Pathogenicity Characteristics of *Proteus Mirabilis* in Patients with UTI

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**ABSTRACT**

A total of two hundred, and fifty urine samples are gathered from hospitals of Thi-Qar governorate over a six-month period, these samples were collected from Al- Nasiriyah teaching hospital, Al-Hussein Teaching Hospital, and Suq Al-Shuyoukh general hospital. Twenty-five *Proteus mirabilis* isolates were identified using their morphology, microscopic features, biochemistry, confirmatory API 20 E tests, and VITEK II system. The twenty-five isolates were examined for antibiotic resistance against 18 different antibiotics. It was found that isolates' levels of antibiotic resistance varied. Amoxicillin-clavulanic acid, azithromycin, nitrofurantoin, and ampicillin were all reported to have high resistance among isolates (100 %) while the resistance to activity of trimethoprim, tetracycline, and cefotaxime was 96%. The resistance to nalidixic acid, cefepime, ceftriaxone, ciprofloxacin, ceftazidime, amikacin, cefixime, gentamicin, norfloxacin, imipenem, and meropenem were (88, 88, 84, 76, 72, 68, 68, 56, 52, 8, 16) % respectively.

**Keywords:** *Proteus mirabilis*; UTI; Antibiotic resistance.

**INTRODUCTION**

Proteus spp. It is a member of the Enterobacteriaceae family. Together with the genera Morganella and Providencia, it is classified with the tribe Proteae (O’Hara et al., 2000). *Proteus mirabilis*, a Gram-negative, dimorphic, motile bacterium, has fascinated scientists for decades due to its ability to transform from small rods into lengthy, multinucleated swarmer cells with thousands of flagella (Armbruster & Mobley, 2012).

*P. mirabilis* is common in many habitats, including sewage, soil, and water as well as the gastrointestinal tracts of animals and humans. (Alqurashi et al., 2022). *Proteus mirabilis* in addition to being able to cause urinary tract infections such as cystitis, pyelonephritis, and asymptomatic bacteriuria, *Proteus mirabilis* infections can also result in bacteremia and life-threatening urosepsis. Moreover, *Proteus mirabilis* can result in the production of urinary stones (Zafar et al., 2019). Patients with long-term indwelling catheters or complex UTIs are also susceptible to this opportunistic pathogen’s infection. (Yuan et al., 2021). Expression of virulence factors in this species, including adhesion molecules, urease, proteases, siderophores, and toxins, contributes to its pathogenicity (Danilo de Oliveira et al., 2021). The bacterium *Proteus mirabilis* is the third most common cause of urinary tract infections. It produces urease, which leads to the production of crystalline biofilm and is regarded one of the most significant virulence features of *P. mirabilis* strains, along with their capacity to swarm on solid surfaces (Filipiak et al., 2020) Clinical strains of *P. mirabilis*, like other Enterobacterales, have evolved significantly resistance to antimicrobial medicines over the past few decades (Literacka et al., 2019)

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MATERIALS AND METHODS

Collection of Samples
Between the 29th of August 2022 and the 29th of January 2023, 250 urine samples from both sex and various ages were collected from hospitals and private clinics in the province of Thi-Qar. The samples were obtained from the patients using sterile containers, and they were subsequently conveyed using Cary Blair swabs.

Cultivation of the samples
Swabs were streaked onto MacConkey’s agar medium and blood agar medium. The inoculated medium was incubated aerobically at 37 °C for 24-48 hours, and then examined for bacterial growth.

Identification of the isolates:
A- Morphological characterization: Examination under the microscope revealed that the isolated bacterial cells were gram-negative, red cocco-bacilli of variable length that frequently occurred individually or in short chains (dimorphic) (Armbruster & Mobley, 2012).

B- Cultural characteristics
The Proteus isolates were recognized by their swarming behavior on blood agar and their pale appearance on MacConkey agar due to their inability to ferment lactose sugar. They also have a characteristic fish odor (Hassoon et al., 2021).

C-Biochemical tests
Catalase, Oxidase, Urease production, Triple Sugar Iron Agar, IMVC Test and API 20E (Tille, 2014).

