Prevalence Of Metallo-B-Lactamase Producing Pseudomonas Spp. (In Tertiary Care Hospital)

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Abstract: Background: Metallo-β-lactamase (MBL) mediated resistance to carbapenem is an emerging threat in Pseudomonas isolates. The aim of this study is to detect metallo-β-lactamase producing isolates of Pseudomonas spp. from various clinical samples from indoor patients in a teaching hospital. Materials and Methods: Total 900 bacterial strains were isolated from different clinical samples from indoor patients. The bacterial strains were isolated and identified as per the standard guidelines. Amongst them 100 isolates of Pseudomonas were taken for the present study. All pseudomonas isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method (CLSIs). In all imipenem resistant isolates of Pseudomonas spp., MBL detection was carried out by Imipenem-EDTA combined-disc synergy test (CDST). Results: Out of 100 isolates of Pseudomonas, 44 (44%) were imipenem resistant. Of these 44 isolates, 30 were producing MBL enzyme. 30 MBL positive isolate included 12 (40%) from surgical wards, 10 (33.33%) from tuberculosis ward, 4 (13%) from medicine ward, 2 (7%) from paediatric ward, 1 (3%) from urology ward and 1 (3%) from neonatal ICU. All MBL positive strains were resistant to β-lactams, aminoglycosides and fluoroquinolones. Conclusion: Prevalence of MBL producing Pseudomonas spp. is 30%. The MBL producing Pseudomonas *spp.* isolates were multidrug resistant. It is important to identify MBL producing pseudomonas isolates in laboratory as may cause serious infections and may cause a nosocomial outbreak. [Prajapati S et al NJIRM 2013; 4(2) : 68-70]

Key Words: Carbapenem-imipenem, Metallo-β-lactamase, Pseudomonas spp.

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Introduction: Pseudomonas spp. is one of the most common pathogen causing nosocomial infection.[1, 2] Pseudomonas causes burn wound infection, iatrogenic infection like post lumber puncture meningitis, post tracheostomy pneumonia, device associated bacteremia and septicemia. [3]

Acquired Metallo-β-lactamase (MBL) in Pseudomonas spp. has recently emerged as one of the most worrisome resistance mechanism. MBL was first detected[Type a quote from the document or the summary of an interesting point. You can position the text box anywhere in the document. Use the Text Box Tools tab to change the formatting of the pull quote text box.] in 1960, in Bacillus cereus which was chromosomal in location. The first plasmid mediated MBL positive Pseudomonas aeruginosa was isolated in Japan in 1991. MBL is an enzyme which requires zinc for their catalytic activity. Their activity is inhibited by metal chelators, such as EDTA and THIOL compounds. MBL hydrolyze all beta-lactam antibiotics including, penicillins, cephalosporins and carbapenems, with the exception of aztreonam (monobactam). [4]

MBLs spread easily on plasmids and cause nosocomial infections and outbreaks. Such infections mainly concern patients admitted to Intensive Care Units with several co-morbidities and a history of prolonged administration of antibiotics. [4] Moreover, MBL producing isolates are also associated with higher morbidity and mortality. [5] Early detection of MBL producing organisms is crucial to establish appropriate antimicrobial therapy and to prevent their interhospital and intrahospital dissemination. [6] The aim of this study is to detect MBL producing isolates of Pseudomonas spp. from indoor patients.

Material and Methods: The study was conducted at Microbiology department, B.J.Medical College, Ahmedabad during the period from 2008 to 2009. In present study, Total 900 bacterial strains were isolated from different clinical specimens like pus, wound swab, urine, sputum, body fluid, and bronchoalveolar lavage of indoor patients.

The bacterial strains were isolated and identified as per the standard guidelines. Amongst them 100 isolates of Pseudomonas were taken for the study. Antimicrobial susceptibility for all Pseudomonas isolates was determined by Kirby-Bauer disc diffusion method as per CLSIs guideline. [7] In all imipenem resistant isolates of Pseudomonas spp. MBL detection was done by Imipenem-EDTA combined-disc synergy test (CDST).

