International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

A Cross-Sectional Observational Study on Antimicrobial Resistance Pattern of Pseudomonas Aeruginosa Isolated from Various Clinical Samples at A Tertiary Care Hospital, South Rajasthan

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OPEN ACCESS

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Received: 02-12-2025
Accepted: 26-12-2025
Available online: 31-12-2025

ABSTRACT

Backgrounds: *Pseudomonas aeruginosa* is an opportunistic pathogen known for its intrinsic resistance to multiple antibiotics and its role in healthcare-associated infections. This research study aims to determine antibiotic resistance pattern of *Pseudomonas aeruginosa* obtained from various clinical samples collected from a tertiary care hospital over a period of six months (from January 2025 to June 2025). Infections caused by *Pseudomonas aeruginosa* are difficult to treat, making surveillance of its antimicrobial resistance essential for guiding effective treatment.
Objectives: To determine the antimicrobial resistance pattern of *Pseudomonas aeruginosa* and also to identify the rate of multidrug resistant *Pseudomonas aeruginosa* in our hospital setting.
Material and Method: Specimens were cultured onto nutrient agar, MacConkey's agar and Blood agar plates. Inoculated plates were then incubated aerobically at 37ºC for 16-24 hrs. *Pseudomonas aeruginosa* was identified by colony morphology, production of pyocyanin pigments, Gram stain, motility, positive oxidase, catalase, urease and citrate utilisation tests.
Results: Out of 86 clinical samples, 30 (53%) - highest isolates were from pus sample, 22(26%) from urine, 13(15%) from sputum/throat swab, tracheal aspirate 7(8%), 6 (7%) ear swab, 5 (6%) BAL, 2(2%) blood and 1(1%) vaginal swab. Out of 86, 58 (67%) *Pseudomonas aeruginosa* clinical isolates were from male and 28 (33%) were from female patients. Higher incidence (30%) was found in 41-60 years age group followed by 27% (24/86) from 21-40 years age group. *Pseudomonas aeruginosa* was 100% (86/86) sensitive to Colistin and least 24% (21/86) sensitive to Ceftazidime. Out of 86, 31(36%) *Pseudomonas aeruginosa* clinical isolates were multidrug resistant.
Conclusion: *Pseudomonas aeruginosa* is a significant pathogen in various clinical settings, with a high prevalence in wound and respiratory samples. Colistin is the most effective antibiotic. The emergence of MDR strains is alarming. Regular monitoring of resistance patterns are essential to guide empirical treatment and control hospital-acquired infections.

Keywords: *Pseudomonas aeruginosa, clinical isolates, antibiotic resistance, MDR*

INTRODUCTION: The WHO has listed *Pseudomonas aeruginosa* as one of the critical pathogens in urgent need of new antibiotics [1]. *Pseudomonas aeruginosa* is a gram negative, aerobic, non-fermentative, motile, rod shaped bacterium and leading cause of nosocomial infections. *Pseudomonas aeruginosa* is one the distinctive ubiquitous pathogen. They can survive with minimum levels of nutrients and grow in temperatures ranging from 4 – 42 ºC. It is found in soil and water [2].

Pseudomonas aeruginosa is more virulent due to production of different virulence factors and development of multidrug resistance. Several mechanisms contribute to multidrug resistance pattern. *Pseudomonas aeruginosa* is multidrug resistant due to acquired and intrinsic determinants [3].

Spread of Pseudomonas infections in Hospital settings is more common through contaminated water. Moist places in the hospital settings are main reservoirs of *Pseudomonas aeruginosa.* Moreover, due to high particulate pollution caused by soil dust, infections caused by pseudomonas present in the dust are also high. Persons carry millions of dust particles and hence Pseudomonas present in them easily spread to patients [4].

The persistent failure to develop, manufacture, and distribute effective new antibiotics is further fuelling the impact of antimicrobial resistance (AMR) and threatens our ability to successfully treat bacterial infections. Emergence of drug resistance in bacteria is one of the burning issue in the world. One of the causes of emergence of drug resistance in bacteria, is higher selection pressure by use of higher amount of antibiotic inappropriately in hospital settings. Many researchers in India have documented high prevalence of drug resistance in *Pseudomonas aeruginosa* isolated from different clinical samples ranging from 8.43% to 32.1%. Infection with multidrug resistant strains of *Pseudomonas aeruginosa,* are of great concern for hospitalised patients [5, 6].

