Original Research Article

Antibiotic sensitivity pattern and imipenem-EDTA double disk synergy test for the detection of Metallo-beta-lactamase producing *Pseudomonas aeruginosa* from clinical samples in a teaching hospital

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A B S T R A C T

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Pseudomonas is widely distributed in nature and in hospital. It is one of the most notorious organisms to cause hospital acquired infections. It shows resistance to several antibiotics because of production of beta-lactamase enzymes. This study was conducted to know the prevalence of MBL(metallo beta lactamase) mediated resistance in *Pseudomonas aeruginosa*. A total of 286 isolates of *Pseudomonas aeruginosa* from different clinical samples were obtained from both out and in patient wards of our hospital. Isolated organisms were subjected to antibiotic susceptibility testing by modified Kirby-Bauer disc diffusion method using Ceftazidime, Gentamicin, Tobramycin, Pipercillin, Amikacin, Cefepime, Ciprofloxacin, Imipenem, Meropenam, Piperacillin-Tazobactum. Further, Imipenem resistant *Pseudomonas* were tested by double disc synergy test using Imepenem – EDTA. The isolates were also subjected to E-test which consists of double side seven dilution range of Imipenem and Imipenem overlaid with constant gradient of EDTA. Of the 286 isolates 134(46.9%) showed resistance to Ceftazidime, 150(52.5%) to Gentamicin, 133(46.6%) to Tobramycin, 142(49%) to Cefepime, 135(46.6%) to Ciprofloxacin, 97(34%) to Amikacin,78(27.3%) to Meropenem, 75(26.3%) to Piperacillin,68(23.1%) to Piperacillin Tazobactum, 67(22.88%) to Imipenem. Our results matched the studies done in India with the prevalence of MBLs in Pseudomonas being 18.85%. The early detection of MBL producing *Pseudomonas aeruginosa* may help in appropriate antimicrobial therapy and avoid the development and dissemination of these multidrug resistant strains.

Introduction

Widely distributed in nature including soil, water and various types of vegetation throughout the world, *Pseudomonas aeruginosa* is a Gram negative, non sporing, non capsulated, straight or slightly curved rod shaped bacterium occurring singly, in

pairs or in short chains (Todar 2011). It is also known to be present in disinfectants, respiratory equipment, sinks, taps, and mops within the hospital as a biofilm. The entry of this organism in the hospital environment could be through visitors and patients or goods that enter in hospital. Contact transmission or vehicle transmission is the common mode of transmission of *Pseudomonas* in hospital (Murray et al 2002). It is therefore reported to be one of the most notorious organisms known to cause hospital acquired infections. It is also known to exhibit intrinsic resistance to several antimicrobials. In addition, it produces an enzyme – β lactamase, which is responsible for the wide spread beta lactam resistance. These β -lactamases hydrolyse the amide bond of the four-membered characteristic β - lactam ring, thus rendering the antimicrobial ineffective [Bradford PA]. The genes , SHV2a and TEM, which encode for the beta lactam resistance have been found in Pseudomonas. aeruginosa and the Enterobacteriaceae family, which suggests that these organisms are widespread reservoir of the ESBL enzymes [Aggarwal R et al 2008].

The introduction of Carbapenems into the clinical practice had revolutionized the treatment of β -lactam resistant bacteria, due to their broad spectrum of activity and stability to hydrolysis by most beta lactamases. They became the drug of choice for extended spectrum beta lactamase (ESBL) producers. However, in recent years, carbapenem resistance has been observed frequently among non fermenting bacilli like *Pseudomonas aeruginosa* and *Acinetobacte*r spp, due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes-carbapenemase. These carbapenemase are class B metallo β -

lactamases (IMP, VIM) or class D-oxacillinases (OXA 23 to OXA 27) or class A - clavulanic acid inhibitory enzymes (SME, NMC, IMI, KPC) (Gladstone P 2005).

The rising antibiotic resistance against commonly used drugs is of great concern. Therefore, *Pseudomonas aeruginosa* must be considered in all infections, regardless of the age or time of onset, so that early, appropriate and often life-saving antibiotic therapy may be instituted (Barton LL et al 1986).

We have performed this study to find out the prevalence of MBL producing *Pseudomonas aeruginosa* in our area and the antibiogram

Materials and Methods

The present study was carried out in the Department of Microbiology, Mallareddy Institute of Medical Sciences, Hyderabad. A total 236 isolates of *Pseudomonas aeruginosa* from various specimens which include pus/wound swab, sputum, throat swab, suction tip, pleural fluid and urine obtained from both out and in patients of our hospitals over a period between Nov 2011 to August 2014 were included in the study. The isolates were identified as *Pseudomonas aeruginosa* by standard microbiological methods and then subjected to antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates as per CLSI guidedlines using Ceftazidime (30µg), Gentamicin (10µg), Tobramycin (10µg), Piperacillin (100µg), Amikacin (30µg), Cefepime (30µg), Ciprofloxacin (5µg), Imipenem (10µg), Meropenem (10µg), Piperacillin-Tazobactum (100/10 µg).

Pseudomonas aeruginosa ATCC 27853 strain was used as the control.

