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Study of Antibiotic Sensitivity of *Pseudomonas aeruginosa* Isolated from Women with Urinary Tract Infection

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

UTIs, or urinary tract infections, are among the most prevalent infections in the world. It is estimated that 7% to 10% of UTIs are caused by *Pseudomonas aeruginosa*. Antibiotic resistance levels in *P. aeruginosa* isolates from UTIs are often greater than in *E. coli* isolates. So, the current study aimed to test the antibiotic sensitivity to *P. aeruginosa* isolated from patients with urinary tract infection. Clinical samples (95) were collected from Kirkuk Hospital in Kirkuk city for the period from May to August 2024 from women who were admitted and hospitalized after consulting the specialist doctor and referring him to the laboratory. The method of collecting samples included the following: Urine samples were taken from women with UTI ranging in age from (5-59 years) of women, after which they were transferred directly to the laboratory to be cultured on the culture media. Of the total samples investigated, 64 (or 67.4%) showed positive findings for *P. aeruginosa* growth when cultured on the blood agar, cetrimide agar, and MacConkey agar. The isolation rate of *P.*

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aeruginosa from women with urinary tract infection was 18.8%. For antibiotic susceptibility test, *P. aeruginosa* showed 81.8%, 93.8% and 94.9% sensitive toward Gentamycin, Imipenem and Amikacin. On the other hand, *P. aeruginosa* was completely sensitive (100%) toward Tobramycin. Its concluded that *P. aeruginosa* is one of the main causes of urinary tract infection in women.

Keywords: Urinary tract infection; Pseudomonas aeruginosa; antibiotic sensitivity; women.

1. INTRODUCTION

One of the most prevalent bacterial diseases that plague people at any point in their lives is urinary tract infection (UTI) (Chang and Shortliffe, 2006, Kucheria et al., 2005). In the US each year, UTIs cause more than 8 million doctor visits, 1.5 million ER visits, and 300,000 hospital hospitalizations (Foxman, 2003, Smith et al., 2006). Moreover, urinary tract infections—which make up 20 to 50% of all monomial infections—are among the most prevalent ailments among hospitalized patients. It is estimated that *P. aeruginosa* causes 7% to 10% of UTIs in hospital settings (Bayyiğit et al., 2023, az-Zarza et al., 2006). A common Gram-negative bacterium that is thought to be the source of healthcare-associated infections is pseudomonas aeruginosa (Klevens et al., 2007). In older patients, *P. aeruginosa* urinary tract infections are linked to increased morbidity and mortality. Antibiotic resistance levels in *P. aeruginosa* isolates from UTIs are often greater than in E. coli isolates (Ironmonger et al., 2005, Newman et al., 2022). One of the most significant bacteria creating challenging clinical issues is *P. aeruginosa* (Estaji et al., 2019). *P. aeruginosa* has resistance against aminoglycosides, quinolones, and β-lactam antibiotics, among other drugs (Hancock and Speert, 2000). The three main types of resistance that *P. aeruginosa* uses to fend off antibiotic attacks are intrinsic, acquired, and adaptive resistance. *P. aeruginosa's* intrinsic resistance is comprised of low outer membrane permeability, the formation of efflux pumps that force antibiotics out of the cell, and the manufacture of enzymes that inactivate antibiotics. Resistance in *P. aeruginosa* can be acquired through horizontal gene transfer or mutational changes (Breidenstein et al., 2011). Because of its capacity to form biofilms and its innate, acquired, and adaptive resistance mechanisms, *P. aeruginosa* poses a serious threat in the clinical setting (Ahmed et al., 2020, Thi et al., 2020). It demonstrates resistance to numerous antibiotics, including as fluoroquinolones, β-lactams, and aminoglycosides, by expressing efflux pumps, producing enzymes that inactivate drugs, and having a low outer membrane permeability (Langendonk et al., 2021). The treatment landscape is further complicated by acquired resistance resulting from mutations and horizontal gene transfer, as well as adaptive resistance demonstrated by the creation of biofilms and the appearance of persister cells (Michaelis and Grohmann, 2023). So, the purpose of the current study was to evaluate Pseudomonas aeruginosa antibiotic sensitivity that was isolated from urinary tract infection patients.

