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Evaluation of Antimicrobial Susceptibility Pattern of Pseudomonas Aeruginosa with Special Reference to MBL Production in a Tertiary Care Hospital

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Materials and methods: 132 isolates of Pseudomonas aeruginosa from various clinical samples were tested for MBL production by double disk synergy method. Antibiotic susceptibility pattern was done by comparing non-MBL producers and MBL producers.

Results: Pseudomonas aeruginosa was isolated from Pus-39.39%, from urine-37.87%, sputum-3.03%. MBL producers were 44(33.34%), non MBL producers were 88(66.6). all MBL producers were 0% sensitive to Imipenem and Meropenem.

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Antibiotic susceptibility and MBL production in Pseudomonas aeruginosa

Dr. B. Anuradha [α], Uzma afreen [σ] & M. Praveena [ρ]

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Conclusion: our study showed that MBL producing Pseudomonas was isolated from all clinical samples and resistant to routinely used antibiotics and also to Imipenem and Meropenem.

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I. Introduction

Pseudomonas aeruginosa is a Gram negative motile bacillus, belongs to the family Pseudomonaceae. It is found in moist environment, disinfectant solutions, water due to its ability to utilize many different organic compounds and survive in nutrient deficient conditions (Nadeem etal 2009). It is a leading cause of nosocomial infection especially critical ill and immune-compromised patients (Hugbo etal 1992). It has been implicated in diverse nosocomial pneumonia, urinary tract infection, surgical site infection, severe burns and infection of patients undergoing chemotherapy for neoplastic diseases or those on antibiotics therapy (Erdem 1999). It has intrinsic resistance to many antimicrobial agents and show resistance to many antibacterial agents. The mechanism of resistance is due to cell wall permeability, production of extracellular chromosomal and plasmid mediated β-lactamases (Livermore 1989), aminoglycoside modifying enzymes, cephalosporinases (Prince 1986), and active multidrug efflux mechanism (Li et al 1994). This multi-drug resistant Pseudomonas aeruginosa causes nosocomial infections which is a global health care problem as it prolongs the duration of hospitalisation and increases the cost of the patient care..

The role of Carbapenems in the treatment of serious bacterial infections caused by β - lactamase resistant bacteria is a great advancement. The Carbapenems available for use in India are Imipenem and Meropenem (Gupta et al 2006). However Carbapenem resistance has been observed frequently in *Pseudomonas aeruginosa* which is due to decreased outer membrane permeability, increase efflux system, alteration of penicillin binding protein and Carbepenem hydrolysing enzyme – carbepenamase (Gladstone et al 2005). They have potent hydrolyzing activity not only against carbepanamase but also against other β lactamase antibiotics (Bush 1998 and Bennet 1999). These MBL producing *P. aeruginosa* strains have been reported to be important cause of nosocomial infection associated with clonal spread (Bush etal 1995).

Therefore detection of MBL producing Gram negative bacilli especially *Pseudomonas aeruginosa* is crucial for the optimal treatment of patients particularly in critical ill and hospitalized patients to control the spread of resistance (Richet etal 2001). Studies about resistant organisms, their impact on health care and cost are important. Detection of emerging resistance to various antibiotics and proper guidelines for empirical therapy are important. Hence the present study is taken up to detect Metallo- beta- lactamase production in pseudomonas aeruginosa in various clinical isolates

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and also to know the susceptibility pattern of MBL and non MBL producers to various antibiotics.

II. Materials and Methods

A cross sectional study was conducted on 132 Pseudomonas aeruginosa strains isolated from different clinical specimens like pus, wound swabs, urine, sputum, body fluids, endotracheal tube secretions, and stool and ear swabs. The following parameters were noted such as age of patient, sex, type of clinical specimen, antibiotic usage and duration of hospital stay, history of Diabetes mellitus, pregnancy, malignancy and alcoholism, history of lung disorders, smoking, any immunosuppressant condition and history of urinary catheterization. The Pseudomonas aeruginosa isolates were confirmed by biochemical reactions as per the standard conventional methods. Standard strain of Pseudomonas aeruginosa ATCC 27853 was used as control.

