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ANTIBIOTIC RESISTANCE PROFILE AND DETECTION OF METALLO – BETA LACTAMASE IN PSEUDOMONAS AERUGINOSA ISOLATES FROM CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

This study aims to analyse the prevalence, antibiotic resistance pattern and detection of metallo beta lactamase in *Pseudomonas aeruginosa* isolates from clinical specimens. A total of 378 *Pseudomonas aeruginosa* isolates were isolated over a period of one year. Antibiotic susceptibility testing was done using Kirby Bauer disc diffusion method following standard guidelines. Inpatient samples contributed to majority of the isolates, proving its nosocomial origin. On the whole there was an increase in resistance percentage in the inpatient isolates reiterating the fact that selection pressure and antibiotic resistance are directly proportional to the use of antibiotics. Highest resistance was noted for Ceftazidime (51.85%). 19 isolates showed carbapenem resistance out of which 11 isolates were Metallo-beta-lactamses producers. Continuous monitoring of antibiogram, revising antibiotic policies periodically, antibiotic stewardship and infection control measures should help us win the war against one of the wisest unicellular bacteria.

KEYWORDS : **Antibiotic resistance, *Pseudomonas aeruginosa*, metallo-beta lactamase, Carbapenem resistance.**

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INTRODUCTION

Pseudomonas aeruginosa is a notorious non-fermenter causing a wide spectrum of infections ranging from otitis media to septicaemia. The minimal nutritional requirements of *Pseudomonas*, as evidenced by its ability to grow in distilled water and its tolerance to a wide variety of physical conditions, contribute to its ecologic success and ultimately to its role as an effective opportunistic pathogen.[1] In the community, the infections are restricted to swimmer's ear and malignant otitis in diabetics. Pseudomonas is well adapted to the respiratory tract environment of Chronic Obstructive Pulmonary disease (COPD), immunocompromised patients and patients receiving intensive care. Septicaemias are found in neutropenic patients, burns patients and patients with decreased immune function as in Acquired Immunodeficiency Syndrome (AIDS). Hospital acquired Urinary tract infection (UTI) is usually secondary to catheterisation, instrumentation or surgery. Pseudomonas causes ophthalmic neonatorum and can cause Meningitis after trauma, surgery or instrumentation.[2] As recorded worldwide, there is a three-fold higher rate of mortality, nine-fold increase in secondary bacteraemia and two-fold increase in length of hospital stay.[2] Pseudomonas acquires antibiotic resistance both intrinsically and extrinsically. The mechanism of resistance centres around efflux pumps and low permeability of outer membrane and drug inactivation.[3] It is noted that Pseudomonas has 10 to 100 fold lower outer membrane permeability compared to Enterobacteriaceae family.[4] In addition it can acquire genes which inactivate Aminoglycosides and cause mutations in quinolone targets. Mostly these mechanisms are present simultaneously, resulting in multidrug resistant phenotypes. More than one mechanism plays a role for each drug becoming resistant. Ceftazidime resistance is due to beta-lactamases and multi-drug efflux pump.[3] Metallo-β-lactamses (MBLs) are group of enzymes which require zinc for their activity. They belong to class B of Ambler classification and 3a, 3b and 3c of the Bush Jacoby classification. MBLs are produced as extracellular or periplasmic enzymes. These enzymes can degrade all of β-lactam antibiotics except monobactams and are not susceptible to therapeutic β-lactamase inhibitors. These enzymes constitute a group of heterogeneous proteins which are divided into subclasses B1, B2 and B3. Subclass B1 exhibits a broad spectrum profile. Subclass B2 hydrolyses only carbapenems. Subclass B3 exhibits broad spectrum activity with preference for cephalosporins and carbapenems. Majority of MBL genes are mobilized by integrons and/or transposons.[5] Since there is increased possibility of horizontal transfer of genes from pseudomonas to Enterobacteriaceae, it imposes risk of spread of multi-drug resistance in nosocomial pathogens.[4-5] Understanding the efficacy of the empirical antibiotics and screening of metallo-beta lactamases would help in antibiotic stewardship to curb the spread of resistance genes. Decreasing the resistant organisms in intensive care setting would directly lead to a decrease in mortality. The periodic testing and analysis of antimicrobial resistance would enable the physicians to detect the trends in the resistance pattern to the commonly prescribed antibiotics for the organism.

