

Isolation and Antimicrobial Susceptibility of *Pseudomonas aeruginosa* Isolated from Urin Samples

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Abstract

The most common kind of bacterial infections are urinary tract infections (UTIs), which are defined by the growth or presence of harmful microorganisms in any part of the urinary system. The goals of the present investigation were to isolate *Pseudomonas aeruginosa* from urinary tract infections and investigate how various drugs impact the bacteria. The pathology laboratory collected urine samples from patients with UTIs. Nutrient agar slants were maintained, and cetrimide agar plates were utilized for isolation. Each nutrient agar plate received a culture of bacterial broth. Next, sterile forceps were used to aseptically and gently push antibiotic disks containing penicillin, amikacin, ampicillin, ceftazidime, nitrofurantoin, gentamicin, and meropenem onto inoculated nutritional agar plates. Antibiotic-disk testing revealed differences between isolates P1, P2, P3, P4, and P5. While p2, p3, and p4 isolates showed resistance to ampicillin, and p2 and p3 isolates showed resistance to nitrofurantoin, meropenem and gentamicin exhibited comparatively broad inhibitory zones. Zones between 10 and 17 mm were created by ceftazidime. The variations imply that the inhibitory profiles of the isolates are not the same.

Keywords: Antimicrobial Susceptibility, Urinary tract infection, *Pseudomonas*

Introduction

Urinary tract infections (UTIs) are the most prevalent type of bacterial infections and characterized by the presence or multiplication of harmful microorganisms in any region of the urinary system (Mancuso et al., 2023; Medina & Castillo-Pino, 2019). Annually, over 150 million new instances of UTIs are documented worldwide (Sujith et al., 2024). Neonates, adult females, sexually active women, and elderly individuals are some of the groups of people most frequently affected. (Akhtar et al., 2021). In addition, a urinary tract infection affects approximately eight percent of expecting mothers as well as if ignored, may result in adverse health effects (Czajkowski et al., 2018). In general, UTIs have a major adverse effect on quality of life and result in substantial economic and public health costs (Flores-Mireles et al., 2015). They may lead to serious medical conditions such bacterial sepsis, impaired urinary tract function, or permanent kidney damage when not treated properly (File & Tan, 1990). *Escherichia coli* remains among the most prevalent cause of illness, accounting for approximately 90% of cases—mostly caused by Gram-negative bacteria—though other fungi or bacteria can rarely be

responsible (Flores-Mireles et al., 2015). Mild pyelonephritis and cystitis can be caused by gram-positive bacteria such *Staphylococcus saprophyticus* and *Enterococcus faecalis* in addition to other types of Enterobacteriaceae like *Klebsiella pneumoniae* and *Proteus mirabilis* (Flores-Mireles et al., 2015). *Pseudomonas*, *Streptococcus*, and other *Klebsiella* and *Proteus* species have also been implicated as uropathogens; the latter two are frequently linked to stone disease (Flores-Mireles et al., 2015; Li et al., 2022).

In view of this, we have studied the isolation and antimicrobial resistance of *Pseudomonas aeruginosa* from urinary tract infections. The aim of the present study was to isolate *Pseudomonas aeruginosa* from urinary tract infection and to study the effect of different antibiotics against it.

Material and Methods

Study Area:

The present study was carried out in the Department of Microbiology at Adarsha Mahavidyalaya, Dhamangaon Rly, District Amravati, State Maharashtra, India. It is located at latitudes 20.7833° and 78.1393°. It has an estimated area of 291 m.

Collection of Samples, Isolation, and Characterization of Bacteria:

Thirty urine samples from UTI patients were collected from the pathological laboratory. Cetrimide agar plates were used for isolation and maintenance were done on nutrient agar slants. For the isolation of UTI-causing strains, a loopful of urine sample was spread onto cetrimide agar plates with the help of a glass spreader. The plates are incubated at 37 °C for 24 to 48 hours. The next day individual colonies were selected and identified on the basis of morphological, cultural, and biochemical characteristics. The individual colony of each plate was taken and inoculated on nutrient agar slants and incubated at 37°C for 24 hours and labelled as master culture. To check morphological characteristics, Gram staining and biochemical tests such as indole, MR, VP, Citrate test was performed. Stock cultures were maintained on nutrient agar slants and stored at 4°C.

