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Detection of *bla*$_{\text{NDM-1}}$ gene encoding for MBL resistance among clinical isolates of Pseudomonas aeruginosa

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Abstract

New Delhi metallo beta lactamase-1 (NDM-1), is a newly identified class B β-lactamase gene and found worldwide. *Bla*$_{\text{NDM-1}}$ producing isolates always shows multidrug resistant patterns, as they possess various genes that encode for resistance to various antibiotics. In this study we aim to elucidate the antibiotic susceptibility pattern of 20 clinical isolates of P. aeruginosa and followed by the detection of *bla*$_{\text{NDM-1}}$ gene by PCR. We found multiple antibiotic resistance even to carbapenem antibiotics. We found only one isolate was confirmed to possess this gene by PCR, this warrants the necessary to screen for this gene and to follow the antibiotic policies. There are only 2 reports of the presence of this gene in patients from Serbia. Though the reports are very limited, knowledge of its prevalence is very important as pseudomonas aeruginosa is purely a nosocomial pathogen with stubborn colonization capability and ability to persist in different environments of clinical setup. Pseudomonas aeruginosa isolates were detected for the presence of *bla*$_{\text{NDM-1}}$ gene by PCR analysis. Detection of the gene was carried out using primer.

Keywords: Pseudomonas aeruginosa, *Bla*$_{\text{NDM-1,}}$ PCR

1. Introduction

New Delhi metallo beta lactamase-1 (NDM-1), is a newly identified class B β-lactamase gene and found worldwide. The first report of this gene has been reported in 2008 in Sweden from a patient who was already hospitalized in India. *Bla*$_{\text{NDM-1}}$producing Enterobacteriaceae are in focus of attention across the world.(Yong et al., 2009) *Bla*$_{\text{NDM-1}}$ producing isolates always shows multidrug

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resistant pattern, as they possess various genes that encode for resistance to aminoglycosides and fluoroquinolones.(Nordmann et al., 2011) The report of Pseudomonas aeruginosa having *bla*$_{NDM-1}$gene is very limited in previous literature.

There are only 2 reports of the presence of this gene in patients from Serbia.(Jovcic et al., 2011) Though the reports are very limited, knowledge of its prevalence is very important as pseudomonas aeruginosa is purely a nosocomial pathogen with stubborn colonization capability and ability to persist in different environments of clinical setup. (Johnson & Woodford, 2013)

Pseudomonas aeruginosa is a Gram-negative, non-fermentative organism found in diverse environmental settings.(Lister et al., 2009) It's an opportunistic pathogen, causing serious infection in patients with weakened immune systems(Coggan & Wolfgang, 2012). This organism was generally intrinsically resistant to a wide variety of antimicrobial agents as well as it having the capacity to develop resistance by mutation or acquisition of foreign resistance genes against different antibiotic classes.(Strateva & Yordanov, 2009) Carbapenem, including imipenem, meropenem and doripenem are often used as a last resort for treatment of infections caused by *P. aeruginosa* and other Gram-negative bacteria. (Giamarellou & Poulakou, 2009)However, carbapenem-resistant *P. aeruginosa* has become prevalent globally.(Nagao et al., 2011) Carbapenem resistance may arise in *P. aeruginosa* via OprD channel deficiencies, up-regulation of efflux pumps and production of various kinds of carbapenemases, including serine β-lactamases of Ambler classes A and D and metallo-β-lactamases (MBLs) of Ambler class B.(Quale et al., 2006)

Among various mechanism of resistance for carbapenem in *P. aeruginosa*, production of MBLs is of particular concern because of their rapid spread, potent carbapenemase activity, resistance to β-lactamase inhibitors and ability to hydrolyze all β-lactam antibiotics with the exception of aztreonam.(Cornaglia et al., 2011) Furthermore, MBLs encoding genes are usually located on integrons, the mobile genetic elements that also carry genes encoding for resistance to aminoglycoside and other antibiotics resulting in multidrug resistance (MDR).(Walsh, 2008)

Several types of MBLs, such as IMP, VIM, SPM, GIM, AIM, FIM and NDM and their variants have been identified in *P. aeruginosa*.(Gupta, 2008) Among them, variants of VIM and IMP types such as *bla* $_{VIM-1}$, *bla* $_{VIM-2}$, *bla* $_{IMP-1}$ and *bla* $_{IMP-2}$ are the most commonly found MBL genes in *P. aeruginosa* and responsible for many nosocomial outbreaks.(Pollini et al., 2013)

Although, production of MBLs in clinical isolates is a serious therapeutic problem, so far limited information is available on MBL production in *P. aeruginosa* clinical isolates from Nepal. The objectives of this study were to determine the prevalence of MBL producing *P. aeruginosa* and to detect MBL-encoding genes (*bla* $_{VIM-1}$, *bla* $_{VIM-2}$, *bla* $_{IMP-1}$ and *bla* $_{IMP-2}$).

2. MATERIALS AND METHODS

2.1 Bacterial isolates:

A total of 20 of non repetitive clinical isolates of Pseudomonas aeruginosa were collected from Saveetha Medical College, Thandalam.

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They were processed for a battery of standard bio chemical tests and confirmed. Isolates were preserved in semi solid trypticase soy broth stock and stored at 4° C until further use.