D-VITEK II Compact system
VITEK-2 is an automated colorimetric system for the identification and susceptibility testing of the most clinically significant bacteria (Conejo et al., 2001). This system's mechanism of action measures the kinetics of bacterial growth using fluorescence-based technologies to detect metabolic changes within 6 hours (Ibrahim, 2019).

Antibiotic Susceptibility Test (AST)
It was performed by Kirby-Bauer procedure on Muller Hinton agar. All isolates were tested against 18 antibiotics, and the results were interpreted according to clinical and Laboratory Standards Institute 2022. Ampicillin 25 μg, Amoxicillin –Clavulanic Acid 20 μg, Cefepime 5μg, Gentamicin 10μg, Amikacin 10 μg, Meropenem 10μg, Imipenem 10 μg, Ceftazidime 30 μg, Cefteriaxone 30 μg, Azithromicin 15 μg, Tetracyclin 10 μg, Ciprofloxacim 10 μg, Nalidacic acid 30 μg, Trimethoprim 10 μg, Ceftotaxime 30 μg, Cefoxime 5 μg, Nitrofurantoin 100 μg, Norfloxacin 30 μg (bioanalyse,Turkey). The inoculum was prepared by growing of Proteus mirabilis on dispersed agar plates, then the colonies grow in the plate transferred by loop into a test tube filled with 3 ml of normal saline. The suspensions density was adapted to 0.5 McFarland standards. The plate surface of Muller- Hinton agar (Himedia India) was inoculated with bacteria by a sterile swab. The swab was soaked into the suspension and pressed into the side of the test tube to discard exuberance fluid, then inoculate the Muller-Hinton agar by streaking method. Antibiotic discs were applied to the inoculated agar and, incubated at 37°C overnight. The diameter of zone of growth inhibition observed was measured and compared to the chart of National Committee for Clinical Laboratory Standards (NCCLS)(CLSI, 2021).

Statistical Analysis
The data of the present study were statistically analysis by using SPSS (Statistical Package of Social Science version 26) based in using both descriptive and, non-parametric Chi-Square at p. value < 0.05.
RESULTS

The present study involved a 250 urine sample which collected from patients with UTI infection, among 250 urine sample the study revealed 25 (13.88%) infected with *P. mirabilis* the results also noted a significant difference according to type of infection at p. value < 0.05 as show in figure 1.

![Figure 1](image1.png)

**Figure 1:** Ratio of *P. mirabilis* to other microorganism in patient with UTI.

<table>
<thead>
<tr>
<th>CalX²=160</th>
<th>TabX²=3.84</th>
<th>DF=1</th>
<th>p. value &lt; 0.001</th>
<th>Sig</th>
</tr>
</thead>
</table>

The study noted 17 (68%) infection in male with *P. mirabilis*, while 8 (32%) infected in female with *P. mirabilis*, the results also noted a non-significant difference in availability of *P. mirabilis* according to sex at p. value < 0.05 as show in figure 2.

![Figure 2](image2.png)

**Figure 2:** A comparison of *P. mirabilis* infection in patients with UTI according to sex.

<table>
<thead>
<tr>
<th>CalX²=3.24</th>
<th>TabX²=3.84</th>
<th>DF=1</th>
<th>p. value 0.072</th>
<th>Non-sig</th>
</tr>
</thead>
</table>
Identification of *Proteus mirabilis* was performed in accordance with (Tille, 2014) Based on the Colonial morphology (Shape, swarming, odor on MacConkey) and microscopic investigation of bacterial cell morphology, the shape, cell organization, and kind of Gram-Stain reaction were determined. After staining, various biochemical tests are performed on every isolate.

**Table 1** Biochemical tests of the *P. mirabilis* isolates.

(-) a negative result, (+) a positive result, (V) variable according to strains.

