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Original Research Article

Antibiotic Susceptibility Patterns of Pseudomonas Sp. Isolated From Various Clinical Samples at a Tertiary Care Hospital: A Cross-Sectional Study

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Abstract

Background: There has been a noticeable rise in the prevalence of multidrug-resistant (MDR) Pseudomonas, which is associated with high rates of morbidity and mortality. Antimicrobial resistance is currently a worldwide problem due to the careless use of antibiotics. The purpose of this study was to determine the distribution rate and patterns of antibiotic susceptibility of Pseudomonas species that were isolated from different clinical specimens at MMCH, Madhubani, and Bihar.

Methods: From January 2025 to June 2025, the Department of Microbiology at Madhubani Medical College and Hospital in Madhubani, Bihar, conducted a descriptive cross-sectional investigation to isolate the bacteria and confirm their pattern of antibiotic susceptibility. Twelve hundred and fifty-two samples were taken from patients of all ages and genders, with the exception of those who were already receiving antibiotic medication. These samples included urine, sputum, swabs (pus, wound, and ear), fluid, and tips (catheter, VP shunt). Samples with appropriate labels were collected in a sterile, leak-proof container. According to CLSI recommendations, the modified Kirby-Bauer disc diffusion method was used to test for antimicrobial susceptibility.

Results: 28 clinical isolates of Pseudomonas species were obtained from 1252 samples. Pus, ear, and wound swab samples included the greatest amount of Pseudomonas spp. (46.4%). Pseudomonas species showed greater sensitivity to piperacillin/tazobactam (92.9%) and significant resistance to cefixime (96.4%).

Conclusion: The findings indicated that Pseudomonas is a common cause of infection in pus, wound exudates, and ear discharge samples. Pseudomonas species showed the highest susceptibility to gentamycin, amikacin, piperacillin/tazobactam, and meropenem. Pseudomonas species' persistent resilience. In order to prevent the rapid spread of resistance strains, the majority of antibiotics must be limited to severe infections and hospital intensive care units.

Keywords: Antibiotic resistance, Cefixime, Kirby-Bauer disc diffusion, Multi drug resistance, Pseudomonas.
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Introduction

One of the most complicated bacterial genera, Pseudomonas has over 140 species, the majority of which are saphrophytic. There are about 25 species that are linked to opportunistic infections in humans. P. aeruginosa, P. fluorescens, P. putida, P. stutzeri, P. mallei, P. pseudomallei, P. maltophila, and P. putrefaciens are a few of the significant clinically significant species [1,2]. The most often found bacteria in clinical collections from hospitalized patients is Pseudomonas aeruginosa [3]. It is a common cause of infections in hospitalized patients, especially those with burns, pulmonary conditions, catheterization, and immunocompromised conditions, despite being a commensal of microflora in healthy persons. It is one of the most prevalent gram-negative bacteria that uses its tissue-damaging toxins to infect people when their immune systems are compromised. Pseudomonas aeruginosa can be isolated from a variety of bodily fluids, including blood, urine, wounds, eye or ear swabs, and sputum, because it can infect any anatomical region. Patients with burns and cancer also have serious concerns about it. Urinary tract infections (UTIs), respiratory infections, including ventilator-associated pneumonia (VAP) in patients with disabilities, bone and joint infections, dermatitis, otitis media, bacteremia, and a wide range of other systemic

diseases can all be brought on by it [4,5]. In nature, it is everywhere. It can flourish and spread to any part of the ecosystem, including soil, vegetation, water bodies, sewage, hospitals, and even the moist areas of healthy people's skin. Additionally, it is more complex due to its resistance to antiseptic and antibacterial medicines. Due to both intrinsic and acquired resistance, this organism is difficult to treat [1, 6]. Pseudomonas aeruginosa's defense strategy renders it resistant to numerous antibiotics through a variety of mechanisms, including chromosomally encoded genes, limited outer membrane permeability, the synthesis of enzymes that inactivate antibiotics, and the efflux system that expels drugs from the cell [7, 8]. his study aimed to isolate and characterize different Pseudomonas species from diverse clinical samples of a tertiary care hospital and to analyze its antibiotic susceptibility profile, taking into account the knowledge of Pseudomonas spp.'s ability to thrive in a variety of habitats, its etiology as well as the pathology and its wide range of mechanisms of resistance to antibiotics.

