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Full Length Research Paper

Assessment of Metallo-beta-lactamase Producing Pseudomonas Species in Soil: Insights from Assam University Campus

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The incidence of multidrug-resistant (MDR) Gram-negative bacteria is increasing, including metallo-β-lactamase (MBL)-producing *Pseudomonas sp.*. The aim of this study was to screen metallo-β-lactamases (MBLs)-producing *Pseudomonas sp.* soil isolates showing susceptibility to imepenem.. MBL production was investigated by phenotypic tests using combined disc diffusion method. During the 3 months period of sampling, 30 isolates of *Pseudomonas sp.* were isolated from soil environments out of which 56.66% revealed MBL-producing isolates, 43.33% non-producers and 10% showed imepenem resistant.

Keywords: Metallo-beta-lactamase, multidrug-resistant, *Pseudomonas sp..*

INTRODUCTION

Pseudomonas is considered as a ubiquitous environmental as well as an opportunistic clinical pathogen with physiologic and metabolic adaptability intricate to treat in today's medical fraternity (Costerton *et al*., 1983; Benett 1985; Henwood *et al*., 2001 and Pitt *et al*., 2001). Considerable resistance mechanisms are exhibited by the antibiotic imepenem belonging to the class carbapenems (Hancock *et al*., 1966). *Pseudomonal* antibiotics is improbable to be devised, it is a regular practice to treat severe infections by combining b-lactam and an aminoglycoside (Hancock *et al*., 2000).

MBL-producing *Pseudomonas aeruginosa* isolates have been reported to be the vital grounds of serious nosocomial infections as septicemia and pneumonia linked with clonal spread (Bush *et al*.,1995; Bashir *et al*.,2011)

,which belongs to a group b-lactamase demanding divalent cations of zinc as cofactors for enzyme action.

Carbapenems are the most effective trustworthy beta lactam treatments against the critical bacterial infections caused by the beta- lactam resistant bacteria today because of its strong bonding to the penicillin binding proteins (Livermore 1995; Livermore 2001). The exploitation and excess of it results in the synthesis of metallo beta lactamase producing bacteria, which proves resistant to most of the beta lactam antibiotics including imipenem and meropenem (Livermore *et al*.,2000).Therapeutic approaches are inadequate or restricted because *Pseudomonas aeruginosa* readily acquires resistance against antimicrobials.

This study aims to analyze the range of incidence of the metallo-beta-lactamases producers amidst few of the isolates which aims at being the imperative cause of the difficulty in treating critical bacterial infections.

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MATERIAL AND METHODS

Study area, Collection of samples, and isolation and maintenance of microorganism:

A total of 62 soil samples were collected randomly from different locations inside the Assam university campus located near Dargahkona, 20 km from silchar, Assam during September to December 2013. Random soil samples were serially diluted upto 10^{-5} CFU to isolate *Pseudomonas sp.*. The isolates were then specifically cultivated on King's B Agar and incubated at 37ºC for 48 hrs considering the aseptic conditions. Biochemical assessments were done as colony morphology, Gram's stain appearance, oxidase reaction. The cultures obtained were maintained at -50ºC on Nutrient Broth with 30% glycerol. For each study an overnight culture was inoculated in fresh Nutrient broth (NB) and further incubated to ensure exponential growth conditions.

Antibiogram test

Antibiotic Susceptibility test was performed by the agar disk diffusion, Kirby Baeur Technique (Kirby *et al*.,2002) in accordance to the protocol recommended by NCCLS (NCCLS 2000) on Mueller-Hinton agar. The plates were incubated at 37ºC for 24 hr to check the zone of inhibition. Bacterial strains that demonstrated resistance to three or more categories of antibiotics were defined as multi drug resistant (MDR). A Sensitive Result obtained was defined as a zone of inhibition that meets the interpretive principles as recommended for inoculation on Mueller Hinton Agar by Standard Method. The antibiotics discs formulated for the susceptibility test were β-lactam[Imipenem(10mg), Ceftadizime(30mg), Ceftriaxone(30mg), Piperacilllin(10mg)], Aminoglycosides[(Amikacin(30mg), Gentamicin[(10mg), Netilmicin(30mg)], Fluoroquinolones [Ciprofloxacin(5mg), Levofloxacin(10mg)].

MBL-determination test

Ethylenediaminetetraacetic acid (EDTA) 0.5 M EDTA (Sigma-Aldrich, India) solution was prepared by dissolving 186.1g of disodium EDTA.2H2O in 1000 ml of distilled water and its pH was adjusted to 8.0 by using NaOH. The mixture was then sterilized by autoclaving. EDTA sol (4 µl) was poured on imipenem and ceftazidime disks to obtain a desired concentration of 750 µg per disk. The EDTA impregnated antibiotic disks were dried immediately in an incubator and stored at -20°C in airtight vials without desiccant until used. An overnight broth culture of test strain (opacity adjusted to 0.5 Mc Farland opacity standards) was inoculated on a plate of Mueller Hinton agar. One 10 µg imipenem and one 30 µg ceftazidime disks were placed on the agar plate. One each of EDTA impregnated imipenem and ceftazidime disks were also placed on same agar plate. The plate was incubated at 37°C for 16 to 18 hours. An increase in the zone size of at least 7 mm around the imipenem-EDTA disk or ceftazidime-EDTA compared to imipenem or ceftazidime disks without EDTA was recorded as an MBL producing strain.

