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A Prevalence Report of IMP and VIM Type Beta-Lactamase Genes in Pseudomonas and Acinetobacter Species.

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ABSTRACT

The major Non-fermenting gram negative organisms isolated are *Pseudomonas aeruginosa* and *Acinetobacter* species. Infections caused by MDR Pseudomonas and Acinetobacter are often severe and life threatening. Metallo beta lactamase genes confer resistance to carbapenem group of drugs, as these pose a last resort of drug for MDR pathogens. The genes have the ability to transfer through mobile genetic elements. Hence the analysis of MBL isolates were screened necessary. 85 Pseudomonas species and 75 Acinetobacter species were collected. Antibiotic susceptibility test was done, 23/85 and 13/85 were meropenem resistant isolate by disc diffusion method and by Minimum inhibitory concentration (MIC) for meropenem none of Pseudomonas isolates were resistant and 11/13 Acinetobacter isolates were resistant to meropenem by MIC. All the organisms were then subjected to PCR irrespective of their susceptibility to detect MBL encoding genes, all the Pseudomonas isolates tested were negative for the presence of IMP and VIM gene and 1/75 and 2/75 showed the presence of bla_{IMP} and bla_{VIM} gene respectively in Acinetobacter species.

Keywords: bla_{IMP} and $bla_{VIM,}$ Carbapenemase gene, Metallo beta lactamase.

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INTRODUCTION

Non-fermenting gram negative organisms are an important opportunistic pathogen which is commonly associated with nosocomial infections. The major organisms isolated are *Pseudomonas aeruginosa* and *Acinetobacter* species. As these strains in the hospital environment have prolonged exposure to various antibiotics, would develop multi-drug resistant (MDR) phenotype [1].

Infections caused by MDR Pseudomonas and Acinetobacter are often severe, life threatening and are difficult to treat. The antibiotic resistance mechanism in these organisms include different enzymes viz the acquisition of extended-spectrum β-lactamases, carbapenemases, aminoglycoside-modifying enzymes and 16S ribosomal ribonucleic acid methylases and mutational changes in the up-regulation of multidrug efflux pump genes, de-repression of ampC, modification of antimicrobial targets in the outer membrane [2,3].

The emergence of carbapenem resistance is of more concern due to its usage as the last line drug of choice for the most important nosocomial agents such as Pseudomonas and Acinetobacter. According to Ambler classification the carbapenemases which include class A enzymes (i.e., KPC types), class B enzymes, or the metallo-beta-lactamases (MBLs [i.e., VIM, IPM, and NDM types]), and class D enzymes, or oxacillinases (i.e., OXA types) [1]. These metallo-beta lactamase genes render the organism more resistant and they have the ability to spread among other isolates by their mobile genetic elements. Therefore, it is necessary to analyse these isolates for MBL resistant phenotype. This would help in use of appropriate antibiotic therapy for the prevention of inter and intra-hospital spread [2]. Hence the present study was carried out to analyse the antibiotic susceptibility pattern of these organisms as well as presence of metallo-beta lactamase genes by PCR.

MATERIALS AND METHODS

A total of 160 isolates of both Pseudomonas and Acinetobacter species were collected at a tertiary care hospital, Chennai during the period of December 2012 to May 2014 from various clinical samples. The isolates were confirmed by the standard biochemical tests. The isolates were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method and the zone of inhibition was measured and interpreted [9]. The ATCC *Pseudomonas aeruginosa* 27853 was used as the positive control.

Determination of Minimum Inhibitory Concentration for Meropenem:

The organisms resistant to meropenem were selected and the minimum inhibitory concentration was determined by E-Strip method. The meropenem E-strip was procured from Hi-sMedia, Mumbai. ATCC *Pseudomonas aeruginosa* 27853 was used as the positive control [9].

Detection of metallo-beta-lactamase gene by PCR:

DNA was extracted from the isolates of both Pseudomonas and Acinetobacter by boiling lysis method. All the 160 isolates were subjected to PCR to detect the presence of metallo-beta-lactamase genes such as bla_{IMP-} and bla_{VIM-}irrespective of their resistance to carbapenem group of drugs by disc diffusion method or by E-strip method. The primers were given in the table 1 [5].The amplified products of the PCR were viewed by 1% agarose gel electrophoresis and the gel was documented in gel documentation system.