In view of all this, the present study was carried out to outline the impact of drug resistance in *Pseudomonas aeruginosa* infections. This would help the treating clinician and the microbiologist for a combined futuristic approach to decrease multi drug resistance.

AIM

The aim of this study is to analyse the prevalence, antibiotic resistance pattern and detection of metallo beta lactamase in Pseudomonas aeruginosa isolates from clinical specimens in a tertiary care hospital.

MATERIALS & METHODS

This cross-sectional study was conducted in the Department of Microbiology in Saveetha Medical College and hospital, Chennai, over a period of 1 year (July 2014 to June 2015). Samples collected were urine, blood, exudate and were inoculated on to Blood agar, chocolate agar and Mac Conkey agar. Pseudomonas strains were confirmed by necessary battery of tests that included gram staining, colony morphology, motility test, oxidase test, sugar fermentation test(oxidative fermentation test) and biochemical tests such as Indole test, urease and citrate test.[6] Antimicrobial susceptibility tests were done by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute(CLSI) guidelines.[7] The panel of drugs tested were as follows: Amikacin (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Ofloxacin (5 µg), Piperacillin/tazobactam (100µg / 10 µg), Cefipime (30 µg), Imipenem (10 µg).

MBL detection method by Imipenem – EDTA (Ethylene diamine tetra acetic acid) combined disc synergy test (CDST-IPM)

A 0.5 M EDTA solution was prepared by dissolving 186.1 gram of EDTA in 100ml of distilled water and adjusting its pH to 8.0 by using sodium hydroxide (NaOH).The mixture was sterilized by autoclaving. Imipenem discs (10 µg) were placed on a sterile plate and 10 µl of EDTA solution was added to each one of them to obtain a desired concentration of 750 µg. The EDTA impregnated antibiotic discs were dried immediately in an incubator and stored at -4˚c and -20˚c in airtight vials without desicant.[8] The test organisms were inoculated on to Muller Hinton agar plates. Two imipenem (10 µg) discs were placed on the surface of an agar plate; one with the EDTA and other without EDTA. Plates were incubated for 16-18 hours at 37˚c. If zone of inhibition of imipenem-EDTA disc was 7 mm more than that of imipenem disc alone, it was considered as MBL positive.[8]

RESULTS

A total of 9755 samples were analysed during the specified period. Out of 9755 samples, 3101 samples were culture positive. 378 *Pseudomonas aeruginosa* isolates were taken up for the study. Of the 378 positive *Pseudomonas aeruginosa* isolates, 92 (24.33%) were from urine cultures, 54 (14.28%) were from respiratory cultures, 217 (57.40%) were from exudate cultures and 15 (3.96%) were from blood cultures.(Table I, Graph I).

Table I
Distribution of Pseudomonas aeruginosa isolates among various samples

Samples	Inpatients with pseudomonas infection		Outpatients with pseudomonas infection		Total & Percentage
	Male	Female	Male	Female	
Exudate (wound swab, pus)	106	84	17	10	217 57.40%
Urine	39	32	16	5	92 24.33%
Blood	8	7	NIL	NIL	15 3.96%
Respiratory (sputum, tracheal aspirates)	31	16	4	3	54 14.28%

Graph I
Percentage Distribution of Pseudomonas aeruginosa isolates in clinical specimens

