Antimicrobial Susceptibility of *Proteus mirabilis* Isolated from Urinary Tract Infections in Duhok City, Iraq, Using VITEK2 System

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Abstract

**Background:** *Proteus mirabilis* is a Gram-negative bacterium, it is renowned for its capacity to swarm across surfaces with strength and create an eye-catching bulls-eye pattern. *Proteus mirabilis* belongs to the Enterobacteriaceae family, which is regarded as one of the most common causes of urinary tract infections (UTIs). UTI is a very common type of infection in the urinary system which can involve any part of the urinary system and mostly can be treated with an antibiotic. This study aimed to isolate *Proteus mirabilis*, estimating its incidence in urinary tract infections among symptomatic patients in Duhok City, Iraq, and assessing its antibiotic susceptibility.

**Materials and Methods:** In this study, 250 midstream urine samples were taken from patients at the Private VIN Hospital in Duhok, Iraq over four months (from April to August 2022) using a sterile urine container. Patients ranged from age (4 to 70) and each sample was cultured on MacConkey and blood agar media. The VITEK2 System is used in the detection of the proteus isolates and also their antimicrobial sensitivity.

**Results:** According to the results in this study, 9.6% (24/250) of the samples collected revealed positive results for *Proteus mirabilis*. Also, the results demonstrated that the most active antibiotics against *Proteus mirabilis* were meropenem, ertapenem, and piperacillin/tazobactam, while the most resistant antibiotics were nitrofurantoin and amoxicillin/clavulanic acid. In this study, females were more infected than males.

**Conclusion:** *Proteus mirabilis* one of the important agent of UTIs, their rate was 9.6%, the most active antibiotics against *Proteus mirabilis* were meropenem, ertapenem, and piperacillin/tazobactam.

Introduction

A urinary tract infection (UTI) is a very common type of infection in the urinary system. Any component of the urinary system, including the urethra, ureters, bladder, and kidneys, might be affected by a UTI. Common symptoms include the desire to urinate frequently, pain during urination, and side or lower back pain [1]. Antibiotics can be used to treat the majority of UTIs [2,3]. Even *Escherichia coli* is the most common organism linked to the etiology of UTIs, but other microorganisms, including *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterobacter*, *Citrobacter*, *Staphylococcus aureus*, *Enterococci*, etc., account for the majority of the rest [2]. Gram-negative, aerobic bacteria that do not ferment are increasingly recognized as significant uropathogens. *Proteus mirabilis* and *Pseudomonas aeruginosa* are the most prevalent and well-known organisms among these non-fermenters [1,4].
Proteus mirabilis is a Gram-negative bacterium that is well known for its capacity to vigorously swarm across surfaces in an arresting bulls-eye pattern. Clinically, this organism most frequently affects patients who are receiving long-term catheterization and is a pathogen of the urinary system [3,5]. These Gram-negative are rods bacteria of the Enterobacteriaceae family belong to the Proteus genus [5]. The swarming phenomenon is the key characteristic that sets Proteus apart from other genera in the Enterobacteriaceae family [6]. Proteus mirabilis, Proteus vulgaris, Proteus penneri, Proteus hauseri, and Proteus myxofaciens were the five species that made up the genus [7]. Proteus is widely distributed in the environment, as an opportunistic human pathogen, it is found in the human and animal gastrointestinal tract, skin, and oral mucosa as well as in feces, soil, and plant [8]. Proteus can be isolated from clinical specimens such as urine, wounds, and blood and it’s also found in normal flora in the intestine of healthy humans, but it is considered an opportunistic pathogen that causes many infections when moves from its usual habitat [9]. Proteus mirabilis biofilm formation in the urinary tract can lengthen the infection and slow antibiotic and immune response activity. As a result, the bacteria are able to adhere to uroepithelial surfaces of host cells resulting in aggregation of ammonia in uroepithelial cells that becomes toxic and this directly leads to tissue damage [10]. Proteus mirabilis is the organism that infects a much higher percentage of patients with complicated UTI, it does not only cause cystitis and acute pyelonephritis in infected patients, but it also causes urinary stones, complicating the problems associated with the urinary tract [11], this bacterium is also the main causes of wound infections, urinary tract infections, rheumatoid arthritis, and infant meningitis [12]. The pathogenicity of Proteus mirabilis is caused by many virulence factors that lengthen their stay in human hosts [13,14].

Proteus mirabilis, particularly in the elderly and those with type 2 diabetes, can cause asymptomatic bacteriuria and symptomatic infections of the urinary tract, such as cystitis and pyelonephritis [15].

