Original article:

Metallo beta-lactamases mediated resistance in Pseudomonas aeruginosa from clinical samples in a teaching hospital of North India.

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Abstract:

Introduction: Metallo beta-lactamases(MBL)are class B type of beta-lactamases and are encoded by genes like VIM,IMP etc.They are enzymes requiring bivalent metal ions, usually zinc, as metal co-factors for their enzymatic activity.MBL production is significant problem in hospital isolates of Pseudomonas aeruginosa.Hence the present study was conducted at our hospital to know the prevalence of MBL amongst Pseudomonas aeruginosa.

Materials and Methods: The study was conducted at our hospital from January 2014 to July 2015.Total of 322 strains of Pseudomonas aeruginosa isolated from various clinical samples like pus,urine,sputum,ET secretions were evaluated for MBL production by Imipenem-EDTA Double Disk synergy test.

Result: Out of 322 Isolates of Pseudomonas aeruginosa 57(17.70%) were MBL producers. All MBL producing isolates showed widespread resistance to different classes of antibiotics.

Conclusion: Hence the study underlines the fact that early detection of MBL producing P. aeruginosa may help in formulating an effective antibiotic strategy and prevent dissemination of these multidrug resistant strains.

Key words: Metallo-betalactamases, Pseudomonas aeruginosa, Imipenem, Double Disk Synergy test.

Introduction:

Nosocomial infections due to drug resistant bacterial pathogens have been associated with increased hospital expenditures and poorer clinical outcomes. *Pseudomonas aeruginosa* is the most frequently isolated troublesome pathogen causing life threatening respiratory tract infections(ventilator associated pneumonia), surgical site and Urinary tract infections in patients from intensive care units(1).

Several mechanisms can contribute to acquired resistance in *Pseudomonas aeruginosa*, including β lactamase production, the up regulation of efflux systems and decreased outer membrane permeability. Metallo-β-lactamases(MBL) mediated resistance is important emerging resistance mechanism in *Pseudomonas aeruginosa* (2).

MBL are carbapenemases which require zinc at the active site.They belong to Ambler's Class B and Bush-Jacoby Mederios Group 3 and hydrolyze virtually all β-lactam agents, including the carbapenems(3,4).Till now seven main types of MBL have been described throughout the world – IMP, VIM, SPM, GIM, SIM, AIM-1 and NDM-1(5).Their continued spread is a major therapeutic challenge.

Thus, this study was undertaken, to determine the antibiotic susceptibility pattern of *Pseudomonas aeruginosa* with special reference to MBL in clinical isolates to serve as a guide for doctors managing

patients.This possibly will help to prevent the associated morbidity and mortality caused by this organism by implementing proper infection control measures as well as formulating an effective antibiotic strategy.

Materials and Methods:

The present study was conducted in the Department of Microbiology at Muzaffarnagar Medical College , Muzaffarnagar,over a period from January 2014 to July 2015. All 322 isolates of *Pseudomonas aeruginosa* obtained from various clinical samples:pus , blood, urine, CSF, ascitic fluid, pleural fluid etc. received in microbiology laboratory from IPD &OPD were included in the study. The isolates were identified as per the standard microbiological procedures(6).Antimicrobial sensitivity testing was performed on Mueller-Hinton agar plates with commercially available disks(Himedia) by Kirby Bauer disk diffusion method and interpreted as per CLSI guidelines(7).MBL producing *Pseudomonas aeruginosa* suspected when the isolate was resistant to meropenem and imipenem.MBL production was further evaluated by Imipenem-EDTA Double Disk synergy test Fig:1(8).

Results:

Out of 1738 culture positive samples obtained during the study period,322(18.25%) yielded the growth of Pseudomonas aeruginosa.Amongst these 223(69.25%) were obtained from male patients and 99(30.75%) from female patients.The male to female ratio was 2.25:1.Out of 322 isolates,57(17.70%) isolates were obtained from the Outpatients Department and 265(82.30%) were from inpatients. Among the inpatient 107(33.23%) isolates were obtained from Surgery ward followed by 70(21.74%) from Medicine ward (Fig:2).Maximum Pseudomonas aeruginosa isolates were obtained from pus 42.86% followed by ET secretions 16.15% (Fig:3).

