

ANTIBIOGRAM OF PSEUDOMONAS AERUGINOSA ISOLATES AT KARAIKAL-PUDUCHERRYM. J. Haja Abdul Nazeer¹, S. Khaja Mohiddin², S. Mohan³, Y. Kavitha⁴, D. Kartikeyan⁵**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: INTRODUCTION: Pseudomonas aeruginosa is the most common gram negative bacteria associated with nosocomial infections. In recent years, a considerable increase in the prevalence and multidrug resistance (MDR) P. aeruginosa has been noticed with high morbidity and mortality. The present study was conducted to find out the current antimicrobial susceptibility pattern of P. aeruginosa isolates obtained from clinical samples at our hospital. **MATERIALS AND METHODS:** Pseudomonas aeruginosa isolated from various clinical specimens during March 2013 to February 2014 were included in the study. Isolates were identified by conventional tests and antibiotic susceptibility was determined by disc diffusion method according to CLSI guidelines. **RESULTS:** A total of 159 Pseudomonas aeruginosa isolates were included in this study. Majority of isolates was found in pus followed by urine. Highest susceptibility was shown towards imipenem followed by amikacin and least susceptibility was shown towards cephalosporins. Multi-drug resistance was shown by 21 isolates tested. **CONCLUSION:** P. aeruginosa showed higher rate of resistance towards commonly used antibiotics which may be due to indiscriminate prescription patterns. To prevent the selection and spread of the resistant bacteria, it is critically important to have strict antibiotic policies.

KEYWORDS: Pseudomonas aeruginosa, Antibiotic sensitivity, Imipenem.

INTRODUCTION: Pseudomonas aeruginosa is an aerobic, motile, gram negative rod that belongs to the family, pseudomonadaceae.¹ Pseudomonas aeruginosa is a ubiquitous and versatile human opportunistic pathogen.² Being an opportunistic human pathogen, and it is the leading cause of nosocomial infections, especially among patients who are admitted to intensive care units (ICU).

It has been implicated in diverse nosocomial infections like nosocomial pneumonias, urinary tract infections (UTIs), skin and soft tissue infections, in severe burns and in infections in immune compromised individuals. Of particular concern is the limited number of effective antipseudomonal agents which are used in the therapeutic practice, due to the constitutive low level resistance to several agents and the multiplicity of the mechanisms of resistance in Pseudomonas aeruginosa.³

Much of the antimicrobial resistance problem stems from the misuse of antibiotics, particularly excessive use. One of the main antibiotic resistance containment strategies is therefore to increase appropriate use and to reduce misuse of antibiotics. Infection prevention and control activities to limit the spread of resistant bacteria are crucial.⁴

The resistance rates of P. aeruginosa are known to vary widely in different settings. Active surveillance of trends in antibiotic resistance of P. aeruginosa is necessary for the selection of appropriate antimicrobial agent for empirical therapy. This study gives an account of the antibiotic sensitivity pattern of P. aeruginosa isolated from various clinical samples.

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MATERIALS AND METHODS: This study was conducted on samples received in Microbiology department, VMMC&H, Karaikal over a period of 1 year (March 2013 to February 2014). A total of 159 clinical isolates from various samples such as pus, urine, sputum, blood, ET tubes, ear swabs and other specimens from in and out patients of VMMC&H were analysed. Only one isolate from each patient was considered in the study.

IDENTIFICATION: The isolates were identified by conventional methods. The strains were identified as *P. aeruginosa*, based on the colony morphology, gram staining, oxidase reaction, production of the pyocyanin pigment, nitrate reduction, use of citrate and malonate as carbon sources, and their ability to grow at 5°C and 42°C.⁵

Antibiotic Sensitivity Testing: Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents by Kirby Bauer disk diffusion method on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute (CLSI) guidelines.⁶

The antibiotics which were tested were Amikacin (AK-30µg), Ceftazidime (Ca- 30 µg), Cefotaxime (Ce-30µg), Cefepime (Cpm- 30µg), Ciprofloxacin (Cf- 5µg), Gentamycin (G-10µg), Piperacillin (Pc-100µg), Piperacillin- Tazobactam (Pt- 100/10µg), Imipenem [I- 10µg], Cefeperazone/Sulbactam (Cfs-10/10µg). *P. aeruginosa* ATCC 27853 strain was used as the quality control. All the analysis was performed using simple percentage method.

RESULTS: A total of 159 *P. aeruginosa* strains were isolated from 1825 clinical specimens. The isolation rate of *P. aeruginosa* was 8.71%. Of these 159 strains of *P. aeruginosa*, 96 (60.38%) were from males and 63 (39.62%) were from females. In our study, most of them belonged to the age group of 31-40 years [21.38%] and above 60 years [21.38%] as shown in Table 1.

SL. No.	Age	Male n=96	Female n=63	Total n=159
1	0-10	2 (2.8%)	4 (6.35%)	6 (3.77%)
2	11-20	2 (2.8%)	1 (1.59%)	3 (1.89%)
3	21-30	11(11.46%)	8 (12.70%)	19 (11.95%)
4	31-40	18(18.75%)	16(25.40%)	34 (21.38%)
5	41-50	19(19.79%)	14(22.22%)	33 (20.75%)
6	51-60	22(22.92%)	8 (12.70%)	30 (18.87%)
7	>60	22(22.92%)	12(19.5%)	34 (21.38%)

Table 1: Age and gender wise distribution of Pseudomonas aeruginosa isolates

Maximum clinical isolates of *P. aeruginosa* were obtained from pus samples 46 [28.93%] followed by urine samples 31[19.50%] as shown in Table 2.