Material and Methods

This descriptive cross-sectional study was undertaken at the Department of Microbiology in Madhubani Medical College and Hospital, Madhubani, Bihar from January 2025 to June 2025 to isolate the bacteria and verify their antibiotic susceptibility pattern. Twelve hundred and fifty-two samples which include urine, sputum, swab (pus, wound and ear), fluid, tips (catheter, VP shunt) were collected from patients of all ages and both genders excluding the patients already on antibiotic therapy. Properly labeled samples were obtained in a clean, sterile, and leak proof container. The antimicrobial susceptibility testing was done using the modified Kirby-Bauer disc diffusion method as per CLSI guidelines.

The samples were inoculated on MacConkey Agar and Blood Agar, and the bacteria were identified by colony morphology, Gram staining, biochemical tests; which included indole test, methyl red test, Voges-Proskauer test, citrate test, triple sugar iron test, oxidase test, and green-blue pigment production test. The antibiotics used were ciprofloxacin ((5 µg), cotrimoxazole (25 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefixime (10 µg), amikacin (30 µg), gentamycin (10 µg), ceftazidime (30 µg), cefoperazone/sulbactum (75/10 µg), meropenem (10 µg), piperacillin/tazobactam (100/10 µg), chloramphenicol (30 µg) (HiMedia, India). Suspension of bacteria maintained to 0.5 McFarland standards was inoculated on Mueller Hinton Agar (HiMedia, India) plates using sterile swabs, and then antibiotic discs were placed on it. The plates were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured and compared with standard strain. The results were interpreted as sensitive, intermediate, resistant according to CLSI guidelines. Pseudomonas (ATCC 27853) was used as standard control strains.

All the results were entered in the worksheet of Statistical Package for Social Science Software (SPSS 25.0) and compared by the chi-square test as percentages with 95 % confidence intervals. A p value of <0.05 was considered to be statistically significant.

Results

Total of 28 (2.2%) Pseudomonas species were identified from 1252 clinical samples. In terms of gender, 12 (42.9%) isolates came from female patients and 16 (57.1%) from male patients. The relationship between the patient's gender and the isolation of Pseudomonas species was not statistically significant. The age group of 16–30 years old had the greatest number of isolates (n=13, 46.4%), while the age group of 46–60 years old had the fewest (n=2, 7.1%). Four (14.3%) of the isolates were older than 60, while five (17.9%) were between the ages of 31 and 45.The correlation between the patient's age and the percentage of Pseudomonas spp. isolations was not statistically significant. (Table 1)

Table 1: Gender and age wise distribution of patients

Gender	**No. of patients**	**Percentage**
Male	16	57.1%
Female	12	42.9%
Total	28	100.0%
Age group (in yrs)	**No. of patients**	**Percentage**
0-15	4	14.3%
16-30	13	46.4%
31-45	5	17.9%
46-60	2	7.1%
>60	4	14.3%

The distribution of Pseudomonas spp. with patient settings was statistically significant, with 18 isolates (1.66%) out of 1083 samples collected from outpatients and 10 isolates (5.91%) out of 169 samples obtained from inpatients (Table 2).

Table 2: Distribution of Pseudomonas spp. in outpatient and inpatient

Patients	No. of cases	Positive Growth		
		Negative (No. %)	Positive (No., %)	p-value
Outpatients	1083	1065 (98.33%)	18 (1.66%)	0.001
Inpatients	169	159 (94.08%)	10 (5.91%)	
Total	1252	1224 (97.77%)	28 (2.23%)	

Pseudomonas spp. isolates from swab samples accounted for 13 (46.4%), urine samples for 8 (28.6%), sputum samples for 4 (14.3%), catheter and VP shunt tips for 2 (7.1%), and fluid samples for 1 (3.6%). Pseudomonas spp. isolates from several samples were statistically significant (Table 3).

Table 3: Distribution of Pseudomonas spp. in different samples

Samples	No. of cases	Positive Growth			
		No.	Percentage	Percentage of Total	p-value
Urine	870	8	28.6%	0.63%	0.002
Sputum	92	4	14.3%	0.31%	
Fluid	92	1	3.6%	0.07%	
Swab	156	13	46.4%	1.03%	
Tips	42	2	7.1%	0.15%	
Total	1252	28	100.0%	2.23%	

Piperacillin/tazobactam 26 (92.9%), amikacin 24 (85.7%), meropenem and gentamycin 19 (67.9%), ciprofloxacin 18 (64.3%), ceftazidime 16 (57.1%), cefotaxime 14 (50.0%), ceftriaxone 7 (25%), and cotrimoxazole 4 (14.3%). As shown in Table 4, Pseudomonas isolates were resistant to cefixime 27 (96.4%), cotrimoxazole 21 (75%), chloramphenicol and cefuperazone/sulbactam 20 (71.46%), ceftriaxone 16 (57.1%), cefotaxime 9 (32.1%), gentamycin 8 (28.6%), ciprofloxacin and ceftazidime 7 (25%), and amikacin 3 (10.7%).