Antibiotic Sensitivity was performed by Kirby – Bauer Disc Diffusion method and the results are recorded as per CLSI recommendation (David Greenwood etal 2008) The antibiotics used were Gentamicin(10ug), Azithromycin(50ug), Ciprofloxacin (5mcg), Cefepime(30ug), Ceftazidime(30ug), ceftriaxone (30mcg), Piperacillin,/Tazobactom(100/10ug), colistin (10ug), Aztreonam(10ug), Meropenem(10ug), Imipenem (10ug). Sensitivity pattern was determined by measuring the zones of inhibition with a calibrated ruler and comparing with the standard reference chart (supplied by Hi media Laboratories).

All Pseudomonas aeruginosa isolates were tested for MBL enzyme production by Double Disk Synergy Test (DDST) as per CLSI guidelines using 10 μg imipenem discs with EDTA- On a plate of Mueller Hinton agar two Imipenem discs (10mcg) were placed and 10mcl of 0.5M EDTA solution was added to one of the discs and incubated over night at 37 degrees. The zones of inhibition around Imipenem and Imipenem-EDTA discs were noted and compared. In case of MBL producers the zone of inhibition around Imipenem and EDTA disc was more than 7mm compared to Imipenem disc alone.

III. Results

Out of 132 Clinical isolates of Pseudomonas aeruginosa 52 (39.39%) were isolated from pus, 50 (37.87%) were isolated from urine, 10 (7.57%) were isolated from sputum, 4 (3.03%) from endotracheal (ET) secretions, 2 (1.54%) from Pleural fluid, 4 (3.03%) from stool sample, 2(1.54%) from Ascitic fluid (ASF), and 6(4.54%) from Broncho alveolar lavage fluid (BAL). (Table: 1)

Table 1 : Clinical sample wise Distribution of Pseudomonas aeruginosa

Type of sample	Number of samples
Pus	52 (39.39%)
Urine	50 (37.87%)
Sputum	10 (7.57%)
Stool	04 (3.03%)
BAL	06 (4.54%)
ASF	02 (1.54%)
ET	04 (3.03%)
PLF	04 (3.03%)
Total	132 (100%)

BAL: Broncho alveolar lavage, ASF: Ascitic fluid, ET: Endotracheal tube, PLF: Pleural fluid

Out of 132 isolates Pseudomonas aeruginosa was isolated from 120 (90.50%) inpatients and 12 (9.50%) out patients.

Age wise distribution of Pseudomonas aeruginosa was as follows. 32 (24.24%) isolates were in the age group of 21 to 30, 11 (16.66%) isolation were in the age group of 31 to 40, 20 (15.15%) isolates were from the age group of 41 to 50, 14 (10.62%) isolates were in the both in 0 to 10 and 51 to 60 years, 10 (7.57%) isolates were in the age group of 61 to 70 and the rest of the 6 (4.54%) isolates were seen in above 70 years of age. This shows that Pseudomonas aeruginosa infections are not confined to particular age group but distributed in all age groups with slight variation.

Out of 132 isolates MBL enzyme production was seen in 44 (33.34%) cases and in 88 (66.66%) cases there was no MBL production. The ATCC 27853 *Pseudomonas aeruginosa* did not show any zone size enhancement by Double Disk Synergy Test (DDST). (*Table 2)*

Table 2 : MBL production in P.aeruginosa

MBL	Number of cases
MBL positive	44 (33.34%)
MBL Negative	88(66.66%)
Total	132 (100%)

Antibiotic sensitivity pattern of both MBL and non MBL producers to various antibiotics is shown in table-3, MBL producers were showing less sensitivity to routinely used antibiotics including imipenem and Meropenem compared to non MBL producers (p value-0.002).

Distribution of Pseudomonas aeruginosa in various clinical samples is shown in table-4

Table 3 : showing antibiotic sensitivity pattern of MBL producers and non producers

Antibiotics (μg)	MBL Positive n=44(33.34%)	MBL Negative n=88 (66.6%)
Gentamicin (10)	0	52(59%)
Ciprofloxacin (5)	5 (11.3%)	29 (65%)
Cefepime (30)	3 (6.8%)	40 (45.4%)
Ceftazidime (30)	5 (11.3%)	52 (59%)
Ceftriaxone (30)	5 (11.3%)	25 (28%)
Azithromycin (50)	3 (6.8%)	10 (11.3%)
Piperacillin/Tazobactam (100/10)	20 (45.4%)	44 (95.4%)
Imipenem (10)	0	20(22%)
Meropenem (10)	0	21(23%)
Colistin (10)	100 (100%)	100 (100%)
Aztreonam (10)	3(6.8%)	23 (25%)