SL. No.	Clinical specimen	No. of <i>P. aeruginosa</i> isolated (%)
1	Pus	46(28.93%)
2	Urine	31(19.50%)
3	Blood	22(13.84%)
4	ET	18(11.32%)

Table 2: Isolation of Pseudomonas aeruginosa from different clinical samples

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The results of antimicrobial susceptibility of *P.aeruginosa* isolates to various antibiotics tested in this study are shown in Table 3.

Antibiotics	Sensitivity (%)
Piperacillin	67 (42.14%)
Piperacillin/Tazobactam	117 (73.58%)
Ciprofloxacin	58 (36.48%)
Amikacin	120 (75.47%)
Gentamycin	96 (60.38%)
Imipenem	148 (93.8%)
Ceftazidime	83 (52.20%)
Cefotaxime	42 (26.42%)
Cefepime	33 (20.75%)
cefeperezone/Sulbactam	114 (71.70%)

Table 3: Antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates to various antibiotics

Highest susceptibility was shown towards imipenem [93.08%] followed by amikacin [75.47%] and least susceptibility was shown towards cephalosporins in a range of 20.75%-52.20%. Multi-drug resistance (resistance to ≥ 3 different classes of antibiotics tested) was shown by 21(13.21%) of *P. aeruginosa* isolates tested (Table 4).

P. aeruginosa isolates, n=159	Resistance to no. of classes of antibiotics tested
39 (24.53%)	0
47 (29.56%)	1
52 (32.70%)	2
21 (13.21%)	≥ 3

Table 4: Antimicrobial resistance patterns of *Pseudomonas aeruginosa* clinical isolates

DISCUSSION: *P. aeruginosa* emerged as an important pathogen and responsible for the nosocomial infections that is one of the important causes of morbidity and mortality among hospitalized patients.⁷ Isolation of *P. aeruginosa* was pre-dominated among males. This observation is in agreement with a previous study conducted by Ahmed et al.⁸ In the present study, isolation rate of *P. aeruginosa* was 8.71%. This was higher than that previously reported by Jamshaid et al as 6.67%.⁷ However, the isolation rate observed in this study is lower than that previously observed by K Prabhat Ranjan et al (29.60%).⁸ In our study, *P. aeruginosa* was predominantly isolated from pus and urine. This is in agreement with previous studies conducted by others.^{9, 10}

The unique feature of *P. aeruginosa* is its resistance to a variety of antibiotics, which is attributed to a low permeability of the cell wall, the production of inducible cephalosporinases, an active efflux and a poor affinity for the target (D N A gyrase).¹¹ In various studies which investigated the resistance of *P.aeruginosa* to ciprofloxacin, the proportion was reported to be 0-89%.¹² In the

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present study, the sensitivity to ciprofloxacin was 36.48%. Because of the increasing resistance to fluoroquinolone in many hospitals, its empirical usage is either banned or restricted to bring the developing resistance rates under control.¹² Higher rate of resistance was found towards cephalosporins. These high values of resistance which were observed were comparable to those of the reports from Gujarat done by Javiya et al, with a resistance value of 75%.¹³

Good sensitivity was shown towards Amikacin (75.47%) compared to Gentamycin (60.38%). This is in comparison with the studies conducted by others.^{14, 15} The aminoglycosides inhibit protein synthesis by binding to the 30S subunit of the ribosome and the inactivation of the aminoglycosides occurs through the production of enzymes which transfer acetyl, phosphate or adenylyl groups to the amino acid hydroxyl substituents on the antibiotics.¹⁶

When Piperacillin alone was tested, a lower rate of susceptibility (42.14%) was found in this study, whereas beta-lactam/ beta-lactamase inhibitor combination such as Piperacillin/Tazobactam showed higher susceptibility (73.58%) followed by cefoperazone/sulbactam (71.70%). This indicates beta-lactamase inhibitor markedly expands the spectrum of activity of beta-lactams.¹³ In the present study, Imipenem was the most active antibiotic with 93.8 % susceptibility. This could be due to its restricted use in our hospital.

This observation is in line with recent studies which reported very good sensitivity to carbapenems.^{17,18} However, other study reported a notable resistance among the isolates of *P. aeruginosa* against carbapenems.¹⁹ The rate of the MDR in *P. aeruginosa* (resistant to ≥ 3 classes of antibiotics) is increasing in many parts of the world and it poses a serious therapeutic challenge.²⁰

In our study, the rate of multi-drug resistance was 13.21%. Other studies conducted in Malaysia (19.6%) and in Iran (100%) showed higher rate of multi-drug resistance.^{21, 22} The increase in occurrence of multidrug resistant strains is caused by a continuous selective pressure of regularly used antibiotics. This selective antibiotic pressure leads to development of bacterial resistance by favoring rapid evolution of the bacterial genome.²³

Multiple antibiotic resistances in bacterial populations are a great challenge in the effective management of infections caused by *P. aeruginosa*. This calls for monitoring and optimization of antimicrobial use. Strengthening of laboratory services at local and national levels will ensure effective surveillance of antimicrobial resistance.²⁴ By this, the rapid dissemination of the antibiotic resistance and its mechanism can be prevented.

CONCLUSION: This study concludes that the clinical isolates of *P. aeruginosa* showed higher rate of sensitivity towards imipenem, followed by amikacin and β lactamase inhibitors. Higher rate of resistance was found towards cephalosporins. In this regard, there is a need to formulate antibiotic policies. It is also necessary to establish the role of antibiotic cycling in reduction of antibiotic resistance by selection of susceptible strains.

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