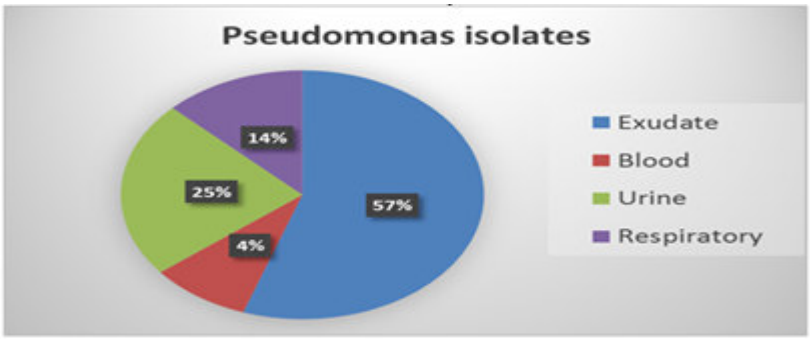
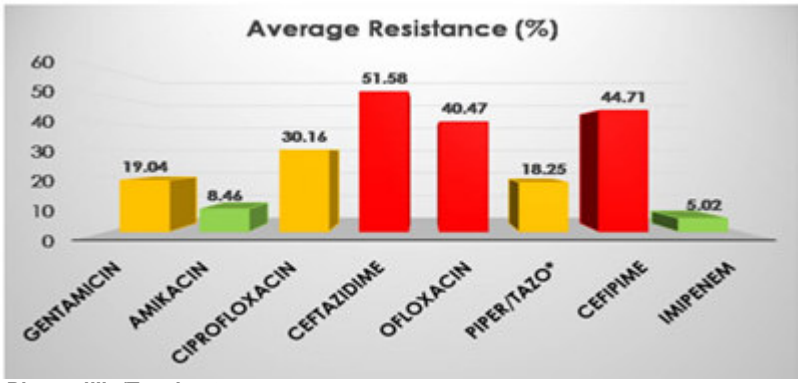


Table II
Percentage of resistant organisms in Inpatient & Outpatient samples

Antibiotics	Exudate		Urine		Blood		Respiratory	
	IP	OP	IP	OP	IP	OP	IP	OP
Gentamicin	**47 (24.7%)**	5 (18.5%)	**15 (21.1%)**	2 (9.5%)	-	-	**2 (4.2%)**	1 (14.2%)
Amikacin	**16 (8.4%)**	3 (11.1%)	**12 (16.9%)**	-	-	-	**1 (2.1%)**	-
Ciprofloxacin	**79 (41.5%)**	9 (33.3%)	**16 (22.5%)**	2 (9.5%)	**1 (6.6%)**	-	**4 (8.5%)**	3 (42.8%)
Ceftazidime	**107 (56.3%)**	14 (51.8%)	**35 (49.2%)**	5 (23.8%)	**15 (100%)**	-	**16 (34%)**	3 (42.8%)
Ofloxacin	**101 (53.1%)**	12 (44.4%)	**28 (39.4%)**	4 (19%)	-	-	**5 (10.6%)**	3 (42.8%)
Piperacillin/ Tazobactam	**43 (22.6%)**	1 (3.7%)	**23 (32.3%)**	-	**1 (6.6%)**	-	-	1 (14.2%)
Cefipime	**93 (48.9%)**	11 (40.7%)	**36 (50.7%)**	3 (14.2%)	**15 (100%)**	-	**10 (21.2%)**	1 (14.2%)
Imipenem	**4 (2.1%)**	1 (3.7%)	**11 (15.4%)**	1 (4.7%)	**1 (6.6%)**	-	**1 (2.1%)**	-

IP - Inpatient, OP - Outpatient

Graph II
Average Resistance percentage of Antibiotics



**** Piperacillin/Tazobactam***

Carbapenem resistance was found in 19 isolates. 12 from urine, 5 from exudates, 1 from respiratory and 1 from blood sample (Table II, Graph II). All 19 carbapenem resistant isolates were subjected to MBL detection test (Ipm – EDTA disc synergy test) (Figure I). Out of 19, 11 (58%) were found to be MBL producers.