Detection of MBL by double disk synergy tests (DDST)

Imipenem resistant *Pseudomonas* isolates were tested by double disk synergy test (DDST) using Imipenem and Imipenem – EDTA, as described by Lee et al (Lee et al 2003). A lawn culture of the organism was inoculated onto MHA plate as per CLSI guidelines. An Imipenem disc(10ug) was placed at a distance of 10mm from a blank disc which contains EDTA (750ug). The plate was then incubated at 37 °C overnight.

An enhancement in the zone of inhibition in the area between the Imipenem and the EDTA discs in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result.

E test

The isolates were also subjected to E test which consist of a double sided seven dilution range of imipenem (4 to 256 µg/ml) and Imipenem (1 to 64 µg/ml) overlaid with a constant gradient of EDTA (courtesy HiMedia). Individual colonies were picked from overnight agar plates and suspended in sterile peptone water to a turbidity of 0.5% Mc Farland's standard. Lawn culture was prepared on MHA plate and the excess moisture was allowed to be evaporate for about 15 min before the E-test MBL strip was applied. The plates were incubated overnight at 37°C. The MIC end points were read where the inhibition ellipses intersected the strip. A reduction of imipenem MIC=3 two folds in the presence of EDTA was interpreted as being suggestive of MBL production.

Results and Discussion

The antibiotic Sensitivity pattern of the 286 *Pseudomonas aeruginosa* isolates is shown in Table:1.

221(77.2%) of the isolates, were sensitive to Imipenem, and 67 (22.8) were resistant. All these 67 were subjected to both DDST (Fig: 1) and E test (Fig: 2). Out of them, 54(18.95%) were MBL producers by both tests showing that both tests are equally effective.

In our study, we observed that our isolates of *Pseudomonas aeruginosa* were more resistance to antibiotics   like Ceftazidime (46.9%) and Cefepime (49%), Gentamicin (52.5%) & Ciprofloxacin (46.4%). Our findings were more or less similar to the study done by Peshattiwar et al, where they observed an increased resistance of this organism to various antibiotics like Cephotaxime- 50.79%, Netilmicin- 45.23%, Gentamicin - 38.09%, Amikacin -36.50%, Ciprofloxacin- 46.82% and Piperacillin- 41.26 % (Prashant dur was et al 2011) and Dwivedi et al, who reported a 63% resistance to Ceftazidime (Dwiwedi M et al 2009).

The 1st MBL producing Pseudomonas was reported from Japan in 1991 & since then it has been described from various parts of the world, including Asia, Europe, Australia, South America & North America. (Gales et al 2003, Lee   et al 2003). In some countries MBLs in *Pseudomonas* constitute about 20% of all nosocomial isolates. (Walsh et al 2005). In India MBL production *by P. aeruginosa* was first reported in 2002 to be 12% by Navaneeth et al.

MBL producing bacteria are associated with a higher morbidity and mortality among the patients. It is not only true that the MBL producers will hydrolyze all classes of ß-lactams but also that we are still several years away from the development of a safe therapeutic antibiotic. Hence, their continued spread would be a clinical disaster posing a serious concern for infection control management.

Table.1 Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*

Antibiotic	Sensitive	%	Resistant	%
Ceftazidime	152	53.1	134	46.9
Gentamicin	136	47.5	150	52.5
Tobramycin	153	53.4	133	46.6
Piperacillin	211	73.7	75	26.3
Amikacin	189	66	97	34
Cefepime	146	51	142	49
Ciprofloxacin	153	53.4	135	46.6
Imipenem	221	77.2	67	22.8
Meropenem	208	72.7	78	27.3
Pip taz	220	76.9	68	19.1

Fig.1 Identification by disk diffusion synergy test

Fig.2 Identification of MBL by E Test

In many studies around the world, there is a wide variation in the resistance pattern of MBLs. In a study conducted by Deshpande et al, 10 − 30% of MBL production was observed in Pseudomonas aerugiosa around India (Deshpande P 2010). Deeba Bashir from Kashmir reported 11.6% MBL positivity while in a study by Lagatolla et al. (2004) in Italy, 70% MBL was found in *Pseudomonas aeruginosa* (Lagatolla C et al 2004). Although our results did not correlate to similar studies done abroad, but the matched the studies done in India.

In the present study, we showed a prevalence of MBL producers in *Pseudomonas* to be around 19%, which was in accordance to similar studies by, Variaya et al (20.8%) (Ami Varaiya et al 2008), Navneeth et al (12%) (Navneeth BV et al 2002), Anil Rajput et al (12%) (Anil Rajput et al 2012). In a study conducted by Khosravi et al in Iran (Khosravi 2008), 19.51% of the Imipenem resistant Pseudomonas were MBL producers.

This emergence of Carbapenemase in this era leads to requirement of strict statutory guidelines implanting intervention for limiting inappropriate uses of antibiotics. Ignorance of rational antibiotics prescribing principles, lack of awareness of the problem of the alarming rise in the multiresistance & pharmaceutical promotion are possible factors leading to unnecessary antimicrobial usage. Inadequate infection control is further compounding the problem. The early detection of MBL producing P aeruginosa may help in appropriate antimicrobial therapy and avoid the development & dissemination of these multidrug resistance strains. So all isolates of P. aeruginosa resistant to imipenem should be screened for MBL production. Combined disk diffusion or disk potentiation test should be introduced in every clinical microbiology laboratory to facilitate better patient care and management of infection control. Since both methods are equally effective, the core cost effective one can be taken into consideration.

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