RESULT

Out of 62 samples tested , 30 were identified as *Pseudomonas sp*. Among the 30 isolates screened for the detection of the MBL producers by the disc potentiation test, 17 was found to be MBL producers and 13 was found to be non- MBL producers (Table. 2).This test was performed to note the frequency of the degree of the production of metallo-beta-lactamase by the isolates as the production of this enzyme renders the organism difficult to be treated .

Susceptibility testing determined by NCCLS agar dilution confirmed resistance to gentamicin, levofloxacin, ceftriaoxne and netilmicin. In addition, 10% isolates showed resistant to imipenem. All the MBL producer *Pseudomonas sp.* were found to be multidrug resistant. Maximum sensitivity was seen with imipenem(90%), followed by moderate activity with piperacillin (50%), amikacin (30%), ciprofloxacin and ceftadizime (20%) and poor susceptibility patterns with the remaining of the drugs. (Table. 1).

CONCLUSION AND DISCUSSION

In this study, we have identified two MBL-producing *Pseudomonas sp.* soil isolates that exhibited low level of imipenem resistance. Consequently, the occurrence of MBL production by the soil isolates of *Pseudomonas sp.* that remain susceptible to imepenem might be unrecognized by the clinical laboratories. It might lead to underestimation of MBL prevalence in the clinical setting. In this manner, these MBL producers might act as silent reservoirs of such resistance determinants, with ability to spread, since MBL-encoding genes are often carried by mobile genetic elements. In addition, failing in detecting MBL production among Imepenem-susceptible isolates may lead to inadequate prescription of this drug and possible therapeutic failure in seriously ill patients.

Identification of carbapenemase producers could also rely upon phenotypic detection, especially in regions where MBL-producing isolates are prevalent. However, the implementation of such recommendation has been exposed by the lack of a consensus regarding the criterion to select isolates for screening, the method, the β-lactam substrates and the best inhibitor combinations to be employed for precise MBL phenotypic detection. Although much progress has been achieved in comprehending the

Table 1. Antibiotic susceptibility pattern of *Pseudomonas sp.*.

Antibiotics Used	No. of susceptible Isolates
Gentamicin (GEN)	02(6.6%)
Imepenem (IM)	27(90%)
Amikacin (AM)	09(30%)
Ciprofloxacin (CIF)	06(20%)
Ceftriaxone (CEF)	04(13.33%)
Levofloxaxin (LE)	02(6.6%)
Piperacillin (PI)	15(50%)
Ceftadizime(CEFTA)	06(20%)
Netilmicin (NE)	03(10%)

Table 2. Screening test of MBL production.

Total No. of samples	Screening positive	Confirmatory Screening	
		MBL producer	Non-producer
62	30	17(56.66%)	13(43.33%)

mechanisms of antimicrobial resistance, it is imperative that the scientific community concentrates efforts in developing means to assess the real prevalence of carbapenemase producers. It is also very important to evaluate its clinical impact and establish whether carbapenems would be appropriate to treat infections due to carbapenemase producers that are still categorized as susceptible to these antimicrobials.

Owing to its physiologic versatility *Pseudomonas sp.* is considered as a life challenging Pathogen, responsible for severe ventilator-associated pneumonia (VAP), invasive infection in hospitalized patients with serious underlying disease, such as leukemia, cystic fibrosis (CF), and extensive burns, ocular infections and other unremitting clinical manifestations as ear otitis, Melioidosis, Glanders, G.I. Infections and so forth (Arora *et al*., 2011; Lister *et al*., 2006). *Pseudomonas sp.* has consistently established itself as a key element or an answer to the antibiotic remedy, attributed to the portentous dimension of its genome. Metallo beta lactamase (MBL) producing *Pseudomonas sp.* are very complicated to eradicate, which results in elevated morbidity and mortality rate amongst patients infected by these opportunistic strains.

Imipenem-resistance in the isolate of *Pseudomonas sp.* was determined by disc diffusion method. Earlier reports suggest that most of the MBL producing isolates have shown resistance to other important groups of antibiotics including third-generation cephalosporin, aminoglycoside and quinolone (Pitout *et al.,* 2005). Acquired MBLs may rapidly emerge and establish a condition of endemicity in certain epidemiological setup (Lagatolla *et al.,* 2004). Reports from various parts of
The world showing emergence of MBL in Enterobacteriaceae is evidence for the spread of these enzymes in this family (Ikonomidis *et al.,* 2005). Emergence of MBLs producing *Pseudomonas sp.* in aquatic ecosystem is alarming and reflects excessive use of antibiotics. Therefore, early detection and prompt infection control measures is important to prevent further spread of MBLs to other gram negative rods. Additionally, it is also important to follow antibiotic restriction policies to avoid excessive use of broad-spectrum antibiotics (Khairnar *et al.,* 2013). This study demonstrated a high prevalence of MBL producing pseudomonas strains in the university campus. Therefore soil isolates can be considered as a potential reservoir for spreading critical infection and thereby rendering themselves resistant to the antimicrobial therapy.

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