Imipenem-EDTA Combined disc synergy test

Figure I
Imipenem-EDTA Combined disc synergy test showing enhancement of zone in IPM with EDTA

Table III
Comparison of resistance profile detected with various studies

	Ciprofloxacin	Ceftazidime	Piperacillin/ Tazobactam	Cefipime
Ansari et al[11]	71.18%	22.03%	16.1%	16.1%
Ravichandra et al[12]	74 to 98%	42 to 100%	27 to 66%	-
Viren et al[9]	69.64%	67.86%	-	69.64%
Chander et al[10]	51.72%	-	55.17% (Piperacillin alone was used)	-
Mohanasoundaram et al[13]	63.1%	57 to 63.3%	-	67 to 72%
Rakesh et al[14]	49%	43%	4%	3% (Cefipime with Tazobactam used)
Our study	30.16%	51.85%	18.25%	44.97%

DISCUSSION

Waging a war against pseudomonas is not an easy job. Its multifactorial drug resistance mechanism and its thriving property in disinfectants poses further threat. The wisest option considering all these factors, it is easier to nip it in the bud. The two major blocks in the transmission would be, to stop plasmid resistance gene transfer and to contain patients with multidrug resistance gene with appropriate therapy. In this study, a total of 378 *pseudomonas aeruginosa* isolates were identified from various clinical specimens from both hospitalized patients and patients treated in the outpatient departments. The prevalence rate of *Pseudomonas aeruginosa* in our study was 12.1% whereas in the other studies, Viren *et al* and Chander *et al* it was 20.2% and 17.05% respectively.[9-10] Majority of the samples, about 57% were from pus and wound swabs which is in line with previous studies of Ansari *et al* (53.8%) whereas Ravichandra *et al* showed a higher percentage of 75.1%.[11-12] Resistance profile of the isolates to a total of eight drugs were tested. The drugs tested were the anti-pseudomonal cephalosporin - Ceftazidime, aminoglycosides - Gentamicin and Amikacin, fluoroquinolones - Ciprofloxacin and Ofloxacin, Beta-lactam(BL) and Beta-lactam inhibitor (BLI) combination - Piperacillin-Tazobactam, Fourth generation cephalosporin - Cefipime and the Carbapenem - Imipenem. The resistance percentage was highest for the drug Ceftazidime (51.85%) which reflects the resistance mechanisms driven by selection pressure. This finding is comparable with the studies of Viren *et al*, Ravichandra *et al* and Mohanasundaram *et al*.[9,12-13] In our study resistance to Cefepime was 44.97% which was low in comparison with about 70% in studies by Viren *et al* and Mohanasundaram *et al*.[9,13] The resistance to fluoroquinolones was 40.97% for Ofloxacin and 30.16% for Ciprofloxacin. All the previous studies showed higher resistance percentage for ciprofloxacin ranging from 50% to 70%. The resistance to Imipenem was the least which was 5.02%. Only Chander *et al* showed 100% sensitivity to Imipenem.[10] All other studies showed higher resistance percentage Ansari *et al* (11.01%), Mohanasundaram *et al* (14 to 27%) and Rakesh *et al* (14%).[11,13-14] This finding reassures the preservation of integrity of high end antibiotics for life-threatening nosocomial infections in the intensive care set up. The resistance to piperacillin-tazobactam was 18.25%. This finding reassures the efficacy of Piperacillin-tazobactam as an anti-pseudomonas drug. In Chander *et al* resistance to piperacillin-tazobactam was as high as 66% in the urine isolates.[10] The resistance to aminoglycosides was considerably low with 19.04% for Gentamicin and 8.46% for Amikacin. They can be used effectively for treatment of *Pseudomonas aeruginosa*. The resistance to Gentamicin was high in previous studies which included Ansari *et al* (52.1%), Mohanasundaram *et al* (around 65%), Ravichandra *et al* (around 77%).[11-13] The resistance profile of the isolates in our study warrants the careful use of ceftazidime, cefipime and the fluoroquinolones - Ciprofloxacin and Ofloxacin. In conditions where these drugs have been used as empirical antibiotics, follow up of the antibiotic susceptibility reports to decide continuation of the

antibiotic or change to a susceptible antibiotic is necessary. In our study, percentage of Metallo-betalactamase producers was found to be 58% of the carbapenem resistant strains. In previous studies done by Rit *et al* and Ranjan *et al*, the percentage was lower which was 41% and 16.5% respectively.[15-16] The study by Rajput *et al* showed higher Metallo betalactamase production of 70%.[17]

