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RESEARCH ARTICLE

Detection of bla_{IMP} gene encoding metallo-beta-lactamase resistance among clinical isolates of *Pseudomonas aeruginosa*

Varshan Raj[1], Gopinath Pa[1], Ashish R Jain[2]

[1]Department of Microbiology, Saveetha Dental College and Hospital, Saveetha University, Chennai, Tamil Nadu, India, [2]Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha University, Chennai, Tamil Nadu, India

Correspondence to: Ashish R Jain, E-mail: dr.ashishjain_r@yahoo.com

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ABSTRACT

Background: In recent decades, *Pseudomonas aeruginosa* has emerged as a multidrug-resistant bacteria by acquiring intrinsic resistance to a number of antimicrobial agents by uses distinctive resistant mechanisms to all the available antibiotics, which include metallo-β-lactamases (MBL) production, extended spectrum β-lactamase production, Amp C production, decreased permeability, altered penicillin binding proteins and rarely, and over expression of efflux pumps. **Aims and Objectives:** The aim of the study was to know the presence of IMP gene among *P. aeruginosa* isolates and to determine the carbapenem resistance mechanisms in carbapenem-resistant isolates of *P. aeruginosa* collected from sputum, blood, urine, pus, in Chennai, India. **Materials and Methods:** A total of 20 of non-repetitive clinical isolates of *P. aeruginosa* were collected and processed for biochemical tests and confirmed. Antibiotic susceptibility testing was determined by Kirby Bauer disc diffusion method. *P. aeruginosa* isolates were detected for the presence of bla_{IMP} gene by polymerase chain reaction analysis. **Results:** Of the 20 clinical isolates of *P. aeruginosa*, 9/20 (45%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, and 3/20 (15%) from pus. Only 2/20 (10%) isolates showed sensitivity to imipenem. Other than that, for all other antibiotics isolates showed complete resistance 20/20 (100%). 17/20 (85%) clinical isolate of *P. aeruginosa* was found to possess bla_{IMP} gene. **Conclusion:** The early detection of MBL-producing *P. aeruginosa* may help in appropriate antimicrobial therapy and avoid the development and dissemination of these multidrug-resistant strains. However, to derive a conclusion, a more number of isolates are recommended and even other types of genes are also screened.

KEY WORDS: Bla_{IMP} Gene; *Pseudomonas aeruginosa*; Polymerase Chain Reaction; Metallo-Beta-Lactamase

INTRODUCTION

In the past few decades, antibiotic resistance ratio has been increased drastically, particularly among non-fermenting bacteria, which became a substantial challenge to treat.[1]

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Among the various nosocomial pathogens, *Pseudomonas aeruginosa* a soil inhabitant, and human saprophyte is well-known opportunistic human pathogen. It causes various life-threatening infections such as ventilator-associated pneumonia, surgical site, and urinary tract infections in patients from intensive care units.[2] Infections become severe when associated with immunosuppressive states mainly diabetes, carcinomas and also in burns injury and cystic fibrosis. Major risk factors are prolonged hospitalization, ventilation, exposure to inadequate antimicrobial therapy and immunocompromised state to get this infection.[3] In recent decades, *P. aeruginosa* has emerged as a multidrug-resistant bacteria by acquiring intrinsic resistance to a

number of antimicrobial agents. Multidrug-resistant status in-turn leads to increased hospital expenditure, prolonged hospitalization, narrowing of therapeutic options, cross infection thus, and landing to increased mortality and morbidity finally. This bacteria uses distinctive resistant mechanisms to all the available antibiotics, which include metallo-β-lactamases (MBL) production, extended spectrum β-lactamase production, AmpC production, decreased permeability, altered penicillin binding proteins and rarely, and overexpression of efflux pumps.[4] As carbapenems are the considered to be the potent antimicrobial agent against multidrug-resistant *P. aeruginosa* (MDRPA), this bacterium has developed resistance even against this group of drugs by producing MBLs (carbapenemase).[5] Imipenem and meropenem in carbapenems have gained increased therapeutic access in many hospital settings against MDRPA. However, as this pathogen has gained already resistance even to these available drugs, identification of nosocomial strains capable of producing MBL has aroused more interest and importance in current status.[6] Acquired MBLs includes the VIM and IMP enzymes, of which there are several variants of the original VIM-1 and IMP-1 MBLs as well as the SPM-1, GIM-1, NDM-1, AIM-1, and SIM-1 enzymes.[7] The VIM and IMP enzymes are by far the most common MBLs found in carbapenem-resistant bacteria, including carbapenem-resistant *P. aeruginosa*.[8] Thus, this study was conducted to know the presence of IMP gene among our *P. aeruginosa* isolates.