2.2 Antibiotic susceptibility testing:

Antibiotic susceptibility testing was determined for this isolates to routinely used antibiotics such as to piperacillin-tazobactam, cefotaxime, ceftazidime, tetracyclin, cotrimoxazole, aztreonam, gentamicin and imipenem by Kirby Bauer disc diffusion method as per CLSI guideline.(Wayne, 2011)

2.3 Detection of *bla*$_{NDM-1}$ gene in pseudomas aeruginosa:

Pseudomonas aeruginosa isolates were detected for the presence of *bla*$_{NDM-1}$ gene by PCR analysis. Detection of the gene was carried out using primer as depicted in table 2. Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. The reaction mixture contained 2mM of $Mgcl_2$0.2mM dNTP mix and 0.8µM of *bla*$_{NDM-1}$ gene with IU of Taq polymerase (New England Biolabs) in a 1x PCR buffered reaction. A positive control of E.coli with *bla*$_{NDM-1}$ gene was also included in this study. PCR amplification was carried out using thermal cycler (Eppendorf) with the following cycling condition. Initial denaturation at 95°C for 5 min and 30 cycles for 30s, 55°C for 30s and 72° C for 40s, followed by a final extension of 5 min at 72°C. PCR products were resolved in 1.5% agarose gel. A 100bp ladder was including in all the gel analysis.(Saikia et al., 2016)

Table 1: Primer detail of *bla*$_{NDM-1}$ gene

Primer	Primer sequence	Product size
*bla*NDM-1	CACTTCCTATCTCGACATGC GGGCCGTATGAGTGATTG	621 bp

3. Results And Discussion

3.1 Sample wise distribution of clinical isolates of *P. aeruginosa.*

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

Figure 1 : Sample wise distribution of clinical isolates of P.aeruginosa

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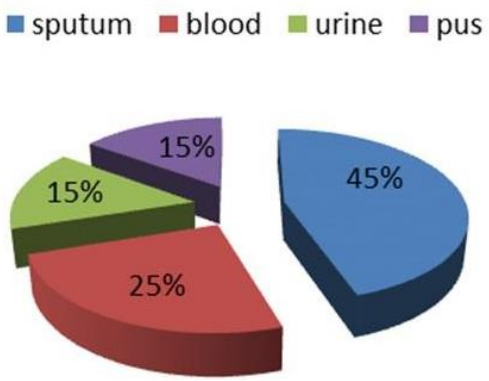

3.2 Results of antibiotic susceptibility testing

In our isolates, we have observed an increased percentage of isolates were 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, aztrionum, gentamicin isolates showed complete resistance 20/20 (100%). The detailed resistant pattern of *P. aeruginosa* isolates were showed in table1.

Table2: 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)

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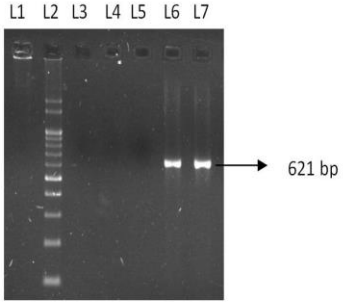
Aztreonam	0(0)	0(0)	20(100)
Gentamicin	0(0)	0(0)	20(100)
Imipenem	2 (10)	1 (5)	17 (85)

3.3 Result of *bla*$_{NDM-1}$ gene in Pseudomonas aeruginosa:

1/20 (5%) clinical isolate of P. aeruginosa was found to possess *bla*$_{NDM-1}$ gene.

Figure 2: Representative gel picture showing *bla*$_{NDM-1}$ gene



L2-100bp ladder; L6 and L7-positive *bla*$_{NDM-1}$.

The prevalence of NDM carrying bacterial isolates is indicative of "superbugs" in the community is common thereby an individual may constrict an infection without being exposed to a hospital environment. The NDM-producing isolates have a comparable antibiotic resistance profile showing resistance against third and fourth generation cephalosporins (cefepime, ceftazidime, cefotaxime, cefoperazone and cefixime), β-lactam/β-lactamase inhibitor combinations, aminoglycosides and carbapenems.

Study conducted by Borah and his colleagues from Assam in 2016 have reported 29% of E. coli and Klebsiella pneumoniae isolates were having *bla*$_{NDM-1}$ gene.(Jovcic et al., 2011) But in our study we found only one isolate out of 20 in P. aeruginosa. Report of Shanthi from Chennai in 2014 stated 61 carbapenem resistant P. aeruginosa isolates tested, only four were found to harbour NDM-1. (Sekar et al., 2013)

Many studies have shown that intensive care unit is the epicenter and the main source of amplification and dissemination of antimicrobial resistance,(Brusselaers et al., 2011) a finding that was also observed in this study among the 24 MBL-positive isolates; 14 (58.4%) were isolated from ICU while 10 (41.6%) were from general ward patients. Since more than 50% of MBL-producing *P. aeruginosa* isolates have been recovered from ICU, it is highly probable that it might be a clonal spread. Unfortunately, because of some limitations, such as lack of more equipped laboratory, we were not able to do genotypic comparisons of the isolates.

4. Conclusion

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NDM-1-producers are now alarmingly increasing worldwide and pose a potential risk for therapeutic failure with the empirical treatments. This finding of NDM-1-producing *P. aeruginosa* highlights the emerging therapeutic challenge. The implementation of strict antimicrobial policies and infection control programs may help to prevent the rapid dissemination of these organisms.

5. Acknowledgment

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6. **Conflict Of Interest**

The authors declare that there were no conflict of interest

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