2. MATERIALS AND METHODS

2.1 Specimen Collection

95 clinical samples from women who had been admitted and hospitalized after consulting a specialist physician and having him referred to the laboratory were gathered from Kirkuk Hospital in Kirkuk City between May and August of 2024. The following was a part of the sample collection procedure: Women suffering from UTIs, ages 5 to 59, had their urine samples collected. The samples were then sent straight to the lab to be cultured on culture media.

2.2 Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis and media characteristics: The *P. aeruginosa* colonies growing on blood agar and cetrimide agar were identified based on their culture characteristics, and they were then incubated for 24 hours at 37ºC.

Direct examination: By using a microscope to examine the morphological characteristics of bacterial cells—specifically, how they contacted the gram stain, which indicates the kind of interaction as well as the shape and arrangement of the germ cells—bacterial colonies were found.

Biochemical reaction and motility test: Numerous biochemical tests, such as the H2S

production, methyl red, citrate, urease, voges-proskauer, catalase, oxidase, and indole test, were carried out in order to identify and diagnose bacteria.

Identification of bacteria isolates via VITEK2 : Advanced colorimetric technology is represented by VITEK 2, the next generation of the gold standard in microbial identification. Procedure: All of the following actions were carried out in compliance with the guidelines provided by the manufacturer, Biomerieux.

Antibiotic susceptibility test (AST): The Kirby-Bauer disc diffusion method employing Muller Hinton (MH) agar was used for the AST for all isolates in compliance with the guidelines set out by the Clinical Laboratory Standards Institute (CLSI, 2020) (Collee et al., 1996, Saleh t al., 2019).

3. RESULTS AND DISCUSSION

3.1 Samples Distribution

Table 1 lists the 95 urine samples that were taken from patients who had UTIs for the current investigation. Based on the best cultured media—such as blood agar, Cetrimide agar, and MacConkey agar—64 (67.4%) of the total samples showed good results for bacterial growth. Thirty-one (32.6%) out of the total samples showed negative results for bacterial growth.

According to the current inquiry, 31 patients (32.6%) had no bacterial infection, while 64 patients (67.4%) tested positive for bacterial infections (Table 1). However, in other trials, the UTI percentages were, respectively, 75.42 percent and 61 percent (Jarjees et al., 2006). The absence of growth in urine samples could be attributed to the impact of antibiotics offered to patients throughout their hospital stay and the use of broad range antimicrobials in their treatment. Furthermore, the rate of bacterial isolations was significantly decreased by applying the outer sterilizer solutions (Krigeret al., 1993). Additional causes of anaerobic bacteria, mold, and other bacteria that cannot be separated using the normal procedures utilized in this study and may require specialized techniques for their isolation and development could be revealed by examining the urine samples (Krigeret al., 1993).

3.2 Identification

On blood agar, Cetrimide, and MacConkey agar, the morphology, diameter, and forms of the bacterial isolates were ascertained. Additionally, the results of the biochemical identification were confirmed by means of the System small Vitek-2 equipment and microscopic and biochemical exams, which comprised the particular tests for each kind. The Vitek-2 results were in line with the findings of the biochemical testing. Table (2) shows that the isolation rate of *P. aeruginosa* from women with urinary tract infection was 18.8%, while the isolation rate of other species from women with urinary tract infection was 81.2%.

3.3 *Pseudomonas aeruginosa*

The shape and diameter of *P. aeruginosa* isolates on Cetrimide agars are depicted in Fig. (1) for the primary isolate, pink-red rods under a microscope, and gram-negative bacteria. Gram stain reaction and other microscopic features were used to diagnose bacterial isolates belonging to the genus. Pseudomonas aeruginosa biochemical tests As illustrated in Fig. (2), it tested negative for urease, indole, and Kligler iron k/k, but positive for citrate, catalase, oxidase, and motility.

