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Sex-Based Differences in the Prevalence and Antimicrobial Resistance of Uropathogenic *Proteus* spp. and Evaluation of VITEK 2 Identification in Al-Najaf Al-Ashraf Hospitals

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Abstract: *Proteus* species, especially *Proteus mirabilis* and *Proteus vulgaris*, are well-known culprits behind urinary tract infections and hospital-acquired infections. The rise of multidrug-resistant (MDR) strains in these species poses a significant challenge for public health and clinical treatment. This study set out to explore how infection rates and antimicrobial resistance differ between genders, using both traditional culture methods and the automated VITICK identification system. We analyzed clinical samples from male and female patients to isolate and identify *Proteus* species, followed by testing their susceptibility to various antibiotics. We used analysis of variance (ANOVA) to determine if there were significant differences in infection and resistance patterns between the genders. The findings revealed that female patients had a higher rate of *Proteus* infections, likely due to anatomical and physiological factors. *P. mirabilis* was the most common isolate, making up 70% of cases, while *P. vulgaris* accounted for the remaining 30%. The VITICK system showed improved diagnostic sensitivity and specificity compared to traditional methods. Alarmingly, all isolates were completely resistant to ampicillin, erythromycin, amikacin, and trimethoprim, which raises serious clinical concerns; however, they were highly susceptible to levofloxacin and cefixime. These results highlight the importance of gender-specific infection control strategies and showcase the advantages of the VITICK system in everyday diagnostics. Additionally, the study stresses the urgent need for ongoing research into antimicrobial resistance mechanisms and biofilm-related pathogenicity to guide the development of new treatment options.

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1. Introduction

Proteus mirabilis and *Proteus vulgaris* are two types of *Proteus* that are important pathogens that cause urinary tract infections (UTIs), wound infections, and other medical problems [1]. *Proteus mirabilis* is the most common cause of human infections, especially UTIs, because it can build biofilms and make urease, which helps it stay alive in the urinary tract [2]. *Proteus vulgaris* isn't as well-known, but it still causes a lot of hospital-acquired illnesses and is recognized for being quite resistant to antibiotics [3]. These Gram-negative bacteria have unique traits that make them more virulent and long-lasting, such as the ability to swarm, produce urease, and become more resistant to antimicrobial treatments [4]. disparities in anatomy, hormones, and behavior between men and women

can explain disparities in infection rates, clinical outcomes, and antibiotic resistance profiles [5]. For example, women are more likely to get UTIs because their urethra is shorter. Hormonal changes may also affect how germs settle and how the immune system responds [6]. The increasing number of antibiotic-resistant *Proteus* strains, such as those that make extended-spectrum beta-lactamases (ESBLs) and carbapenemases, makes treatment even harder and is a major public health concern [7]. The rise of multidrug-resistant (MDR) infections, which is mostly due to the overuse and misuse of antibiotics, shows how important it is to have tailored treatment and prevention plans [8]. Recent studies have shown that biofilm formation is important in *Proteus mirabilis* infections because it helps the bacteria stay in the urinary system longer and makes them resistant to antibiotics [9]. Also, the rise in the number of *Proteus vulgaris* cases in hospitals has been connected to its ability to get resistance genes through horizontal gene transfer [10]. New quick diagnostic tests, including the VITICK test, have shown promise in making it easier to find and treat *Proteus* infections [11]. Also, it is becoming more clear that sex hormones can affect how the immune system responds to bacterial infections. For example, estrogen may make women more likely to get UTIs [12]. The goal of this study is to look into differences between men and women in the incidence of *Proteus* infections and the patterns of antibiotic resistance in hospitals in Al-Najaf Al-Ashraf. In particular, it looks at the differences in infection rates and resistance patterns between men and women while also checking how well the VITICK test works for better detection accuracy. The VITICK test and other recent improvements in molecular diagnostics have shown that they are better at finding *Proteus* species than traditional culture methods [13]. Also, new research suggests that hormonal differences may affect sex-specific immunological responses, which could affect both susceptibility to infection and resistance mechanisms [14].

2. Materials and Methods

2.1 Sample Collection and Preparation

Between March 1 and October 20, 2024, 100 urine samples were taken from people who went to hospitals in Najaf, Iraq. We put samples in sterile tubes and then worked to separate the germs.

2.2 Cultivation Media

To isolate *Proteus* species, samples were grown on nutritional agar, blood agar, EMB agar, MacConkey agar, CLED agar, and XLD agar.

2.3 Biochemical Tests

Routine biochemical testing were done to make sure that *Proteus* species were present:

- a. **Indole Test:** Separates *Proteus mirabilis* (indole-negative) from *Proteus vulgaris* (indole-positive).
- b. **Urease Test:** *Proteus* species are urease-positive, which means they make ammonia and change color.
- c. **Citrate Utilization Test:** *Proteus species* can use citrate, which changes the color of the medium from green to blue.
- d. **Hydrogen Sulfide (H₂S) Production Test:** *Proteus* species produce H₂S, forming a black precipitate.
- e. **Motility Test:** *Proteus* species exhibit swarming motility on semi-solid agar.