Table 4: Antimicrobial susceptibility pattern of Pseudomonas spp. isolates against first-line and second-line drugs

Class of Antibiotics	Potency (µg)	Susceptibility Patterns of Pseudomonas spp. (N=28)					
		Sensitive		Intermediate		Resistant	
		Total No.	Percentage	Total No.	Percentage	Total No.	Percentage
Fluoroquinolones							
Ciprofloxacin	5	18	64.3%	3	10.7%	7	25%
Sulfonamides							
Cotrimoxazole	25	4	14.3%	3	10.7%	21	75%
Aminoglycosides							
Gentamycin	10	19	67.9%	1	3.6%	8	28.6%
Amikacin	30	24	85.7%	1	3.6%	3	10.7%
Cephalosporins							
Ceftazidime	30	16	57.1%	5	17.9%	7	25%
Ceftriaxone	30	7	25.0%	5	17.9%	16	57.1%
Cefixime	10	0	0	1	3.6%	27	96.4%
Cefotaxime	30	14	50.0%	5	17.9%	9	32.1%
Carbapenem							
Meropenem	10	19	67.9%	0	0	9	32.1%
Combination Drugs							
Cephoperazone-Sulbactam	75/10	6	21.4%	2	7.1%	20	71.4%
Piperacillin-Tazobactam	100/10	26	92.9%	0	0	2	7.1%
Miscellaneous							
Chloramphenicol	30	8	28.6%	0	0	20	71.4%

Discussion

Swab samples had the highest percentage of Pseudomonas spp. identified during the study period (46.4%), followed by urine samples (28.6%) and sputum samples (14.3%). Pseudomonas spp. isolation from several samples was statistically significant ($p < 0.05$). The results are consistent with prior data that indicated a greater prevalence in pus swabs, followed by urine samples, and the highest number of clinical isolates from pus swabs (57.64%), followed by urine samples (24.2%).[9, 10] The results were inconsistent with the study, which found that sputum and tracheostomy samples were the primary source of Pseudomonas spp. (28.57%), followed by pus (24.13%), urine (19.04%), and fluids (15.38%). The patient is susceptible to both community-acquired and hospital-acquired Pseudomonas spp. infections because wound and ear infections occur on exterior body surfaces that are susceptible to contamination with dust, dirt, and contaminated water.[11] Additional factors that may contribute to Pseudomonas spp. infection in India include poor health, illiteracy, malnutrition, underlying disorders, and improper use of medical treatments.[12]

Patients in the 16–30 age group had the largest number of Pseudomonas isolates (46.4%), followed by those in the 31–45 age group (17.9%). Pseudomonas spp. infection did not significantly correlate with patient age ($P>0.05$). The biggest numbers of Pseudomonas isolates were found in various age groups, according to earlier research findings. This age group's participation in various practices, extended hospital stays, or lowered immunity could be the cause.[13]. Pseudomonas isolates were more frequently found in males than in females in this investigation, with a male to female ratio of 1.5:1. Pseudomonas spp. infection did not, however, significantly correlate with gender ($P>0.05$). This outcome is consistent with a prior study [9] that found Pseudomonas infections in 61.78% of males and 38.22% of females. Numerous studies have indicated that males are more likely than females to be infected with Pseudomonas species.[14]

Pseudomonas is still common today, and new resistant forms of the bacteria continue to cause illnesses in both the community and hospitals.[15] Compared to outpatients, inpatients had the greatest number of cases in this study. Pseudomonas spp. infection and outpatient/inpatient status were significantly correlated ($p < 0.05$).

The current study's findings are consistent with those of earlier research [10], which indicated the highest rates of inpatient cases in 2008, 2009, and 2010 (35.86%, 57%, and 40.7%, respectively). Hospital-acquired infections are caused by Pseudomonas spp., which thrive in hospital environments. Additionally, underlying illnesses, weakened immunity, and extended hospital stays put inpatients at danger. The bacterium is especially likely to colonize equipment that needs a moist, body-temperature environment, such respiratory therapy equipment and dialysis tubing.[15] Because of their low cell wall permeability, synthesis of inducible cephalosporinases, active efflux, and poor affinity for the target DNA gyrase, Pseudomonas species are unique in that they are resistant to a wide range of antibiotics.[10] Pseudomonas spp. isolates were more susceptible to the aminoglycosides gentamycin and amikacin in this investigation. These results are consistent with those of Savas et al. [15], who stated that for Pseudomonas spp. At 26%, the resistance rate to amikacin was quite low. These results are comparable to those of Kumar et al. [16] and Tripathi et al. [17], who found lower rates of amikacin resistance (26.66% and 10.79%, respectively). According to other research, amikacin had stronger antipseudomonal effects than gentamycin.[18]