Table 1. Distributed of study samples according to UTI

	No. (%) +ve culture	No. (%) -ve culture	Total No.(%)
Women	64(67.4%)	31(32.6%)	95 (100.0%)

Table 2. Isolates percentages of gram negative bacteria

Gram negative bacteria	No.	%
P. aeruginosa	12	18.8
Other types	52	81.2
Total	64	100

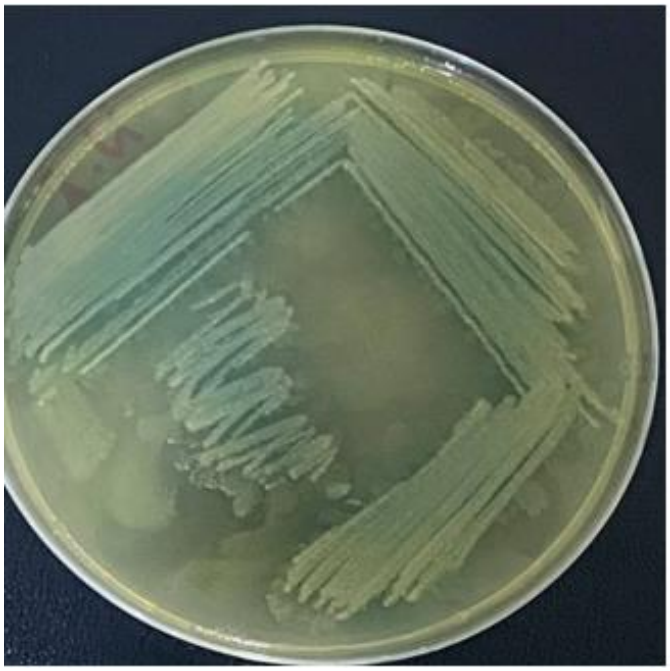

Fig. 1. *P. aeruginosa* colonies on Cetrimide agar

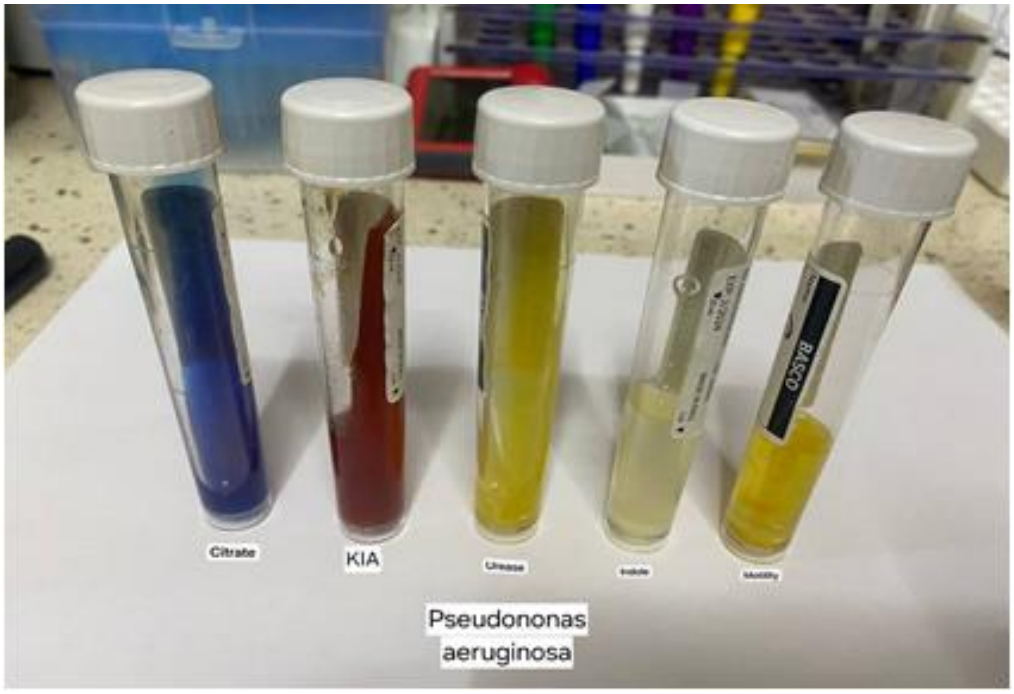

Fig. 2. The biochemical tests for *P. aeruginosa* isolates

3.4 Antibiotic Susceptibility Test

P. aeruginosa showed 81.8%, 93.8% and 94.9% sensitive toward Gentamycin, Imipenem and Amikacin. On the other hand, *P. aeruginosa* was completely sensitive (100%) toward Tobromycin respectively.

