Research, Adesh University for a period of two years from July 2014 to June 2016.

A total of 116 clinical isolates of *A. baumannii* were recovered from various clinical samples like- Endotracheal secretions, tracheal aspirates , blood, pus, urine and sputum samples of patients admitted in ICU and various wards of the hospital. *A. baumannii* isolates were identified on the basis colony characteristics, Gram staining morphology and conventional biochemical methods [7]. The isolates were further subjected to antimicrobial sensitivity testing by Kirby Bauer method [8] and results were interpreted according to CLSI guidelines.[9]

MBL production was determined phenotypically by Disc poterntiation test using Imipenem and Imipenem+ EDTA discs. An increase in zone size of at least 7mm aound Imipenem + EDTA disc as compared to Imipenem was recorded as a MBL positive isolate. [10] Molecular characterisation for phenotypically positive was done by PCR for the prescence of bla VIM genes. The genomic DNA was extracted from all isolates and was used as atemplate for amplification of specific gene. The amplification for the gene was carried out by PCR to check fior the presence or absence of MBL genes.

Extraction of genomic DNA:

The strains selected for gene detection were inoculated into Mueller Hinton Agar and incubated at 37° C for 18 to 24 hours. One colony from this fresh culture was resuspended in 200 micro litre sterile water and heated at 95 C for 10 minutes. This was followed by centrifugation at 13,000 xg for 10 minutes. The supernatant obtained served as a DNA template for PCR.[11]

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Amplification of bla VIM gene:

The primer sequence required for amplification of VIM genes was ordered from Chromous Biotech Pvt. Ltd. Bangalore, India. Following sequence of bla VIM forward primer and bla VIM reverse primer was used:

Forward primer (5' - 3'):

ATTGGTCTATTTGACCGCGTC

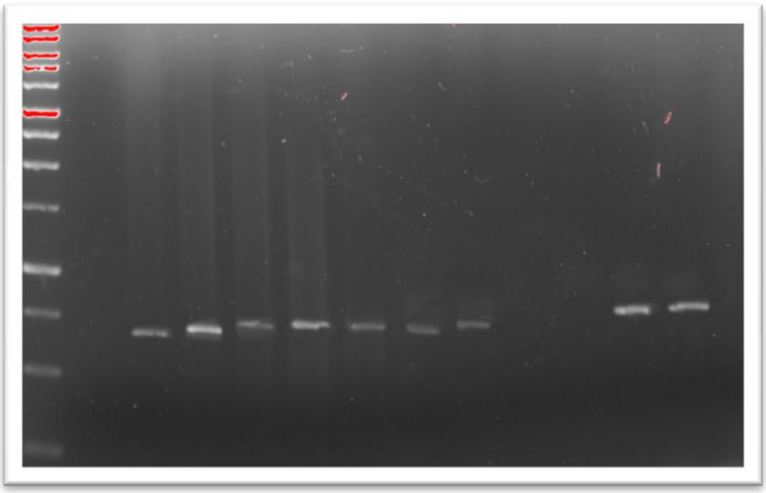
Reverese primer (5' - 3'):

TGCTACTCAACGACTGACCG

10µl PCR mixture was prepared by mixing DNA template (4 µl); Forward primer (0.5 µl) ; Reverse primer(0.5 µl) and master mix 5 µl. The PCR mixture was subjected to PCR thermocycler (BIO-RAD T100) for gene amplification under standardised conditions. Agarose gel electrophoresis was carried out in 1.2% agarose gel for one hour; at 100 V and 100 mA and then the bands (680 bp) were visualised under Gel Doc Imager (BIO-RAD)

Results

A total of fifty-two isolates of A. baumannii showing MBL screening test positive by disc- potentiation test were tested for presence of blaVIM gene. Thirty two isolates (62%) showed the presence of blaVIM genes and twenty isolates (38%) were found to be negative for bla VIM gene.



Agarose gel electrophoresis (1.2%) of PCR amplified product of blaVIM gene in *A. baumannii*; Lane M: 1 kbp ladder, Lane 2, 3, 4, 5, 6, 7, 8, 11, 12: The PCR amplicon sizes for VIM gene (680 bp)

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Discussion

In recent years, there has been an increase in carbapenem resistance genes among Gram negative bacteria in the Indian- Subcontinent.[13] In this study, although 52 *A. baumannii* strains were found to be MBL positive by phenotypic test ut MBL resistance genes were not detected in all of them. The results of our study are close to Amudhan et al in which 54 out of 92 i.e 58.6% *A. baumannii* showed presence of bla VIM gene.[14] In a study by Safari et al (2015), 30 % isolates of *A. baumannii* were positive for presence of this gene.[15] In a study by Atkas and Kayacan (2008)[16] and Marjani et al (2013)[17], bla VIM gene was not detected in any isolate though they were positive for MBL production by phenotypic detection methods.The discrepancy between phenotypic and genotypic results was due to false results reported by use of EDTA disc. The membrane permeabilising effect of EDTA can increase the susceptibility of Gram negative bacteria such as *A. baumannii* and *P. aeruginosa*.[18,19,20] Carbapenem hydrolyzing class D genes are widespread over multiple continents , as opposed to the class B MBL genes of *A. baumannii*.[3] In case of genotypically negative isolates some other mechanisms of resistance like- mutations leading to loss of porins, overexpression of efflux pump mechanisms or presence of other MBL genes (bla IMP, bla SPM, bla SIm and bla GIM) might be responsible for resistance to carbapenems.

Conclusion

It is evident from this study that despite high prevalence of phenotypic MBL production and higher resistance of *A. baumannii* isolates against carbapenems, lower rates of MBL coding genes have been detected. Continuous research and surveillance is necessary to monitor the prevalence and spread of antibiotic resistance genes that are associated with *A. baumannii* in clinical settings. MBL resistance detected by phenotypic methods must be confirmed by genotypic methods like multiplex PCR.

Acknowledgement
We express our sincere thanks to Dr R. G. Saini, Chairperson, Centre for Interdisciplinary and Biomedical Research (CIBR), Adesh University and Dr. Prabhjot Kaur Gill, Associate Professor, CIBR for providing infrastructure and guidance at each and every step.

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