Antimicrobial activity by using the agar disk diffusion method:

Nutrient agar and nutrient broth were prepared as per the labeling of the box. About 15-20 ml of sterilized molten nutrient agar was poured on each petri plate. After pouring the agar medium, all petri plates were kept at room temperature so that the medium can properly solidify. About 5 ml of nutrient broth was added to test tubes and sterilized in an autoclave. After cooling, nutrient broth test tubes were inoculated by *Pseudomonas aeruginosa* isolates with the help of an inoculating needle under aseptic conditions and incubated at 37°C for around 24 hours. A hundred microliter bacterial broth culture was transferred to each nutrient agar plate by using a sterilized micropipette and spread with the help of a sterilized glass spreader. After spreading, allow it to settle for about 5 minutes. Then antibiotics disc such as penicillin, amikacin, ampicillin, ceftazidime, nitrofurantoin, gentamicin, and meropenem were placed on inoculated nutrient agar plates by using sterile forceps aseptically and gently pressed. Then the plates were incubated at 37 °C for 24-48 hours. After incubation the diameter of the zone of inhibition around each antibiotic disc was measured and recorded in mm.

Results

Colony characteristics and preliminary identification:

Pseudomonas aeruginosa was isolated on cetrimide agar plates by the spread plate method, and then the plates were incubated at 37°C for 24-48 hours. After 24 hours the growth was observed on cetrimide agar

plates. On cetrimide agar plates, off-white colored colonies were observed. Out of 30 samples, only 5 samples showed typical *Pseudomonas aeruginosa* like colonies on cetrimide agar plates. The selected colonies were inoculated on a nutrient agar slants for further study. Then for the identification of bacteria, the Gram staining was performed, and the rod-shaped, gram-negative bacteria was observed under a microscope. For the confirmation of bacteria, the IMVIC test was performed. Then the antimicrobial activity against antibiotics such as amikacin, ampicillin, ceftazidime, nitrofurantoin, gentamicin, penicillin, and meropenem was done by the disc diffusion method. For the antimicrobial activity, nutrient agar plates were inoculated with bacterial culture of *Pseudomonas aeruginosa*, and then the antibiotic discs were placed on them. Then the plates were incubated at 37°C for 24-48 hours.

Antimicrobial activity against bacterial isolates:

Zones of inhibition in millimeters for five isolates tested against seven antibiotics are shown in the table. A larger zone generally indicates greater inhibition under the conditions used, while “R” is recorded in the source as resistance.

Table. 1. Antimicrobial susceptibility of bacterial isolates						
Sr. No.	Antibiotic	Isolates and zone of their inhibition (mm)				
		p1	p2	p3	p4	p5
1.	Amikacin	25	18	20	21	23
2.	Ampicillin	19	R	R	R	21
3.	Ceftazidime	10	16	15	17	14
4.	Nitrofurantoin	21	R	R	16	15
5.	Gentamicin	29	22	24	24	27
6.	Penicillin	30	11	10	10	12
7.	Meropenem	33	27	27	28	24