Pseudomonas aeruginosa is one of the common members of normal flora of nasopharynx, and is capable of colonizing the respiratory tract. *Pseudomonas aeruginosa* commonly infects immunocompromised patients. *Pseudomonas aeruginosa* can cause severe infections like pneumonia, meningitis, Urinary tract infections, dermatitis, bone and joint infections, gastrointestinal infections, soft tissue infections, and endopthalmitis [7]. *Pseudomonas aeruginosa* is responsible for many systemic infections, especially in patients with severe burns and those suffering from cancer or AIDS [8]. Almost, 10 % of the infections contracted during hospital stay of patients are caused by *Pseudomonas aeruginosa* [9].

Pseudomonas aeruginosa is one of the major organism responsible for drug-resistant nosocomial infections. Day by day, *Pseudomonas aeruginosa* is becoming a common pathogen causing drug resistant hospital acquired infections. Intrinsic resistance of microorganism, instrumentation and inappropriate administration of broad-spectrum antibiotics, contribute to make *Pseudomonas aeruginosa* a major virulent nosocomial pathogen [10].

Keeping this genuineness in mind, this research study was undertaken to study the antibiotic resistance pattern of *Pseudomonas aeruginosa* among the various clinical isolates to guide clinicians for providing empirical treatment and control hospital–acquired infections.

OBJECTIVES: Objectives of this study were to determine the antimicrobial resistance pattern of *Pseudomonas aeruginosa* and to identify the rate of multidrug resistant *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

The present cross sectional observational research study was conducted at the Department of Microbiology in a tertiary care hospital - Ananta Institute of Medical Sciences and Research Centre, Rajsamand, from January 2025 to June 2025 on 86 *Pseudomonas aeruginosa* clinical isolates.

INCLUSION CRITERIA: *Pseudomonas aeruginosa* obtained from OPD and IPD patients, in different clinical samples of all age groups and of both sexes, received in the Microbiology laboratory were included.

EXCLUSION CRITERIA: Clinical specimen's yielding growth of gram negative bacilli other than *Pseudomonas aeruginosa* and all gram positive bacteria were excluded.

Isolation & identification of *Pseudomonas aeruginosa*- Different clinical samples collected as per standard procedures, from OPD and different wards of both sexes with different ages suffering from clinical infection were processed to isolate *Pseudomonas aeruginosa*. Samples were cultured onto nutrient agar [Image 1], MacConkey's agar and Blood agar plates. Plates were then incubated aerobically at 37ºC for overnight. The strains were subjected to different biochemical tests to identity the isolate. *Pseudomonas aeruginosa* was identified by colony morphology, production of pyocyanin pigments, Gram stain, motility, positive catalase, oxidase, urease and citrate utilisation tests [11].

Determination of antibiotic susceptibility -Antibiotic susceptibility pattern was studied on Muller-Hinton agar by standard disc diffusion (Kirby-Bauer) method [12]. Antimicrobial agents used to study were Piperacillin/Tazobactum (100/10ug), Ceftazidime (30ug), Aztreonam (30ug), Imipenem (10ug), Meropenem (10ug), Amikacin (30ug), Gentamicin (10ug), Ciprofloxacin (5ug), and Colistin (10ug) [Image 2]. Standard inoculums size was prepared using turbidity standards (0.5 McFarland=$1.5X10^8$ CFU) as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. *Pseudomonas aeruginosa* ATTC 27853 was used as the control strain. Zone of inhibition of all the antibiotics were measured with scale in reflected light against a black background, to the nearest mm. Interpretation was done according to the Clinical Laboratory Standards Institute guidelines [12].

RESULTS:

Out of 86 *Pseudomonas aeruginosa* clinical isolates , 58 (67%) were from male patients and 28(33%) were female patients (Table 1). Higher rate of *Pseudomonas aeruginosa* (26/86,30%) was found in the age group of 41-60 years (Table 2).

