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Detection of *bla*NDM-1 and *bla*VIM Mediated Carbapenem Resistance in *Pseudomonas aeruginosa* in a Tertiary Care Hospital in South India.

Chitralekha Saikumar*, and K Mahesh kumar.

Sree Balaji Medical College & Hospital, Works Road, Chromepet, Chennai, Tamil Nadu, India.

ABSTRACT

100 clinical isolates of pseudomonas aeruginosa obtained from various clinical samples from tertiary care hospital, is screened for carabapenem resistance by disk diffusion method using Meropenem disk (10 µg).All the meropenem resistance strains were further subjected to phenotypic methods like Modified Hodge test (MHT),Combined disk diffusion test and E-test.All the carbapenem resistant strains were further subjected to polymerase chain reaction(PCR) to identify the resistant genes. In our study majority of the genes detected were blaVIM rather than blaNDM-1.
Keywords: Pseudomonas aeruginosa, Carbapenems, Meropenem, Modified Hodge test, Combined disk diffusion test,E-test

**Corresponding author*

Introduction

Pseudomona aeruginosa is primarily an opportunistic pathogen that causes infection in hospitalized patient and it is often difficult to treat due to its resistant drug profile.Carbapenems are often used as antibiotic of last resort for treating infections due to multidrug-resistant gram negative bacilli, because they are stable even in response to extended spectrum and AmpC- β-lactamases[1].There is a recent evidence of, increasing case of carbapenem resistance in Pseudomonas aeruginosa.Carbapenem resistance in pseudomonas is attributed mainly to Metallo-Beta-Lactamase(MBL) production[2] .The MBLs efficiently hydrolyze all beta lactams, except for aztreonam, in vitro [3] therefore detection of MBL producing Pseudomonas aeruginosa is crucial for optimal treatment of patients and to control the spread of resistance [4].

MATERIALS AND METHODS

The study was conducted in a tertiary care hospital from June 2015 to December 2015. It included 100 Pseudomonas aeruginosa from various clinical specimens. The isolates were obtained from sputum,urine,pus,wound swab, blood and endotracheal aspirate. The organism is identified up to species level by the conventional biochemical tests and confirmed the atypical organisms by vitek-2(bio Merieux) automated identification system.

Antimicrobial Susceptibility testing

The antibiotic disc used are as follows Amikacin (30 µg), Ciprofloxacin (5 µg), ceftazidime (30 µg),Aztreonam (30 µg),Piperacillin-tazobactam (100/10 µg),imipenem (10 µg), meropenem (10 µg),colistin (10 µg) and polymyxin B (300 units).The antimicrobial susceptibility test is performed in accordance with CLSI guidelines by using disc diffusion method [5]

Phenotypic Methods

The carbapenemase production was screened by the Modified Hodge test (MHT) [6] and MBL production by combined disk diffusion test using Imipenem and ethylene diamine tetra acetic acid (EDTA) [7]. Further, the phenotypic presence of MBL enzymes was detected by imipenem/imipenem + EDTA strips (Ezy MICTM strips, Himedia, India). The MIC ratio of MBL- test of ≥8 mg/L was interpreted as indicative of MBL activity. The test was performed according to the manufacturer's instructions.

Polymerase Chain Reaction

DNA was extracted from all the MBL positive strains by heat boil method and this DNA strains were subjected to PCR using primers targeting blaNDM-1 followed by blaVIM. Primers used are given in Table 1.The amplication was done under the following conditions for 35 cycles :denaturation at 94 °C for 1 min, annealing for 1 minute at 66 °C for bla VIM and at 56 °C for blaNDM-1 and extension at 72 °C for 1 minute. Cycling was followed by a final extension at 72°C for 10 minutes. For optimization of PCR, strains previously confirmed by PCR and gene sequencing were used as positive control and P. aeruginosa ATCC 27853 was used as negative control. The PCR product of 442bp(blaVIM) was visualized by agarose gel electrophoresis (Fig1)

Table 1

Primer	Primer sequence(5'-3')	Product size (bp)
*bla*VIM^{-F} *bla*VIM^{-R}	5'GTGCTTTGACAACGTTCGCT-3' 5'TCCACGCACTTTCATGACGA-3'	442
*bla*NDM-1^{-F}	5'GGGCAGTCGCTTCCAACGGT-3'	475
*bla*NDM-1^{-F}	5'GTAGTGCTCAGTGTCGGCAT-3'	

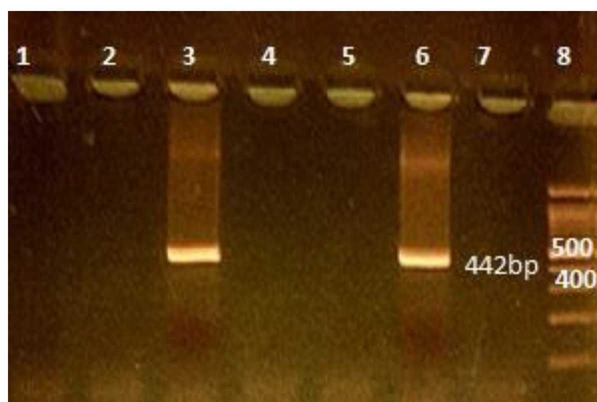

Fig 1:PCR, electrophoresis gel image demonstrating blaVIM-1 gene L- 8: molecular mass marker (100bp DNA ladder), L- 7: Negative control,L- 6: Positive Control,L- 3: Test sample- Positive ,L- 1,2, 4 & 5: Test sample-Negative

RESULTS

Out of 100 clinical isolates of Pseudomonas aeruginosa, carabapenem resistance was detected in 26 isolates by disk diffusion method using meropenem (10 µg). All the 26 meropenem resistant strains were further subjected to Phenotypic methods.Out of 26 meropenem resistance strains,24 strains were positive for Modified hodge test, 22 for combined disk diffusion test and 20 E-test.

All the 24 strains positive positive for phenotypic methods were further subjected to molecular analysis (PCR) for detection of resistant genes blaVIM and blaNDM-1.Among the 24 isolates blaNDM-1 was detected in none of the isolates. Whereas blaVIM was detected in 11 isolates

DISCUSSION

Our study shows that blaNDM-1 is not a major mechanism mediating carbapenem resistance in P.aeruginosa in our set up, however the blaVIM gene appears to be the predominant gene involved in carbapenem resistance in P.aeruginosa.Since the study is limited to only two genes i.e blaVIM and blaNDM-1,the reason for other MBL strains may be due to left out genes like SPM-1,GIM-1,SIM-1 or IMP-1.Resistance to the carbapenems in P.aeruginosa other than MBL production is often attributed to impermeability, which arises via the loss of the opr DPorin,the upregulation of an active efflux pump [8].

Though CLSI has not recommended any standardized phenotypic methods for screening MBL in clinical isolates.The results of E-test are more consistent with molecular analysis results.

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