Table 1: shows the primer sequence of IMP and VIM type beta-lactamase genes

Primers used	Sequence	Product size
IMP-F’	5’-GAAGGCGTTTATGTTCATAC-3’	581 bp
IMP-R’	5’-GTATGTTTCAAGAGTGATGC-3'	
VIM-F	5'-GTTTGGTCGCATATCGCAAC-3’	382 bp
VIM-R’	5’-AATGCGCAGCACCAGGATAG-3'	

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RESULTS

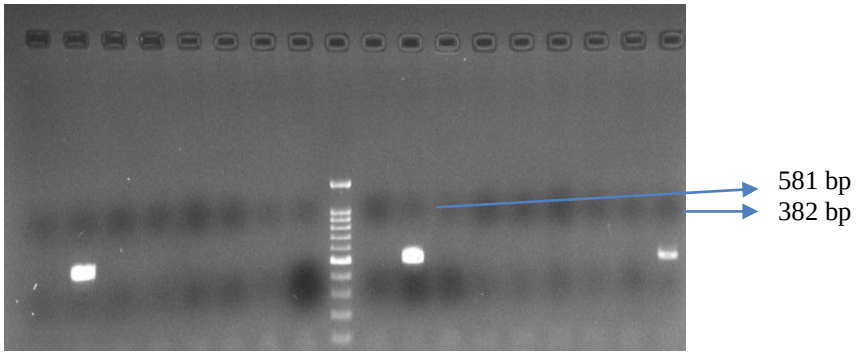
Out of the total 160 isolates 85 were *Pseudomonas aeruginosa*, 36 were *Acinetobacter baumannii*, 20 were *A.haemolyticus*, 6 were *A.junii*, 9 were *A.calcoaceticus* and 4 were *A.lowfii*. Out 85 *P.aeruginosa* (23/85) 27.05% and out of 75 Acinetobacter species (13/75) 17.33% of isolates were resistant to meropenem. The antibiotic sensitivity profile shows 75% of the Acinetobacter were sensitive to colistin and Polymyxin B and 82.53% of Pseudomonas isolates were sensitive to meropenem, Aztreonam, colistin and Polymyxin B by Kirby Bauer disc diffusion method.

Minimum inhibitory concentration for meropenem was tested for all the isolates by E-strip method. none of the AST resistant Pseudomonas isolates were resistant to meropenem by MIC, out of 13 AST resistant isolates of Acinetobacter, 11 were resistant to meropenem with a breakpoint at >32µg/ml.

All the strains of Pseudomonas and Acinetobacter were subjected to PCR for the presence of Metallo-beta-lactamase gene such as bla_{IMP-}, and bla_{VIM-}, out of 85 isolates of Pseudomonas none of them showed the presence of bla_{IMP-} and bla_{VIM-} gene. In Acinetobacter species, 1/75 and 2/75 showed the presence of bla_{IMP} and bla_{VIM} gene respectively.

Figure 1: shows the presence of blaIMP and blaVIM gene in Acinetobacter

L1 L2 L3 L4 L5 L6 L7 L8 M L9 L10 L11 L12L13L14L15L16L17



L2, L10 – shows the presence of blaIMP gene
L17 – shows the presence of blaVIM gene

DISCUSSION

Acinetobacter species and *Pseudomonas aeruginosa* are noted for their intrinsic resistance to antibiotics and for their ability to acquire genes encoding resistance determinants. This study shows the differences and similarities between Acinetobacter and Pseudomonas infections, Carbapenem resistance is a significant clinical problem and is a cause of majority of nosocomial infections in hospitalized patients. *Acinetobacter baumannii* possess outer membrane porins which play a major role in the carbapenem resistance. In 2007, from Karnataka the authors had reported that 14% resistance to meropenem by disc diffusion method [4]. And in 2010, a study from Puducherry reported that 22% and 57% of pseudomonas and Acinetobacter isolates were resistant to meropenem respectively [7]. In the present study 17.43% of pseudomonas isolates and 25% of the Acinetobacter isolates were resistant to meropenem. The MICs of the disc diffusion resistant isolates were found to be >32µg/ml, these results were in contrast to the study conducted from Karnataka [4].

In our study, the carbapenem susceptible isolates identified by phenotypic method also possessed MBL genes by PCR. A study from Maharashtra during 2012 showed that the prevalence of MBLs in Pseudomonas was 11.42% and in Acinetobacter species it was 10.40% [6] similarly, our results also correlated as 13.04% prevalence in Pseudomonas and 9.09% in Acinetobacter.

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CONCLUSION

The rate of resistance to beta lactams was very high and all the resistant isolates were found to harbor the resistant genes. Even though the prevalence of *bla*$_{IMP}$ and *bla*$_{VIM}$ type MBL genes was low, the other mechanisms are also involved in resistance to beta-lactams [8]. By understanding the mechanisms underlying in resistance to antibiotics, can facilitate the development of preventive measures and control strategies of nosocomial infections.

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