<table>
<thead>
<tr>
<th>No.</th>
<th>Biochemical test</th>
<th><em>p.mirabilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxidase</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Catalase test</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Indole Production</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Urease Production</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>production of H2S</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>lactose Fermentation</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Maltose Fermentation</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Methyl red</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Voges-Proskauer</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Simmons’Citrate</td>
<td>v</td>
</tr>
</tbody>
</table>

The isolated bacteria were tested against eighteen antibiotics with different mechanism of action, and the study showed 75.78% of isolated *P. mirabilis* were resistant for antibiotics, 7.55% of isolated bacteria were get moderate response for action of antibiotics, and 16.67% were susceptible for antibiotics activity, also noted all isolated *P. mirabilis* were resistant for activity of AMC, AZM, NIT, and AM, also noted 96% of isolated were resistant for activity of both TMP and CTX, also the study showed not isolated *P. mirabilis* were sensitive for AMC, AZM, TE, CTX, and NIT as show in figur3 and table 2.
Figure 3: Antibiotics susceptibility of isolated *P. mirabilis*
Table 2: Antibiotics susceptibility of isolated *P. mirabilis*.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>AMC</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CAZ</td>
<td>5</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>CRO</td>
<td>2</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>IPM</td>
<td>20</td>
<td>80</td>
<td>3</td>
</tr>
<tr>
<td>MEM</td>
<td>15</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>AK</td>
<td>1</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>AZM</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TE</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CIP</td>
<td>5</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>FEB</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>NA</td>
<td>3</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>TMP</td>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>CTX</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CFE</td>
<td>5</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>GM</td>
<td>8</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>NIT</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NOR</td>
<td>9</td>
<td>36</td>
<td>3</td>
</tr>
<tr>
<td>AM</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Susceptibility %</td>
<td>16.67</td>
<td>7.55</td>
<td>75.78</td>
</tr>
</tbody>
</table>

Cal$X^2$=213.1, Tab$X^2$=48.96, DF $= 34$, p. value $0.072^{\text{sig}}$
DISCUSSION

The prevalence rate of *P. mirabilis* was (13.88%) that slightly more when compared with findings of (Ram et al., 2019) who reported a prevalence rate of 10.7% in human urine samples and disagree with (Kareem et al., 2017) who reported a prevalence rate of *P. mirabilis* was (19.3%). Variation in the prevalence rate can be attributed to a variety of factors, including geographic region and antibiotic use (Ram et al., 2019) or it may be attributable to variations in sample size and the number of hospitals surveyed (Kamil & Jarjes, 2019).

The present study showed that there were no significant differences between males and females at getting the infection with *P. mirabilis* (P value 0.05). Our results (68% for males) and (32% for females) converged with (Suhartono et al., 2022) who found that the pathogens were more frequently detected in men (70.24%) than women (28.92%), but, disagreed with (Abdelkreem et al., 2018), (Mirzaei et al., 2019) and (Owaied & Jabur, 2022) This is up to that the male patients predominance is on the base of the fact that males more susceptible to infection due to the fact that male exposure is greater as they are representing the majority of workforce, so they are exposed more to acquiring infectious diseases (Bahashwan & Shafey, 2013) and, the fact that the majority of the samples were taken from males with urinary catheters in intensive care wards.

The majority of the *P. mirabilis* strains isolated from our hospitals were resistant to the most of penicillins and cephalosporins that used in this study, which produced difficulties with the treatment of UTIs caused by *Proteus mirabilis*. The increasing prevalence of antibiotic resistance mechanisms, particularly beta lactamase and efflux pumps, makes treating UTIs more difficult (Foxman, 2010).