MATERIALS AND METHODS

Bacterial Isolates

A total of 20 of nonrepetitive clinical isolates of *P. aeruginosa* were collected from Saveetha Medical College, Thandalam. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semi-solid trypticase soy broth stock and stored at 4°C until further use.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was determined for this isolates to routinely used antibiotics such as to piperacillin-tazobactam, cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztreonam, gentamicin, and imipenem by Kirby Bauer disc diffusion method as per CLSI guideline.[9]

Detection of bla_{IMP} Gene In *Pseudomonas Aeruginosa*

P. aeruginosa isolates were detected for the presence of bla_{IMP} gene by polymerase chain reaction (PCR) analysis. Detection of the gene was carried out using primer [Table 1].

Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. The reaction mixture contained 2 mM of $Mgcl_2$ 0.2 mM dNTP mix and 1µM of bla_{IMP} gene with 0.5U of Taq polymerase (New England Biolabs) in a 1× PCR buffered reaction. A positive control of *P. aeruginosa* with bla_{VIM} gene was also included in this study. PCR amplification was carried out using thermal cycler (Eppendorf) with the following cycling condition. Initial denaturation at 98°C for 6 min and 30 cycles for 40 s, 57°C for 30 s, and 76°C for 30 s followed by a final extension of 5 min at 74°C. PCR products were resolved in 2% agarose gel. A 100 bp ladder was including in all the gel analysis.[10]

Table 1: Primer detail of bla^{IMP} gene

Primer	Primer sequence	Product size
bla^{IMP}	5'-GTT TGG CCG CAT TTC CA AC-3' 5'-AAT GCG GAG CAC AAG GAT AG-3'	393 bp

RESULTS

Of the 20 clinical isolates of *P. aeruginosa*, 9/20 (45%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, and 3/20 (15%) from pus [Figure 1].

In our isolates, we have observed that an increased percentage of isolates have shown to be resistant to most of the routinely used antibiotics. Only 2/20 (10%) isolates showed sensitivity to imipenem. Other than that, for all other antibiotics such as piperacillin-tazobactam, cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztreonam, and gentamicin isolates showed complete resistance 20/20 (100%) [Table 2].

17/20 (85%) clinical isolate of *P. aeruginosa* was found to possess bla_{IMP} gene. L3-100bp ladder, L4- bla_{IMP} gene positive [Figure 2].

DISCUSSION

Multidrug-resistant *P. aeruginosa* is a major cause of hospital acquired infections and known to cause a wide spectrum of life-threatening diseases. These organisms are resistant to almost all commonly available antibiotics with limited treatment options. Several mechanisms such as carbapenemase production, oprD mutation, AmpC, and efflux pumps overexpression are involved in carbapenems resistance among *P. aeruginosa* strains. However, the emergence of carbapenem resistant *P. aeruginosa* isolates has become a serious clinical concern because of its intrinsic and acquired resistance mechanisms, limiting the treatment option.

Study conducted by Ramakrishnan *et al*. from Puducherry reported that MDRPA isolates showed the highest resistance to carbapenems such as meropenem (84%) and imipenem (40%), which were found to be the precious weapon against MDRPA infections and this is an alarming sign. All the isolates

Table 2: Results of antibiotic susceptibility pattern of ***P. aeruginosa***

Antibiotics	Sensitivity (20) (%)	Intermediate (20) (%)	Resistant (20) (%)
Piperacillin-Tazobactam	0 (0)	0 (0)	20 (100)
Cefotaxime	0 (0)	0 (0)	20 (100)
Ceftazidime	0 (0)	0 (0)	20 (100)
Tetracycline	0 (0)	0 (0)	20 (100)
Cotrimoxazole	0 (0)	0 (0)	20 (100)
Aztreonam	0 (0)	0 (0)	20 (100)
Gentamicin	0 (0)	0 (0)	20 (100)
Imipenem	2 (10)	1 (5)	17 (85)

