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Detection of Metallo-Beta-Lactamase Encoding Genes by Multiplex RT-PCR among Clinical Isolates of Pseudomonas Aeruginosa

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ABSTRACT

Introduction: Metallo-beta-lactamase producing *Pseudomonas aeruginosa* is an important emerging nosocomial pathogen, due to its ability to acquired drug resistance.
Aim & objectives: The aim of this study was to detect MBL encoding genes by genotypic method among clinical isolates of *Pseudomonas aeruginosa*.
Material & Method: A total of 50 non-repetitive isolates of P. *aeruginosa* recovered from various clinical samples were included in this study. All these isolates were screened for MBL production by RT-PCR irrespective of imipenem sensitivity and the presence of bla_{VIM}, bla_{IMP} & bla_{NDM} was determined.
Results: Out of 50 isolates of *P. aeruginosa*, MBL were detected in 32 (64%) isolates by PCR. bla_{VIM} gene was detected in 21 isolates, followed by bla_{NDM} in 11 isolates and bla_{IMP} in 5 isolates.
Conclusion: The prevalence of MBLs among *Pseudomonas aeruginosa* has been increasing. This study confirms a high rate of MBL production & dissemination of bla_{VIM} gene among Pseudomonas aeruginosa.

Keywords: *Pseudomonas aeruginosa*, Metallo-beta-lactamase, bla_{VIM}, bla_{IMP}, bla_{NDM}, PCR.

INTRODUCTION

Pseudomonas aeruginosa is the most common and important pathogen causing nosocomial infections. The genome of *P. aeruginosa* is relatively large as compared to other sequenced bacteria, so it has great metabolic versatility and high adaptability to environmental changes [(1), (2)]. *Pseudomonas aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones, β-lactams & carbapenems. Emerging resistance towards expanded spectrum cephalosporins & carbapenems among *Pseudomonas aeruginosa* is a major concern. Carbapenems are the most potent agents for treatment of gram negative infections including *Pseudomonas aeruginosa*, because these agents are stable against the majority of beta-lactamases. Metallo-β-lactamases enzymes have the ability to hydrolyze carbapenems and are resistant to the commercially available β-lactamase inhibitors clavulanic acid, avibactam or tazobactam and developmental penicillanic acid sulfones and diazabicyclooctanes, belonging to Ambler's class B. bla_{VIM}, bla_{IMP} & bla_{NDM} genes

responsible for MBL production are plasmid associated. Enzymes related to bla_{VIM} & bla_{IMP} possess the broadest substrate of hydrolysis range [3]. Strains having bla_{NDM} exhibit multidrug resistant profile because these also harbour other genes resistant to aminoglycosides & fluoroquinolones [4].

MATERIAL AND METHODS

The study was conducted in Department of Microbiology at R.N.T. Medical College, Udaipur (Raj). Ethical approval was obtained from the Institutional Ethical Committee (IEC). A total of 50 non repetitive isolates of *Pseudomonas aeruginosa* recovered from various clinical samples like Pus, Urine, Blood, Sputum, Swabs (Ear, Nasal, Tracheal), Fluids (Bronchial, Pleural, Endotracheal) were included in this study. Clinical specimens submitted for routine culture & antibiotic susceptibility. Antibiotic susceptibility test for all these isolates were done by Kirby Bauer disc diffusion method. P. aeruginosa ATCC 27853 was used as control in the susceptibility testing.

Detection of MBL encoding genes by RT-PCR

P. aeruginosa strains were subjected to DNA extraction with HiPurA Bacterial Genomic DNA Purification kit, code MB505, Himedia (Himedia Laboratories Pvt. Ltd) [5, 6] and amplification by RT-PCR with Hi-PCR Carbapenemase Gene (Multiplex) Probe PCR kit, code MBPCR132 Himedia (Himedia Laboratories Pvt. Ltd). PCR assay was performed to detect the occurrence of bla_{VIM}, bla_{IMP} & bla_{NDM} genes. The test was performed as recommended by the manufacturer (Himedia).

The DNA purification procedure using the miniprep spin columns comprises of three steps- adsorption of DNA to the membrane, removal of residual contaminants & elution of pure genomic DNA. The columns have a high binding capacity & high quality DNA is obtained from various species.