The β-lactam antibiotics are a class of bactericidal medicines whose chemical structure contains the β-lactam ring. These antibiotics are categorized as Penicillins, cephalosporins, monobactams, carbapenems, and penems (also called thiopenems), this classification is based on the molecular composition of the ring fused to the β-lactam pharmacophore unit, which produces a noncoplanar bicyclic scaffold. (Lima et al., 2020) All isolates show high resistance to penicillin group (ampicillin and amoxicillin-clavulanic acid). Were resistant (100%) to ampicillin that concen with (Al-shibly et al., 2017) and (Algburi et al., 2020) who found the resistant rate to ampicillin is (100%), but disagree with (Owaied, 2022), (Jawad & Al-ramahi, 2017) and, (Gomaa et al., 2019) who found the resistance rate to ampicillin (71.4, 84.05, 91.5) respectively. Our findings showed that the rate of resistance to (amoxicillin-clavulanic acid) was Absolute compatibility with (Al-shibly et al., 2017) and (Algburi et al., 2020) but dissent with (Mirzaei et al., 2022), (Owaied, 2022) and (Sokhn et al., 2020) who found the resistance rate (2.7, 69, 88) respectively. The results show that these antibiotics are being used inappropriately to treat bacterial infections (Algburi et al., 2020) Regarding extended-spectrum cephalosporins, ceftriaxone, cefotaxime, ceftazidime, cefixime, and cefepime substantially higher bacterial resistance (84, 96, 72, 68, 88) respectively was reported in this study. The result for Ceftriaxone was slightly agreed with (Owaied, 2022) from Thi-Qar province, (Algburi et al., 2020) and (Rout et al., 2014) who demonstrated that (78.8%, 90% 90.3%) respectively of isolates were resistant. This study disagree with (Mirzaei et al., 2022), (Sokhn et al., 2020) and, (Kassim Ghaima et al., 2017b) their result was (10, 95.8, 58.8) respectively.

Our result of cefotaxime resistance was 96% this agree with (Algburi et al., 2020) that found the resistance rate to cefotaxime was (90%) but, the results of (FM et al., 2018), (Al-Shibly et al., 2017) and (Owaied, 2022) were (51.1%, 86%, 59.5%) differ with the study results.

The recent study revealed that the resistance to ceftazidime was 72% that differ slightly with (Jawad & Al-ramahi, 2017) who found the resistance rate to ceftazidime was (66.66%). But contradicted with other studies by (Mirzaei et al., 2022), (Gomaa et al., 2019) and, (Kamil & Jarjes, 2019) (11.8, 44.7, 97.8) respectively.

The study also reported a moderate resistance to cefixime (68%) this agree with (Kassim Ghaima et al., 2017) who found the cefixime resistance rate was (65.8%) but, contradicted with (Algburi et al., 2020) with (90%) resistance rate.

The study indicate the finding to cefepime resistance rate was corresponds with other studies such as (Owaied, 2022) and (Kamil & Jarjes, 2019) their results were (83.8%, 91%) respectively. But, contrast to previous investigations by (Rout et al., 2014), (Sokhn et al., 2020), (Gomaa et al., 2019) and (FM et al., 2018) their result were (67.7%, 96%, 72.3%, 53.2) respectively.
The most widely used antibiotics for treating a variety of diseases, including UTIs, are fluoroquinolones (FQs) (Nakano et al., 2019). Regarding the resistance to fluoroquinolones (ciprofloxacin 76%, norfloxacin 52%, nalidixic acid 88%). The resistance of ciprofloxacin disagree with the findings of (Wadee & Najm, 2021), (Kamil & Jarjes, 2021), (Gomaa et al., 2019) and (Hammadi, 2023) their finding results were(100%, 91.4%, 53.2%, 38.9%) respectively and this not comparable with our result.

The study also revealed a high resistance to nalidixic acid (88%) compared to (Jawad & Al-ramahi, 2017), (Hassan, 2018), (Jamel et al., 2020), (Hussein et al., 2020) and, (Alabi et al., 2017) they found the resistance rate of nalidixic acid (75.36%, 30%, 60%, 46%, 53.7%) respectively.

The outcomes of the current study showed a high resistance to norfloxacin (52%) compared to (Mirzaei et al., 2019), (Hussein et al., 2020) and (Jamel et al., 2020) who found the resistance rate to norfloxacin (13.6, 11.1, 8.4). These findings demonstrate the uncontrolled use of these antibiotics to treat bacterial infections (Algburi et al., 2020).

The aminoglycosides are commonly prescribed, broad-spectrum, bactericidal antibiotics for children, primarily for Gram-negative bacterial infections (Germovsek et al., 2016).