P<0.05

Graph : showing antibiotic sensitivity pattern for both MBL and non MBL producers

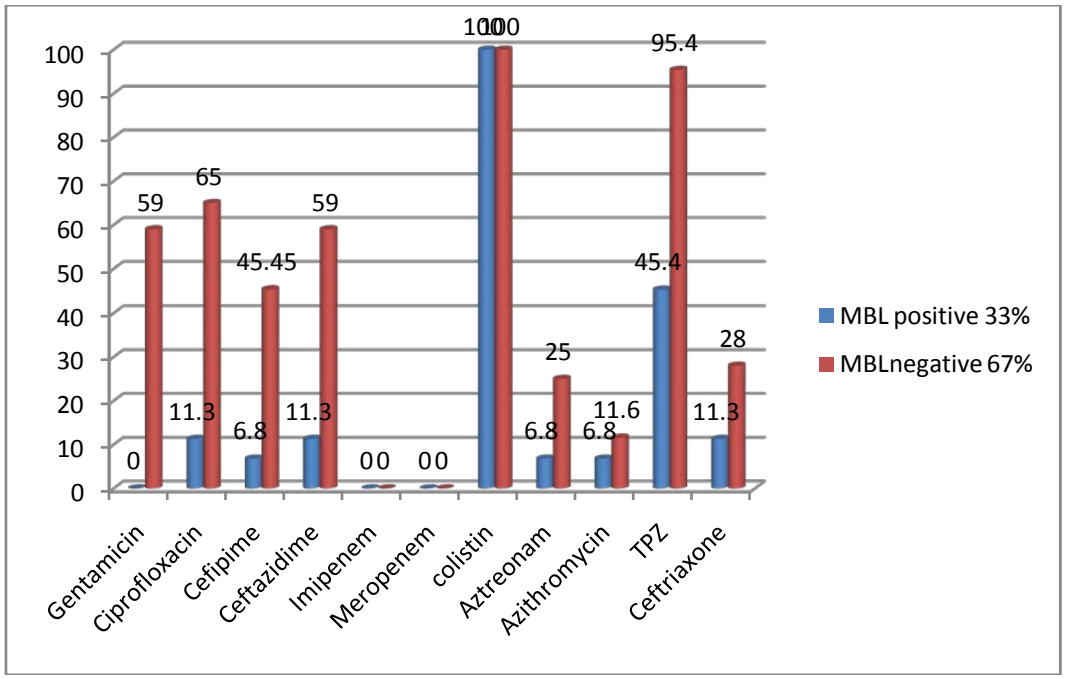

Table 4 : Distribution of MBL producing P.aeruginosa according to clinical specimen

Clinical specimen	P.aeruginosa (n-132)	MBL Producers(n=44)
Pus	52	18 (40.90%)
Urine	50	16(36.36%)
Sputum	10	04(9.09%)
ASF	02	02 (4.45%)
PLF	04	02 (4.45%)
ET	04	02 (4.45%)
Stool	04	00
BAL	06	00
Total	132	44(100%)

IV. Discussion

Out of 132 clinical isolates of Pseudomonas aeruginosa 52 (39.39%) were from pus samples followed by 50 (37.87%) urine samples. In one study by Bashir etal (Bashir etal 2011), among MBL producers 27.3% from urine, followed by 24.2% from wound infections. In 2008 Javiya et al(Javiya etal 2008) reported the highest number of Pseudomonas infection was found in urine followed by pus and sputum which indicates that wound infections and Urinary tract infections are the most common hospital acquired infections. These are the most important cause of morbidity in general population and also in hospitalized patients. In our study 90.5% of isolates were from inpatients and 9.5% were out patients, all MBL producers were from in-patients which show that Pseudomonas aeruginosa mainly causes nosocomial infections. Our study is correlating with Bashir etal 2011. Pseudomonas aeruginosa infections were seen almost equally in both males and females with 53.03% and 46.96% respectively. Among the isolates of Pseudomonas aeruginosa 24.24%cases were in the age group of 21 to 30 followed by 16.66% were in the age group of 31 to 40 only 4.54% were above 71 years. There was a slight variation with other studies in age but pseudomonas infections are distributed in all age groups.