Out of 86 *Pseudomonas aeruginosa* clinical isolates, 30 (35%) were from pus followed by urine 22(26%), Sputum 13 (15%) , tracheal 7(8%), ear swab 6(7%), BAL fluid 5(6%),Blood 2(2%), and vaginal swab 1(1%) as shown in Table 3.Highest sensitivity was observed with Colistin (86/86,100%) and least with Ceftazidime (21/86,24 %) (Table 4). Multidrug resistance found in 31 (36%) *Pseudomonas aeruginosa* clinical isolates (Table 5).

Table 1: Gender wise distribution of clinical isolates of *Pseudomonas aeruginosa* (n= 86)

Gender	Number	Percentage (%)
Male	58	67
Female	28	33
Total	**86**	**100**

Table 2. Age wise distribution of clinical isolates of *Pseudomonas aeruginosa* (n= 86)

Age group (Yrs)	No of isolates	Percentage (%)
0-20	7	8
21-40	24	28
41-60	26	30
61-80	25	29
Above 81	4	5
Total	**86**	**100**

Table 3. Specimen wise distribution of *Pseudomonas aeruginosa* (n=86)

Clinical Specimen	Number	Percentage (%)
Pus/Wound swab	30	35
Urine	22	26
Sputum/Throat swab	13	15
Tracheal Aspirate	7	8
Ear swab	6	7
BAL fluid	5	6
Blood	2	2
Vaginal Swab	1	1
Total	**86**	**100**

Table 4 Antimicrobial Susceptibility pattern of *Pseudomonas aeruginosa* (n=86)

Antimicrobial agent	Sensitive (%)	Resistant (%)
Piperacillin/Tazobactum (100/10ug)	70(81%)	16 (19%
Ceftazidime (30ug)	21(24%)	65 (76%)
Aztreonam(30ug)	39(45%)	47 (55%)
Imipenem(10ug)	72(84%)	14 (16%)
Meropenem(10ug)	69(80%)	17 (20%)
Amikacin(30ug)	63(73%)	23 (27%)
Gentamicin(10ug)	62(72%)	24 (28%)
Ciprofloxacin(5ug)	31(36%)	55 (64%)
Colistin(10ug)	86 (100%)	0.0 (0 %)

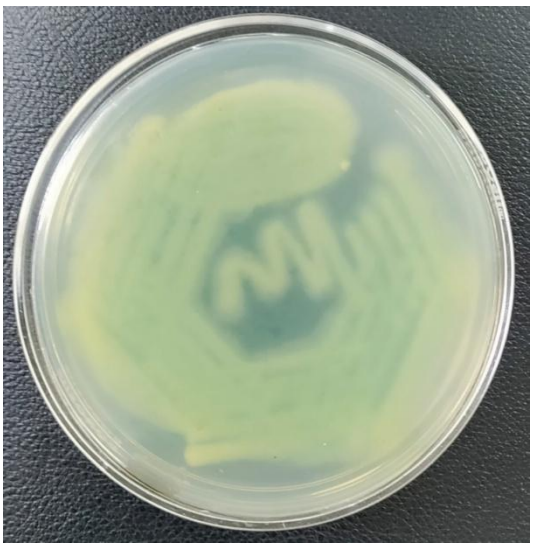

Image 1

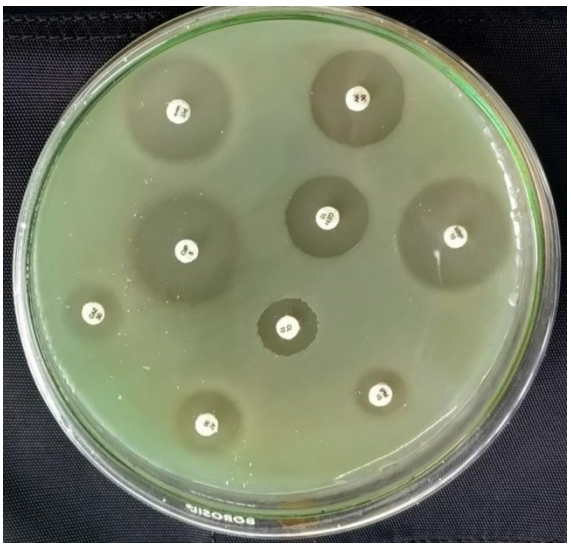

Image 2

Image 1: Growth of *Pseudomonas aeruginosa* on nutrient agar. **Image 2:** Antibiotic susceptibility test of *Pseudomonas aeruginosa*