The resistance pattern of Pseudomonas aeruginosa was noted as follows,ceftazidime-226(70.19%), ceftriaxone-224(69.57%),tobramycin-154(47.83%),gentamicin-198(61.49%),amikacin-196 (60.86%), ciprofloxacin-204(63.35%),imipenem & meropenem-57(17.70%),piperacillin-tazobactam-73(22.67%) and piperacillin-204(63.35%)(Fig:4).All 57(17.70%) isolates resistant to imipenem/meropenem were MBL positive by Imipenem-EDTA Double Disk synergy test.

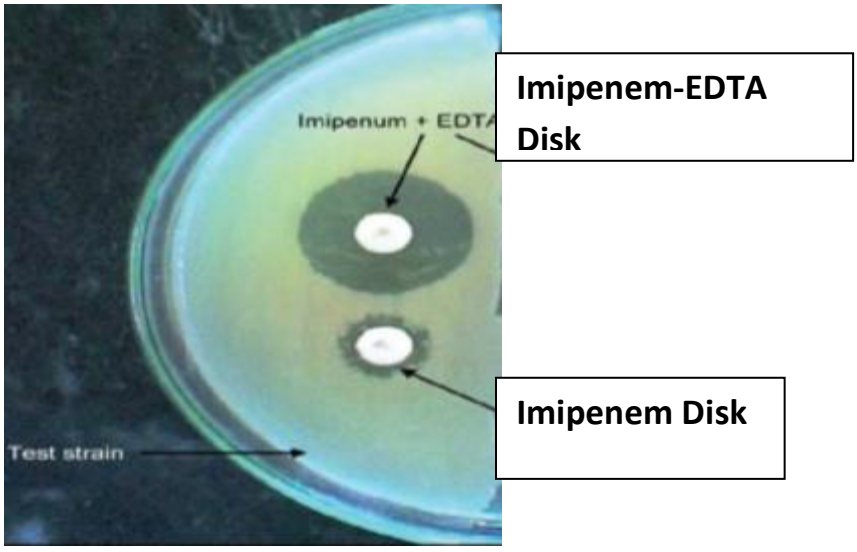

Fig 1: Imipenem-EDTA Double Disk synergy test.

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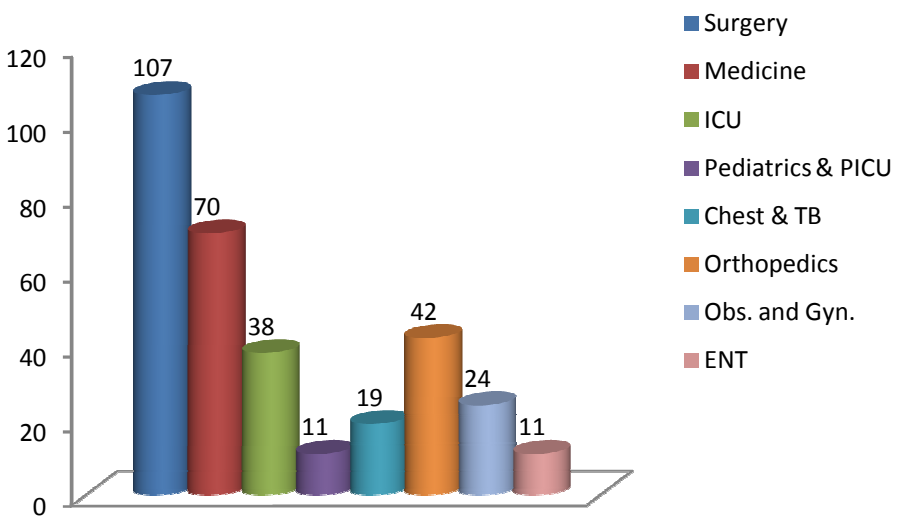

Fig 2: Ward wise distribution of *Pseudomonas aeruginosa* producers:

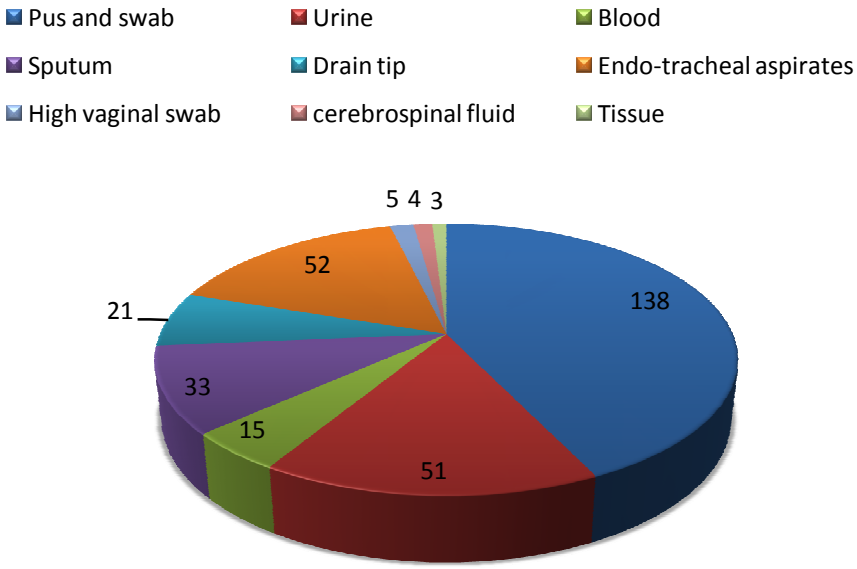

Fig 3: Percentage distribution of *Pseudomonas aeruginosa* producers from various clinical samples.

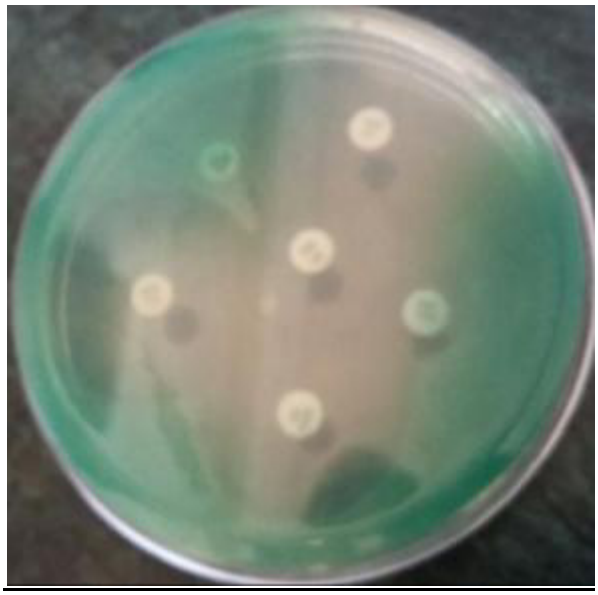

Fig 4: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*.

Discussion:

Metallo-betalactamase producing *Pseudomonas aeruginosa* is on a rise threatening the effectiveness of many antimicrobial agents.

In the present study, the rate of isolation of *Pseudomonas aeruginosa* was higher in indoor patients (82.30%) as compared to that in the outdoor patients (17.70 %). A similar observation was made by Prashant et al who reported the rate of isolation of *Pseudomonas aeruginosa* as (84.93%)in indoor patients and (15.07 %) in outdoor patients(9). Pus (42.86%) was the main source of *Pseudomonas aeruginosa* followed by Endotracheal aspirate (16.15%) and urine (15.83%) in our study.Similar results had been obtained in different studies in India reported by Mohanasoundaram and Arora et al(10,11). Shanthi et al isolated 41.8% of specimen from respiratory tract , followed by 25.5% from urinary tract(12). In another study by Pal Ramprasad Balikaran et al the clinical specimen from which *Pseudomonas aeruginosa* was commonly isolated was urinary tract followed by pus(13).

In present study all *Pseudomonas aeruginosa* isolates showed high resistance to Ceftazidime (70.19%) followed by ceftriaxone (69.57%) , piperacillin (67.08%), ciprofloxacin (63.35%), Gentamicin (61.49%) and tobramycin (47.83%).Our findings were more or less similar to the studies done by Shrivastava G et al and Peshattiwar et al(14,15).

Out of 322 isolates 265 were sensitive to imipenem and meropenem and 57 were resistant. All these 57 were subjected to by Imipenem-EDTA Double Disk synergy test.. All of them were found to be MBL producers .

There is a wide variation in the resistance pattern of MBLs. In the present study, we showed a prevalence of MBL producers in Pseudomonas to be around 18 %, which is in accordance to similar studies by Shrivastava G et al(20%) Variaya et al (20.8%), and Anil Rajput et al (12%) (14, 16,17).All of these isolates were sensitive to Polymyxin B.

Hence the study underlines the fact that early detection of MBL producing *P. aeruginosa* may help in formulating an effective antibiotic strategy and

prevent dissemination of these multidrug resistant strains.So all isolates of Pseudomonas aeruginosa resistant to imipenem should be screened for MBL production .

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