MBL producing Pseudomonas aeruginosa is emerging as nosocomial pathogen and cause of concern for clinicians. It has the ability to acquire resistance to broad spectrum β - lactam antimicrobial agents which include 3rd generation Cephalosporins, Cephamycin, Carbepenems, Gentamicin and Fluoroquinolones. This MBL enzyme production has a potential to spread rapidly and to different Gram negative bacilli such as E.coli, Klebsiella and no suitable antimicrobial agents are available. In our study among the 132 isolates of Pseudomonas aeruginosa 44(33.34%) were MBL producers and 88 (66.66%) were non producers. Among the MBL producers majority were Pus samples 18 (40.90) followed by urine samples 16(36.36%) and 4 (9.09%) sputum. This indicates that MBL production among the isolates of Pseudomonas aeruginosa significant problem in wound infection followed by urinary tract infection. In our study prevalence of MBL producing Pseudomonas aeruginosa strains was 33% as the studies from other parts of India showing variable prevalence rate. In one study by Attal Ro in 2010 showed 11.4%, Navaneeth et al in 2000 12%,[Mendiratta et al in 2005 8.2% ,[Hemalatha et al in 2005 14%, Agraval et al in 2008 8.5%. Since then the incidence of MBL producing by Pseudomonas aeruginosa has been reported to be 10 to 30% from various clinical specimens across the country (Taneja etal 2003). Mary et al reported 42% MBL production; Sarkar et al reported 54.54%. This shows that prevalence of MBL producing Pseudomonas aeruginosa is increasing day by day.

Among the MBL non producers maximum sensitivity was seen to Colistin (100%), Piperacillin/Tazobactam (95.4%) followed by Ciprofloxacin (65%), Ceftazidime (59%) and Gentamycin (59%). Imipenem(22%) and Meropenem (23%) and Aztreonam (25%). Among MBL producers majority were sensitive to Colistin (100%), Piperacillin/Tazobactam (45.4%) and none of them were sensitive to Imipenem and Meropenem (0%) Gentamicin (0%), Cefepime and Azithromycin (6.8%), ciprofloxacin, Ceftazidime, ceftriaxone (11.3%) and Aztreonam (6.5%). The reason for Carbapenem resistance would be due to reduced uptake of drug. Our study is correlating with Bashir etal 2011 where MBL producers were resistant to gentamicin and 9.1% sensitive to Ciprofloxacin, 18.1% to Piperacillin/Tazobactam. Similar results were observed by Navneet etal 2002 and Agarwal etal 2006 in one study by Rakesh Kumar etal 2014 showed that very low sensitivity was observed to 3rd generation Cephalosporins, Ceftazidime 5%, Amikacin 10%, Ciprofloxacin 20%. They also observed that MBL producing Pseudomonas aeruginosa strains were 95% sensitive to Colistin. Our results are nearer to the above study. Our results are similar to Seema etal 2012 where both MBL and non MBL producers were 100% sensitive to colistin. , MBL producers were 0% sensitive and non MBL producers were 20% sensitive to Aztreonam. This shows that MBL producing Pseudomonas aeruginosa is developing resistance to routinely used antibiotics compared to non MBL producers. A 'p' value <0.05 was considered to be significant. This emerging drug resistance in Pseudomonas aeruginosa is causing problems in treatment, increasing the mortality rates and prolonged hospitalization.

V. Conclusion

Pseudomonas aeruginosa continues to be leading cause of serious infections particularly nosocomial infections mainly effecting the inpatients. The predominant infections observed were wound infections and urinary tract infections. The most common age group affected was between 21 to 30 years and Pseudomonas aeruginosa infections were equally distributed between males and females. The present study has demonstrated that 33% of pseudomonas aeruginosa are MBL producers and developing resistance to commonly prescribed antimicrobial agents such as Azithromycin, Cefepime, Ciprofloxacin, Gentamicin and Ceftazidime. MBL producers were resistant to Imipenem and Meropenem. The emergence of multidrug resistant Pseudomonas aeruginosa due to the indiscriminate use of antibiotics is a challenging clinical problem which leads to the

development of resistance to the routinely used antibiotics. There is a need to do surveillance to know the susceptibility pattern and to detect the MBL producers. MBL producers cause problems in treatment and in infection control. Revised guidelines on rational antibiotic usage are to be reinforced. Infection control measures must be intensified to minimize costs of patient care.

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