Imipenem-EDTA combined-disc test (CDST): The test organisms were inoculated on Mueller Hinton agar as recommended by the CLSIs. A 0.5 M EDTA solution was prepared by dissolving 18.61 gm of EDTA in 100 ml of distilled water and adjusting its pH 8.0 by using NaoH. The Mixture was sterilized by autoclaving. Two imipenem (10ug) discs were placed on the surface of an agar plate at distance of 30 mm and 4 µl EDTA solution was added to one of them to obtain a desired concentration of 750 µg. The inhibition zones of imipenem and imipenem-EDTA discs were compared after 16 to 18 hours of incubation in air at 37˚C. In the combined disc test, if the increase in inhibition zone with the imipenem-EDTA disc was ≥7 mm than the imipenem alone, it was considered as MBL positive. [5, 8, 9]

Result: A total of 100 isolates of Pseudomonas included 46 from swab, 20 from urine, 16 from pleural fluid, 7 from pus, 7 from sputum, 2 from drain, 1 from ascitic fluid and 1 from bronchoalveolar lavage (BAL).

Out of total 100 isolates of Pseudomonas, 44 (44%) isolates were resistant to imipenem, 68 (68%) to ceftazidime, 42 (42%) to aztreonam , 54 (54%) to piperacillin-tazobactam, 72 (72%) to amikacin , 70 (70%) to gentamicin, 71 (71%) to levofloxacin and 79 (79%) to ciprofloxacin. 30 out of 44 impenem resistant isolates showed ≥7 mm zone enhancement in imipenem-EDTA combined-disc test (CDST) (Table-1).

Table-1: Pseudomonas isolates producing MBL.

Total Pseudomonas spp. Isolates	100
Screening test positive isolates	44 (44%)
MBL producing isolates	30 (30%)

Table-2 shows ward-wise distribution of MBL producing Pseudomonas strains. Maximum isolates were from surgical wards and least were from urology ward and neonatal ICU.

Table-2: Ward-wise distribution of MBL positive pseudomonas isolates.

Ward	Total MBL isolates	%
Surgical wards	12	40
Tuberculosis ward	10	33.33
Medicine ward	04	13
Pediatric ward	02	7
Urology ward	01	3
Neonatal ICU	01	3

All MBL producing Pseudomonas spp. were resistance to all β lactam antibiotics (except aztreonam), aminoglycosides, fluoroquinolones.

Discussion: Pseudomonas spp. is one of the most frequent nosocomial pathogen and the infections due to these are often difficult to treat due to antibiotic resistance. Various studies have reported MBL production ranging from 7% to 65%. [10]

A total 44 isolates of Pseudomonas spp. were found to be resistant to imipenem and 30 of 44 isolates were MBL positive. In present study MBL production in pseudomonas isolates was 30%. There is high prevalence of MBL production among imipenem resistant Pseudomonas spp. as reported by various studies (Table-3).

Table-3: Comparison of MBL production.

Studies	Imipenem Resistant (%)	MBL positive (%)	MBL positive in Imipenem resistant (%)
Present study	44	30	68.18
Mathur purva at al [10]	69.23	52.74	47.61
Mehulchaudhary at al [11]	05	05	100
Bashier at al [12]	13.42	11.66	86.84
Hemlatha et al [13]	16	14	87.55

In present study, MBL Positive Isolates were mostly isolated in Surgical wards (40%), Tuberculosis ward (33.33%), Medicine ward (13%), while less in Pediatric ward (7%), Urology ward (3%) and Neonatal ICU (3%). In Mehul chaudhary at al [11] study, 77% MBL positive isolates were isolated from surgical ward. All MBL positive isolates of Pseudomonas were resistant to βetalactams (except aztreonam), Fluoroquinolones and Aminoglycosides.

Conclusion: Prevalence of MBL production in pseudomonas isolates was 30% in the study. There was high prevalence of MBL production among imipenem resistant Pseudomonas spp. All MBL producing Pseudomonas spp. were multidrug resistant. It is important to identify nosocomial infection producing MBL positive isolates of Pseudomonas spp. because it poses not only therapeutic problem, but also a serious concern for infection control management.

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