Certain European nations have been reported to have the highest rates of resistance to aminoglycosides (AL-Taee et al., 2019). According to the findings, every *P. aeruginosa* sample tested positive for amikacin (90.9%), and all isolates were very sensitive (100%) to imipenem and tobramycin. The study's obtained

results, which are displayed in Table (3), indicate a notable increase in pseudomonal resistance to beta-lactam antibiotics. These findings concurred with research published by (Golshani et al., 2013). Between cefixime, levofloxacin, ciprofloxacin, and vancomycin, the percentage of resistance to other antibiotics was 46.2%. Modifications in the permeability of the outer membrane through changes in porin protein channels constitute a component of many resistance mechanisms. *P. aeruginosa* can acquire resistance to this antibiotic through the outer membrane, which offers an efficient intrinsic barrier to accessing the targets, which are located in the cytoplasm, cell wall, or cytoplasmic membrane (Al-Saffar et al., 2019). The present findings were in contrast to those of Hegazy et al. (2018), who reported that 74.4% of *E. coli* isolates were cefotaxime-resistant. The results of this investigation were less than those of theirs since 38.5% of the bacteria were resistant to ceftazidime. 6.8% of Iranian bacteria were resistant to ceftazidime, compared to 15.5%, 42.2%, and 30% to cefotaxime. The researchers in those investigations hypothesized that the bacteria's resistance may be due to the presence of natural efflux pumps in those organisms (Maleki et al., 2016, Suresh et al., 2016). The current study's findings indicated that there was 30.8% and 23.1% resistance to aztreonam and nalidixic acid, respectively. Primarily, these resistances serve as a significant marker for the existence of ESBLs. Unquestionably, one of the most significant etiological culprits of many serious and potentially fatal nosocomial infections is ESBL-producing Gram negative rods (Kang et al., 2015). In Gram-negative bacilli, the genes producing ESBLs are typically found on sizable, transportable plasmids that are readily able to proliferate (Franiczek et al., 2017). Nalidixic acid is a well-established antibiotic that is still a top choice for treating urinary tract infections (UTTIs) in patients since E. coli isolates show only a moderate level of resistance to it. The results of this study were consistent with other previous studies, including those carried out in Iran by Tajbakhsh et al. (2016) and India by Mittal et al. (20140. After isolating bacteria from patients with urinary tract infections, the results showed that the rate of bacterial resistance to this antibiotic was rather low, reaching 6.6% and 6.25%, respectively.

Table 3. Antibiotic susceptibility test of *P. aeruginosa*

Antibiotics	Sensitive %	Intermediate %	Resistant %	P value
AMP	41.1	20.4	38.5	
VN	53.8	0.0	46.2	0.0001
DA	69.2	0.0	30.8	
TMP	72.3	9.1	18.2	
CAZ	69.2	0.0	30.8	
CTX	61.5	0.0	38.5	
CFM	53.8	0.0	46.2	
CN	81.8	18.2	0.0	
IMI	93.8	0.0	6.2	
NA	76.9	0.0	23.1	
CIP	53.8	3.1	43.1	
LEV	53.8	3.1	43.1	
AZT	61.2	8.3	30.5	
AK	94.9	5.1	0.0	
TOB	100.0	0.0	0.0	

AMP= Ampicillin, VN= Vancomycin, DA=, Clindamycin, TMP=Trimethoprim, CAZ= Ceftazidime, CTX=, Cefotaxime, CPM=Cefepime, CN=Gentamicin, IMI=Imipenem, NA=Nalidixic acid, CIP=Ciprofloxacin, LEV= Levofloxacin, AZT = Azithromycin, AK=Amikacin, TOB = Tobramycin

4. CONCLUSIONS

The results of the current work showed that *P. aeruginosa* is one of the main causes of urinary tract infection in women, and that *P. aeruginosa* has high resistance against many antibiotics, but Tobromycin was the best drug in treating *P. aeruginosa*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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