Proteus is considered to be the third most prevalent bacteria associated with UTIs and UUTI illnesses, behind Escherichia coli and Klebsiella pneumoniae. This bacterium can also cause meningitis or bacteremia, as well as infections of the digestive tract, respiratory system, joints, bones, and skin [12]. The bacteria are harmful because of a variety of virulence factors, including adhesion molecules, flagella, toxins, and enzymes. Proteus mirabilis, which is more common in cases of asymptomatic bacteriuria in the elderly and people with type 2 diabetes, can cause symptomatic urinary tract infections such as cystitis and pyelonephritis [15]. Additionally, bacteremia and potentially fatal urosepsis can develop from these infections. Urinary stones may also occur as a result of Proteus mirabilis infections [16]. This present study aimed to determine the rate of Proteus mirabilis from patients suffering from UTIs in Duhok City, Iraq and to study the antibiotic susceptibility properties of the Proteus isolates by using VITEK2 system.

Methods

Sample Collection

In this study, 250 midstream urine samples were taken from patients at the Private VIN Hospital in Duhok, Iraq over four months (from April to August 2022) using a sterile urine container. Patients ranged from age (4 to 70) years, and from both genders were enrolled in this study. All patients did not receive antibiotics within three days ago, and all of these samples were examined microscopically for UTI by pyuria and the presence of bacteria in the urine.

Identification of Proteus mirabilis Isolates from Urine Sample

Proteus mirabilis was isolated from a urine using common microbiological methods to study colony characters, staining reactions, and biochemical tests. In this study, MacConkey and blood agar media were used for culturing the urine samples and then were incubated at 37 °C overnight. All media were prepared according to company instructions (Difco, USA).

After the incubation period, a smear was prepared from suspected colonies for microscopic examination and stained with gram stain to examine cell shape, grouping, reaction, and non-spore formation under the microscope. Also, the oxidase test was done as a biochemical test for identification, and then the isolates were confirmed as proteus bacteria by using the VITEK2 System (BioMérieux, France).

Antibiotic Sensitivity Test

In this study, the effect of antibiotics against the Proteus mirabilis isolates was tested by using the VITEK2 compact system (BioMérieux, France). According to the method described for VITEK2 Device, by using AST-N card, which contains (Amoxicillin, Amoxicillin/clavulanic acid, Piperacillin/Tazobactam, Cefuroxime, Cefuroxime Axetil, Cefoxitin, Cefixime, Cefazidim, Ceftriaxone, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Fosfomycin, Nitrofurantoin, & Trimethoprim/Sulfamethoxazole) Within 10 hours, the VITEK 2 ID-GN card can identify the proteus.

Result and Discussions

Isolation and Identification of Proteus Isolates

Two hundred fifty (250) midstream urine samples were collected from patients who have symptoms of urinary tract infection. The identification was done depending on the Gram stain, microscopic examination, morphological characteristics, and VITEK2 system. The Proteus bacteria showed pale colonies on MacConkey
agar and non-lactose fermenters. It also produced swarming motility on blood agar after 24 hours of incubation. The suspected isolates are considered to be Proteus. Microscopic examination of the bacterial fixed smear showed a rod-shaped, pink-colored (gram-negative) stain, non-spore-forming, motile bacteria, and oxidase-negative characteristics, which give a good indication that these cells belong to the Proteus species, which was then confirmed by the VITEK 2 system.

Prevalence of Proteus mirabilis
A total of 250 urine samples from patients with urinary tract infection symptoms were screened. It was found that 9.6% (24/250) of the samples collected gave positive for Proteus mirabilis.

The present result indicated that Proteus was found in both sexes, and among different ages. The isolation percentage was (70%), among the females and (30%) among the males, as shown in Table 1. The result of the current study is relatively similar to that observed by Schaffer et al. [5] who found that Proteus isolates represented 10.5% of the UTI cases tested. On the other hand, the present results conflicted with the study [17] which found a higher percentage of a positive culture of urine samples (23%), and the study [18] which reported that the rate of Proteus mirabilis in UTI was about 17.9%, and also at the same time the study disagreed with our in the prevalence of Proteus UTIs in males and females which mention that the rate of infection slightly higher in females than in the males and this opposite to the rate obtained in this study, but even that in most studies done on UTI, Women get UTIs more often because a woman’s urethra (the tube from the bladder to where the urine comes out of the body) is shorter than a man’s, and this makes it easier for bacteria to get into the bladder [14].

The resistance observed toward Tigecycline may be due to the loss of outer membrane porins and LPS modifications by the bacteria [20]. Fosfomycin antibiotic revealed a high activity against the proteus isolates, this antibiotic can inhibit bacterial cell wall synthesis and now is regarded as one of the first-line antibiotics in many countries for uncomplicated UTI in women [21].

Ciprofloxacin is a member of the fluoroquinolone group of antibiotics, which inhibit bacterial DNA and protein synthesis [24], this antibiotic has a sensitivity of 75% to the proteus isolates, while others reported less than this rate (40%) in a study done by [25]. Antibiotics like Trimethoprim/sulfamethoxazole, are combined due to their synergistic effect, they act by inhibiting folic acid synthesis, thereby inhibiting bacterial nucleic acid synthesis.