The recent study's findings demonstrated amikacin resistance (68%) approach the results of (Sokhn et al., 2020) who found (72%) of P. mirabilis isolates were resistant to Amikacin But contradicted with other studies by (Owaied, 2022), (FM et al., 2018), (Gomaa et al., 2019) and, (Al-shibly et al., 2017) their finding were(50%, 53.2%, 31.9%, 31.25%) respectively. While the resistance of P. mirabilis isolates to gentamicin (56%) this result agree with (Al-shibly et al., 2017) and (Gomaa et al., 2019) who found (54.86%, 53.2%) respectively, of Proteus mirabilis isolates were resistant to gentamicin. But disagree with (Hussein et al., 2020), (Hassan, 2018), (Jamel et al., 2020) and, (Al-Bassam & Al-Kazaz, 2013) their results were (20.6%, 65%, 60%, 50%) respectively.

The resistance to the carbapenems was shown by the resistance to imipenem and meropenem with percentage (8%, 16%) respectively, as a result of the infrequent use of imipenem and meropenem in our country, there is little increase in antibiotic resistance. Our finding imipenem resistance rate was conform with (FM et al., 2018), (Gomaa et al., 2019), (Algammal et al., 2021) and (Mirzaei et al., 2019) who found that (8.5%, 10.6%, 8.6%, 11.8%) respectively, of Proteus mirabilis isolates were resistant to imipenem. Our findings are in opposition to those of (Al-shibly et al., 2017), (Hammadi, 2023) and (Al-Bassam & Al-Kazaz, 2013) who found (18.75%, 27.8%, and 15%) respectively.

Meropenem resistance rate was in agreement with (Ibrahim, 2019) and (Al-shibly et al., 2017) reported that (20% and 15.62%) of P. mirabilis isolates subsequently were resistant to meropenem. These findings differ with some of the previous reports (Gomaa et al., 2019), (Owaied, 2022), (Algammal et al., 2021) and (Jawad & Al-ramahi, 2017) reported that (8.5%, 42.8%, 8.6% and 5.6%) of P. mirabilis isolates, respectively were resistant to meropenem.

In the recent study (100%, 96%) of Proteus mirabilis isolates were resistant to azithromycin and tetracycline respectively, this observation to azithromycin was in accordance with result of other study conducted by (AL-Jeelizy, 2022) who demonstrated that 90% of isolates were resistant to azithromycin but dissent with (Hassan, 2018) who found 18% of P. mirabilis isolates were resistant to azithromycin. The observation to tetracycline was in accordance with results of other studies conducted by (Gomaa et al., 2019), (Jamel et al., 2020), (Kamil & Jarjes, 2021), (Talebi et al., 2023), (FM et al., 2018) their results were(100%) and (Umar et al., 2016) who found (95%) of Proteus mirabilis isolates were resistant to tetracycline but diverge with (Jawad & Al-ramahi, 2017), (Raheem et al., 2017) and (AL-Jeelizy, 2022) their finding were (82.60%, 87.5%, 80%) respectively. Likewise, in this study, result high resistance rate (96%) was reported against trimethoprim, the resistance rate was comparable with the study result with (Raheem et al., 2017) who show 100% of Proteus mirabilis isolates were resistant to trimethoprim while, this result dissent with (AL-Jeelizy, 2022), (Jawad & Al-ramahi, 2017) and (Hassan, 2018) who found (87.5%, 75.63%, 74%) of Proteus mirabilis isolates were resistant to trimethoprim.

The extra outer cytoplasmic membrane of Proteus isolates, which contains a lipid bilayer, lipoproteins, and lipopolysaccharides, may be responsible for their multidrug resistance. Resistance of Proteus to antibiotics was the result of selection for drug resistance, which has been linked to an increase in inappropriate antibiotic use. Iraq's use of antimicrobial agents is inconsistent (Al-Bassam & Al-Kazaz, 2013).

The existence of a single drug with activity against such a wide variety of resistances seems improbable. Responding to the challenges of Gram-negative resistance will require a multifaceted approach, including the use of current antimicrobial agents with caution, improved diagnostics (including the rapid detection of resistance) and, surveillance.
better adherence to basic infection prevention measures, the development of new antibiotics, and research into non-antibiotic treatment and preventive strategies (Zowawi et al., 2015).

REFERENCES