Table 5: Multi-drug resistant *Pseudomonas aeruginosa* (MDRPA)(n=86)

Total *Pseudomonas aeruginosa* clinical isolates	**Multi-drug resistant *Pseudomonas aeruginosa***	**Percentage (%)**
86	31	36

DISCUSSION:

The *Pseudomonas aeruginosa* is a leading as well as recognised nosocomial pathogen. Even with advances in sanitation establishments and availability of wide range of antibiotic agents with antipseudomonal activities, severe infections caused by *Pseudomonas aeruginosa* continue to be challenge in treatment of nosocomial infections.

In this research study, we aimed to assess the antimicrobial resistance pattern and to identify the rate of multi-drug resistant *Pseudomonas aeruginosa* obtained from various clinical samples.

In this study, the rate of isolation in male was higher (58/86, 67 %) than females (28/86,33%). Equivalently, in other studies, Andhale J.D et al, reported the higher prevalence of *Pseudomonas aeruginosa* in males (76.66 %) than females (23.33%)[13]. The reasons may be hormonal and immune system differences, behavioural and exposure risks. Males may be more frequently exposed to hospital settings and indwelling devices which are risk factors for *Pseudomonas aeruginosa* infections.

In this research study, prevalence of *Pseudomonas aeruginosa* was higher (26/86,30%) in 40-61 years age group. Other researchers have documented similar finding in India [14]. The reason may be the more exposure of this age group. In our study, the most common sample was pus/wound swab (30/86,35%), urine (22/86,26%), and sputum (13/86, 15%). In other studies, equivalent findings are reported by Andhale JD and Pathi et al [13,15].

Pseudomonas aeruginosa is intrinsically resistant to many antimicrobial agents and can acquire resistance genes via mutation, horizontal gene transfer, efflux pumps, and porin channel changes. In our study, *Pseudomonas aeruginosa* clinical isolates were 100% sensitive to Colistin (86/86,100%) followed by Imipenem (72/86,84 %), Piperacillin/Tazobactum (70/86, 81%), Meropenem (69/86,80%), Amikacin (63/86, 73%), Gentamicin (62/86, 72%), Aztreonam (39/86, 45%), ciprofloxacin (31/86,36%), and least sensitive to Ceftazidime (21/86,24%). Our findings are in agreement with other researchers where the pathogen was 90 % sensitive to Imipenem [16]. Colistin is the most effective antibiotic and there is no resistance to Colistin. This is similar to study conducted by Rachana et al [17], who has also reported 100% sensitivity to Colistin. Therefore, Colistin should be used to treat the patient judiciously to avoid development of drug resistance in *Pseudomonas aeruginosa* pathogen.

Multidrug resistance in *Pseudomonas aeruginosa* is a growing concern, especially in hospital-acquired infections. In our research study, 31 out of 86 *Pseudomonas aeruginosa* clinical isolate showed 36% multidrug resistance pattern. The higher MDR pattern indicates urgent need for combination therapy and to implement the antimicrobial stewardship program to avoid further development of drug resistance in this pathogen. In other similar study, researchers have reported higher incidence (60 %) of MDR pattern in this pathogen [14]

CONCLUSION:

Pseudomonas aeruginosa is a significant pathogen in various clinical settings, with a high prevalence in wound and respiratory samples. Colistin is the most effective antibiotic. The emergence of MDR strains is alarming. Regular monitoring of susceptibility patterns is essential to guide empirical treatment and control-hospital-acquired infections.

RECOMMENDATIONS:

To strengthen Antimicrobial Stewardship Programme in various hospitals. Regular antimicrobial surveillance studies and strict infection control policies in healthcare settings should be followed.

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