P. aeruginosa: Pseudomonas aeruginosa

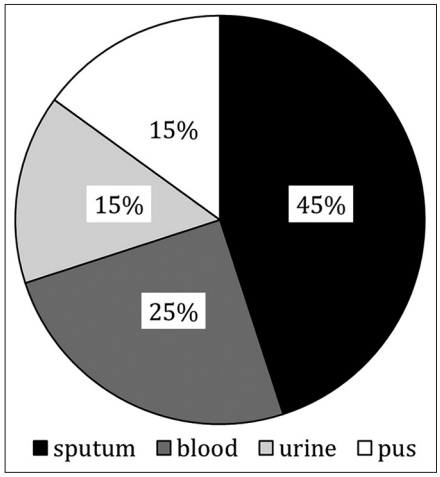

Figure 1: Sample wise distribution of clinical isolates of *Pseudomonas aeruginosa*

showed 100% sensitive to polymyxin B and colistin.[11] In this study, we have observed complete resistance to all the antibiotics tested except imipenem which showed 85% isolates were resistance, which is in concordance with other earlier studies, however, we did not include colistin in our study. A report by Aliskan *et al*., with 1071 MDRPA, reported resistance to imipenem (22.5%) and meropenem (31%).[12] Deepak *et al*. during 2009–2010 with 193 *P. aeruginosa* reported resistance to imipenem (3.7%), which is less compared with the present study.[13] A study conducted by Manoharan and his colleagues in 2010, found 30% of their isolates were found to harbor IMP gene by PCR,[10] we also seen similar kind of result as we observed 85% of positivity of having this gene. They have already reported novel IMP type of MBL producing Pseudomonas spp. detected in India during 2006 in which strains were clustered in 33 ribotypes with clones found in multiple hospitals.[14]

It seems that chronic underlying conditions, prolonged period of intensive care unit stay and the use of invasive techniques and devices predispose patients to infection with these resistant isolates. However, to derive a conclusion, a more number of isolates are recommended and even other types of genes are also screened.[15,16] This indicates the important role of clinical microbiology laboratories to distinguish

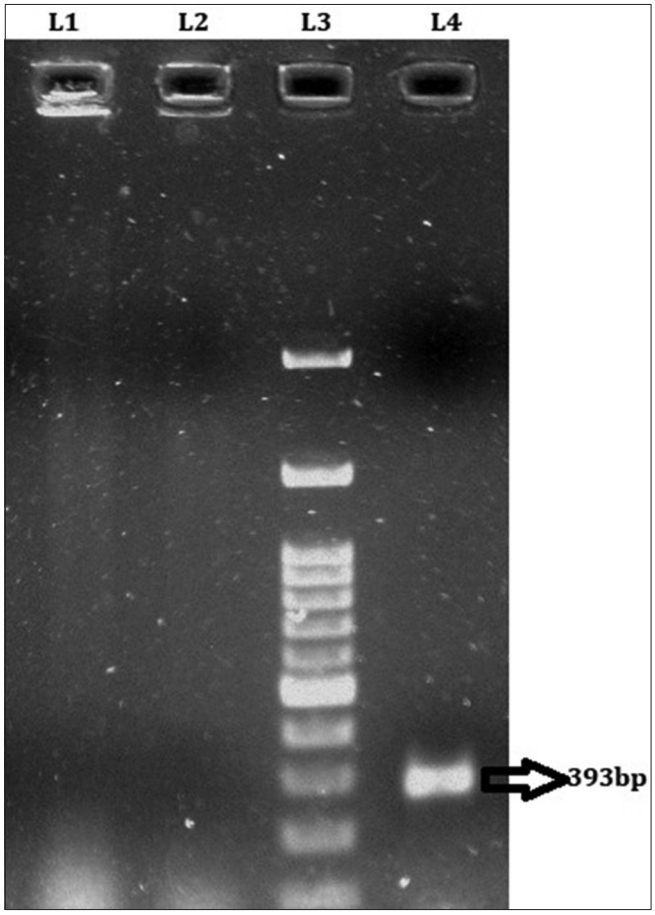

Figure 2: Representative gel picture showing bla_{IMP} gene

MBL-producing *P. aeruginosa* from strains with other mechanisms responsible for carbapenem resistance.

CONCLUSION

Therefore, in our study, we observed increased percentage of IMP mediated carbapenem resistance in our isolates, which indicates that this gene may be found in most of the *P. aeruginosa* carbapenem isolates. The early detection of MBL-producing *P. aeruginosa* may help in appropriate antimicrobial therapy and avoid the development and dissemination of these multidrug-resistant strains.

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