These results are similarly consistent with those of Chand et al. [19], who found that gentamycin sensitivity was higher and that the resistance rate was only 20.68%. The results contradict those of Agbo et al. [20] and Motbainor et al. [21], who found that Pseudomonas had a high rate of gentamycin resistance (73.43% and 54.5%, respectively). It may be because these medications have poor substrate qualities for the enzymes that cause phosphorylation, acetylation, and adenylation. Similar to studies by Anil and Shahid [13] and Ramana and Chaudhury [22], which found 27.59% and 39% of Pseudomonas spp. resistant to ciprofloxacin, respectively, this investigation found only 25% of Pseudomonas spp. The outcome is similarly similar to recent research by Agbo et al. [20], Chand et al. [19], and Kumar et al. [16], which revealed 21.68%, 31.03%, and 35.55% resistance to ciprofloxacin, respectively, indicating that it may be the standard treatment for Pseudomonas spp. infection.

Gram-negative bacteria, particularly P. aeruginosa, have recently shown enhanced resistance to third-generation cephalosporins. Additionally, the current study revealed a high percentage of isolates that were resistant to ceftriaxone (57.1%) and cefixime (96.4%). These high resistance levels are similar to those reported by Chand et al. [19] and Motbainor et al. [21], who demonstrated 100% resistance to ceftriaxone and cefixime. These results are also consistent with research by Anil and Shahid [13], which showed a greater ceftriaxone resistance rate (68.96%). Enzymatic inactivation, biofilm formation, and greater use of beta-lactam antibiotics like amoxicillin are all linked to the rise in cephalosporin resistance.

Additionally, the current study revealed a greater risk of co-trimoxazole resistance (75%). A study conducted in Nepal revealed lower levels of co-trimoxazole resistance (51.72%).[13] Studies by Motbainor et al. [21] and Kumar et al. [16] revealed high resistance to co-trimoxazole (100% and 64.44%, respectively). The pathogen may have altered the metabolic route, avoiding the folic acid production processes where the medication acts. The majority of Pseudomonas isolates were susceptible to meropenem (67.9%) and piperacillin/tazobactam (92.9%). Meropenem (32.1%) and piperacillin/tazobactam (7.1%) resistance were found in a small number of Pseudomonas isolates. These findings are comparable to those of a research conducted by Tripathi et al. [17], which revealed isolates that were 65.68% sensitive and 20.59% resistant to meropenem and 89.22% sensitive and 4.9% resistant to piperacillin/tazobactam. Kumar et al. [16] and Chand et al. [19] showed low rates of piperacillin/tazobactam resistance (11.1% and 19.4%, respectively). Agbo et al. also observed a low rate of meropenem resistance [20]. For future use, these medications must be kept as antipseudomonal agents. Pseudomonas isolates in this investigation exhibited a high level of resistance to both chloramphenicol and cefoperazone/sulbactam (71.4%), which was consistent with a report from India that revealed a 75% resistance rate of Pseudomonas spp. to chloramphenicol.[23] Kumar et al.'s work [16], which revealed only 20% resistance to cefoperazone/sulbactam, is inconsistent with these findings. According to certain statistics, P. aeruginosa antimicrobial resistance to cefoperazone/sulbactam varied from 10.4% to 56.9% in Asia-Pacific regions.[24] This indicates that Pseudomonas' resistance to this medication is growing daily, which could be caused by extended-spectrum and plasmid-mediated transferable enzymes. One of the main causes of the selection and persistence of antimicrobial resistance in bacterial pathogens is the widespread use of antibiotics.

Conclusion

The findings indicate that Pseudomonas is the cause of the infection in pus, wound exudates, ear discharges, and urine samples. Cefixime did not affect any of the Pseudomonas spp. isolates. The isolates of pseudomonas exhibit the highest susceptibility to amikacin, gentamycin, piperacillin/tazobactam, and meropenem. Since Pseudomonas species are progressively developing resistance to carbapenem and other critical antibiotics in many regions of the world, these medications should only be used for severe infections in hospital intensive care units in order to prevent the rapid spread of resistant strains.

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