RT-PCR is used to amplify a targeted DNA sequence by use of hydrolysis probes that are short oligonucleotides that have a fluorescent reporter dye attached to the 5' end & a quencher dye to the 3' end. During polymerization reaction, a fluorescent reporter (R) dye and a quencher (Q) dye are attached to the 5' & 3' end of the probe respectively. The probes are designed such that they anneal within a DNA region amplified by specific set of primers. During PCR amplification, these probes hydrolyze to the target sequences located in the amplicon, the DNA. As the Taq DNA polymerase replicates the template with the bound probe, the 5'-nuclease activity of the polymerase enzyme cleaves the fluorescent probe. The end result in cleavage of the probe is separation of the reporter dye from the quencher dye & increasing the reporter dye signal. As the probe is removed from the target strand, primer extension continues to the end of the template strand. The fluorescence detected in the quantitative PCR thermal cycle is directly proportional to the fluorophore released & the amount of DNA template present in the PCR. The PCR reaction consisted of a final reaction volume of 25µl, containing 5µl of templet DNA & 20µl of Master mix. Qualitative PCR amplification for the detection of bla_{VIM}, bla_{IMP} & bla_{NDM} genes were carried out on CFX BioRAD instrument.

PCR Program

Initial denaturation : 95°C for 10 minutes
Denaturation : 95°C for 05 seconds
Annealing & Extension: 60°C for 1 minute (plate read)
Hold : 4°C for ∞
No. of cycles for denaturation, annealing & extension – 45

Dyes / Photophores for target genes

Fam : NDM gene

Texas Red : IMP gene
Cy 5 : VIM gene
Cy5.5 : IC

Data interpretation

Ct value	Result
≤ 40	Detected (+)
> 41 or N/A	Not Detected (-)

Data Analysis: - Conditions for a valid diagnostic test

	VIM (FAM)	IMP (Texas Red)	NDM (Cy5)	IC (Cy5.5)
Positive Control	+	+	+	+
Negative Control	_	_	_	_

The presence or absence of a signal in the Cy5.5 channel is not relevant for the validity of the test run due to competition between the test template and Internal Control template.

RESULTS

A total of 50 non repetitive isolates of *Pseudomonas aeruginosa* were subjected to RT-PCR for detection of MBL encoding bla_{VIM}, bla_{IMP} & bla_{NDM} genes irrespective of imipenem sensitivity. Out of 50 isolates, 32 (64%) isolates were positive of MBL genes. The most prevalent MBL gene was bla_{VIM}, which was detected in 21(42%) isolates, followed by bla_{NDM} in 11 (22%) isolates and bla_{IMP} in 5 (10%) isolates. 03 (1.5%) isolates harboured both bla_{VIM} & bla_{NDM}. bla_{VIM} & bla_{IMP} were found co-exist in one (0.5%) isolate and bla_{NDM} & bla_{IMP} genes were found to co-exist in two (01%) isolate.

DISCUSSION

P. aeruginosa represent a serious therapeutic challenge for dissemination of hospital-acquired infections. The production of MBL enzymes, particularly related to bla_{VIM}, bla_{NDM} & bla_{IMP} have been identified as the most predominant determinants of carbapenem resistance and becoming highly distributed in various countries [(7)].

In our study bla_{VIM} was the most prevalent gene (21/50, 42%) in *P. aeruginosa*, followed by bla_{NDM} gene (11/50, 22%). In majority of Indian studies bla_{VIM} & bla_{NDM} are the most prevalent carbepenemase gene [(8), (9), (10)]. The results are nearly similar to M. K. Alkhudhairy et al, 33.3% bla_{VIM}[(11)] , Nishu Verma et al, 29.1% bla_{VIM} & 28.4 % bla_{NDM} [(12)], Johann D. D. Pitout et al, 43% bla_{VIM} & 2% bla_{IMP} [(13)], they had reported MBL production by PCR in *P. aeruginosa*. In the study carried out by Vijeta Bajpai et al, the most prevalent gene was bla_{NDM} (46.06%), followed by bla_{VIM} (33.95), which were different from current study [(14)]. In current study co-existence of bla_{VIM} & bla_{NDM}, bla_{VIM} & bla_{IMP} and bla_{NDM} & bla_{IMP} was 1.5%, 0.5% and 01% respectively. Co-existence of MBL genes were also observed in different studies in India [(15)].

CONCLUSION

The present study indicated an increase in MBL production & dissemination of bla_{VIM} gene among *Pseudomonas aeruginosa*. Such strains pose therapeutic dilemmas for clinicians. This calls for strict infection control practices and judicious use of antibiotics with implementation of antibiotic polices in all hospitals and early detection of MBL by phenotypic and genotypic methods in all laboratories to prevent the emergence & spread of the resistance mechanism.

Declaration by Authors
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