CONCLUSION

The carbapenem resistance in our study is comparatively less which reflected the restricted use of the carbapenem group. Ceftazidime, Cefipime and fluoroquinolones should be used judiciously with follow up of susceptibility results. According to our results, targeted therapy with BL – BLI combination like Piperacillin-tazobactam and aminoglycosides helps in decreasing the magnitude of drug resistance. Detection of MBLs producing strains and taking steps to reduce the rate of transfer between different strains are important for treating *P.aeruginosa* infections. Continuous monitoring and antibiotic stewarding will pave the way for infection control.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Paranjothi S, Dheepa R. Screening for Multidrug resistance bacteria Pseudomonas aeruginosa in hospitalized patient in Hosur, Krishnagiri (Dt). Int J Pharm Biol Sci. 2010 Jul-Sep;1(3):1-15.
2. Mesaros N *et al*. *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. Clin Microbiol Infect. 2007; 13:560–78.
3. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa.* J R Soc Med. 2002; 95(41):22–6.
4. Hancock REW *et al*. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. Drug Resistance Updates. 2000;3:247–55.
5. Sacha P *et al*. Metallo-beta-lactamases of *Pseudomonas aeruginosa*--a novel mechanism resistance to beta-lactam antibiotics. Folia Histochem Cytobiol. 2008;46(2):137-42.
6. Mackie, McCartney. Practical Medical Microbiology: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. 14th ed. India: Elsevier; 2006.p.414-8
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
8. Noyal MJC *et al*. Simple screening tests for detection of carbapenemases in clinical isolates of non-fermentative gram negative bacteria. Indian J Med Res. 2009 Jun;129:707-12.
9. Viren AJ *et al*. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian J Pharmacol. 2008 Oct; 40(5):230–4.
10. Chander A, Shahid RM. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates at a tertiary care hospital in Kathmandu, Nepal. Asian J Pharm Clin Res. 2013 Jul;6(3):235-8.
11. Ansari A *et al*. Antibiotic Resistance Pattern in *Pseudomonas aeruginosa* Strains Isolated at Era's Lucknow Medical College and Hospital, Lucknow, India. Int.J.Curr.Microbiol.App.Sci. 2015 Special Issue;1:48-58.
12. Ravichandra Prakash H *et al*. Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* strains isolated from clinical sources. J Pharm Biomed Sci. 2012;14(14):1-4.
13. Mohanasoundaram KM. The Antimicrobial Resistance Pattern in the Clinical Isolates of *Pseudomonas aeruginosa* in a Tertiary Care Hospital; 2008–2010 (A 3 Year Study), J Clin Diag Res 2011 June;5(3):491-4.
14. Rakesh MR *et al*. Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care hospital, Ahmadabad. Nat J Med Res. 2012 June;2(2):156-9.
15. Rit K, Chakraborty B, Dey R, Chakrabarty P, Naha A, Saha R. Prevalence of *Pseudomonas aeruginosa* and Acinetobacter spp producing metallo-β-lactamase in a tertiary care hospital. J Dr NTR Univ Health Sci 2013;2:18-21..
16. Ranjan S *et al*. Comparison of Epidemiological and Antibiotic Susceptibility Pattern of Metallo‐Beta‐Lactamase-Positive and Metallo‐Beta‐Lactamase-Negative Strains of *Pseudomonas aeruginosa.* J Lab Physicicans 2014 Jul-Dec;6(2):109-13.
17. Rajput A *et al*. Prevalence of Metallo-betalactamases (MBL) producing *Pseudomonas aeruginosa* in a Tertiary care Hospital. Ind J Bas App Med Res. 2012 Sep;1(4):304-8.