Table 1: Prevalence of Proteus mirabilis according to the age and sex

<table>
<thead>
<tr>
<th>Patient sex and age group</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (15-75) years</td>
<td>70%</td>
</tr>
<tr>
<td>Male (15-75) years</td>
<td>30%</td>
</tr>
</tbody>
</table>

Antibiotic Susceptibility Test of Proteus mirabilis Isolates
Twenty-four proteus isolates were tested depending on VITEK2 compact system for sensitivity tests toward 18 selected antibiotics as illustrated in Table 2. It was observed that there was a difference in the antibiotic resistance of the isolates, as. Proteus mirabilis showed very high sensitivity (100%) toward some antibiotics which involve Meropenem, Ertapenem, Piperacillin/Tazobactam, and 96 %, and 92% for Amikacin and Imipenem respectively, while sensitivity gradually decreased for, Fosfomycin, Cefoxitin, Gentamicin, Cefixime, Ceftriaxone, Cefuroxime, Ciprofloxacin, and Cefazidime. Less sensitivity was observed toward, Amoxicillin/Clavulanic Acid and Trimethoprim/Sulfamethoxazole (30% and 40%, respectively) with the lowest sensitivity to Tigecycline (4%).

The cephalosporin group is also a member of the beta-lactam family, which showed good sensitivity to Proteus mirabilis, the study demonstrated that the resistance to members of this group ranged from 10% to 35%, which was much lower than those obtained by Al-Jebouri and Al-Taiee (2002), who found that the resistant percentages to the same antibiotic were 80% and 72%, respectively [10].

Piperacillin/Tazobactam showed very high sensitivity (100%), this antibiotic is a beta-lactamase inhibitor, and it can be effective against proteus strains that resist other beta-lactamases. Aminoglycosides inhibit protein synthesis by binding to bacterial 30s ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth [22]. This group includes Amikacin and Gentamicin, Amikacin has a potent broad spectrum antibiotic effect used to treat different types of bacterial infections, in this study, it showed a sensitivity of 96% for the isolates, which was similar to the results obtained by [23], and this percentage was lower for Gentamycin.

Ciprofloxacin is a member of the fluoroquinolone group of antibiotics, which inhibit bacterial DNA and protein synthesis [24], this antibiotic has a sensitivity of 75% to the proteus isolates, while others reported less than this rate (40%) in a study done by [25]. Antibiotics like Trimethoprim/sulfamethoxazole, are combined due to their synergistic effect, they act by inhibiting folic acid synthesis, thereby inhibiting bacterial nucleic acid synthesis.
formation. This study reported a resistance rate of 62.5%, which is close to the rate of 66.7% reported by [26].

*Proteus mirabilis* was resistant to Ampicillin and Amoxicillin/Clavulanic acid at 70%, while the highest resistance was reported toward Nitrofurantoin at 92%, and this agrees with the study done by [17] which reported very high resistance of Proteus isolates toward Nitrofurantoin, and Amoxicillin with rates of (100%) for both of them.

Antibiotics have been used for a long time and also the misuse of antibiotics, makes the drugs less effective and more resistant [27,28]. The more antibiotics are used, the more resistant the bacteria can become, making treating that infection more difficult [29], therefore, updating information about antibiotic resistance is very necessary.

<table>
<thead>
<tr>
<th>The Used Antibiotics</th>
<th>S (%)</th>
<th>R (%)</th>
<th>I (%)</th>
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<tbody>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>8 (33)</td>
<td>16(67)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>24 (100)</td>
<td>0 (0%)</td>
<td>8 (33%)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>18 (75)</td>
<td>6 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>16 (67)</td>
<td>8 (33%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cefixime</td>
<td>19 (79)</td>
<td>5 (21%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>16 (67)</td>
<td>8 (33%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ceftiraxone</td>
<td>18 (75)</td>
<td>6 (25%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>24 (100)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>22(92)</td>
<td>1(4%)</td>
<td>1(4%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>24 (100)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>23(96)</td>
<td>1(4%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>85</td>
<td>15</td>
<td>1(4%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18(75)</td>
<td>4 (17%)</td>
<td>2(8%)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>20 (83)</td>
<td>4 (17%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>23(96)</td>
<td>1(4%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>2(8%)</td>
<td>22(92)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>(37.5%)</td>
<td>15(62.5%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1(4%)</td>
<td>22(92)%</td>
<td>1(4%)</td>
</tr>
</tbody>
</table>

**Table 2: The Rate of Susceptibility Test for 24 Proteus mirabilis Isolates to the Different Types of Antibiotics**

**Conclusions**

*Proteus mirabilis* is an important cause of UTI with a rate of 9.6% and in females more than in males, also in this study, *Proteus mirabilis* isolates showed absolute sensitivity toward Piperacillin/Tazobactam, Ertapenem, Meropenem, while the most resistant antibiotics in the study were Nitrofurantoin.

**Conflict of Interest**

The authors declare that there is no conflict of interest regarding the research data and tools used in this study.

**Acknowledgements**

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**Author Contributions**

All authors conceived this work and drafted and finalized this study

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