Note: “R” = resistance

For isolate p1, the largest recorded zone was with meropenem (33 mm), followed by penicillin (30 mm) and gentamicin (29 mm). The smallest zone was with ceftazidime (10 mm). Ampicillin and nitrofurantoin had recorded zones of 19 and 21 mm, respectively. For isolate p2, largest recorded zones were with meropenem (27 mm), followed by gentamicin (22 mm). Resistance was recorded to ampicillin and nitrofurantoin. Ceftazidime and penicillin zones were 16 and 11 mm, respectively. For isolate p3, Meropenem produced the largest recorded zone (27 mm), followed by gentamicin (24 mm). Resistance was recorded to ampicillin and nitrofurantoin. Ceftazidime and penicillin zones were 15 and 10 mm, respectively. For isolate p4, Meropenem produced the largest recorded zone (28 mm), followed by gentamicin (24 mm). The table records zones for all antibiotics except none; ampicillin is marked resistant. Nitrofurantoin was recorded at 16 mm and ceftazidime at 17 mm. For isolate p5, the largest recorded zone was with meropenem (24 mm), followed by gentamicin (27 mm) and amikacin (23 mm); note that gentamicin is numerically larger than meropenem in this isolate. Ampicillin was recorded at 21 mm, while nitrofurantoin was 15 mm.

Table 2: Photographs for Zone of Inhibition of *Pseudomonas aeruginosa* against Antibiotics

	Fig. 1: PS-1 zone of inhibition of <i>Pseudomonas aeruginosa</i> against antibiotics
	Fig. 2: PS-2 zone of inhibition of <i>Pseudomonas aeruginosa</i> against antibiotics.
	Fig. 3: PS-3 zone of inhibition of <i>Pseudomonas aeruginosa</i> against antibiotics.
	Fig. 4: PS-4 zone of inhibition of <i>Pseudomonas aeruginosa</i> against antibiotics.
	Fig. 5: PS-5 zone of inhibition of <i>Pseudomonas aeruginosa</i> against antibiotics.

Out of the five isolates, meropenem and gentamicin show comparatively large recorded zones overall. The largest single zone in the table is 33 mm for meropenem against p1. Gentamicin zones range from 22 to 29 mm, and meropenem zones range from 24 to 33 mm. Ampicillin resistance is recorded for three isolates (p2–p4), while nitrofurantoin resistance is recorded for two (p2–p3). Ceftazidime zones are comparatively smaller (10–17 mm).

Discussion

Urinary Tract Infection (UTI) is caused by pathogenic invasion of the urinary tract, which leads to an inflammatory response of the urothelium. Proliferation of bacteria in the urinary tract is the cause of

urinary tract infection. The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organism, the severity of the infection, and the patient's ability to mount an immune response to it (Foxman et al., 2014). Urinary tract infection is one of the most common frequently occurring nosocomial infections. Normally UTIs are caused by a variety of Gram-positive and Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa*, etc. (Ouno et al., 2013).

Antibiotic-disk testing showed variation among isolates p1, p2, p3, p4 and p5. Meropenem and gentamicin produced relatively large inhibition zones, whereas resistance to ampicillin was observed in p2, p3, and p4 isolates and to nitrofurantoin in p2 and p3 isolates. Ceftazidime produced zones between 10 and 17 mm. The differences suggest that the isolates do not have identical inhibition profiles.

Antibiotics usually are the first treatment for urinary tract infections. Your health and the type of bacteria found in your urine determine which medicine is used and how long you need to take it. For treating UTI, certain antibiotics are used, such as amikacin, ampicillin, ceftazidime, nitrofurantoin, gentamicin, penicillin, meropenem, etc. Some antibiotics are resistant to certain bacteria. Antibiotic resistance is a huge problem for UTIs, compared to other infections, because UTIs are so common. So, we see the effects of antibiotic resistance much more immediately.

In the present study we studied the effect of some antibiotics on bacteria that cause UTI. After 24 hours the zones of inhibition were observed around the antibiotic disc, and the zone of inhibition was measured. Some antibiotics show susceptibility, and some show resistivity. Ampicillin and nitrofurantoin show resistance against this bacteria, and the most effective antibiotic was meropenem, as it shows a 33 mm zone of inhibition that shows high susceptibility, and also other antibiotics that were used.

Conclusion

The results indicate that five bacterial isolates produced different antibiotic inhibition profiles. Meropenem and gentamicin showed comparatively large zones in the reported measurements, while resistance to ampicillin and nitrofurantoin was noted in selected isolates. The preliminary colony, Gram-stain and other biochemical characteristics confirms *Pseudomonas* sp. at preliminary level.

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