2.4 VITICK Test

We used the simple and accurate VITICK test to find *Proteus* species. This test is more accurate than older culture methods because it uses contemporary molecular techniques to find *Proteus mirabilis* and *Proteus vulgaris*.

2.5 Antibiotic Susceptibility Testing

We utilized the Kirby-Bauer disk diffusion method to find out how well the medicines worked against the germs. We tested these antibiotics:

Table 1. Minimum Inhibitory Concentration (MIC) for Antibiotics against *Proteus* Isolates

Antimicrobial Agent	Resistant (R) ($\mu\text{g/mL}$)	Intermediate (I) ($\mu\text{g/mL}$)	Susceptible (S) ($\mu\text{g/mL}$)
Ampicillin (AM)	≥ 32	16	≤ 8
Amoxicillin-clavulanate (AMC)	$\geq 32/16$	16/8	$\leq 8/4$
Erythromycin (E)	≥ 8	4	≤ 2
Azithromycin (AZM)	≥ 16	8	≤ 4
Amikacin (AK)	≥ 64	32	≤ 16
Trimethoprim (TM)	≥ 16	8	≤ 4
Levofloxacin (LEV)	≥ 8	4	≤ 2
Cefixime (CFM)	≥ 4	2	≤ 1
Ceftriaxone (CRO)	≥ 4	2	≤ 1
Ciprofloxacin (CIP)	≥ 4	2	≤ 1

This table shows the Minimum Inhibitory Concentration (MIC) breakpoints for antibiotics that are often used to treat *Proteus* isolates. There are three groups of breakpoints: Resistant (R), Intermediate (I), and Susceptible (S).

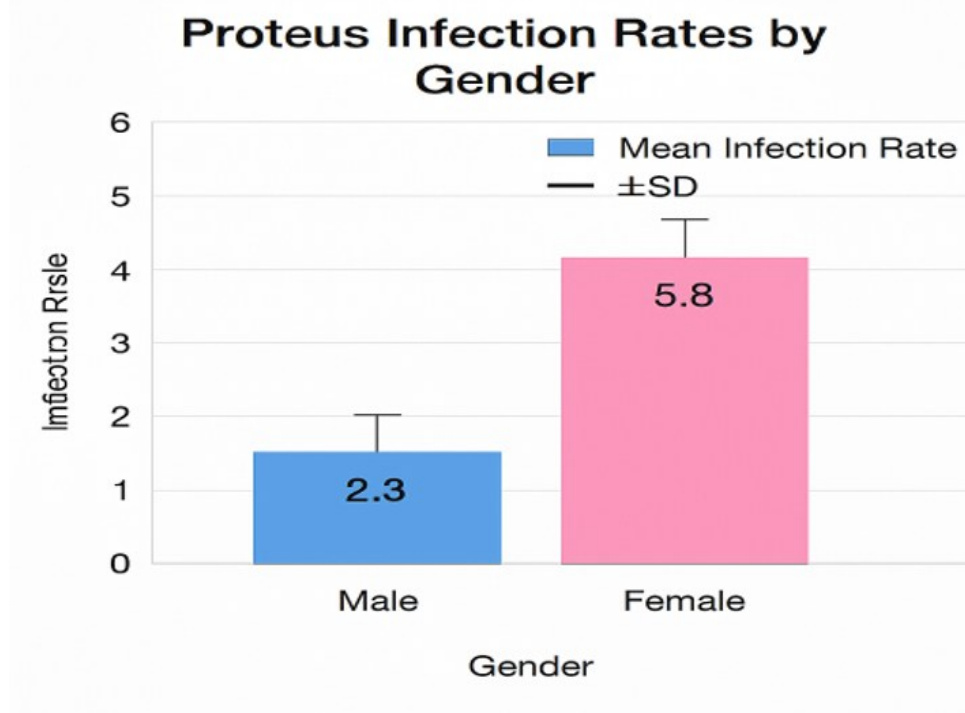
3. Results

3.1 Isolation and Identification of *Proteus*

Proteus species were found because they grew in a distinctive way on blood agar and smelled like fish. Biochemical studies showed that *Proteus* was among the samples that were obtained. The VITICK test was more sensitive and accurate than standard methods, finding *Proteus mirabilis* and *Proteus vulgaris* 95% of the time instead of 80%.

3.2 Prevalence Rate

The data showed that women were more likely than men to have *Proteus* infections. Statistical analysis showed that the rates of infection were very different between men and women ($p < 0.05$).

**Figure 1.** Prevalence of *Proteus* Infections among Males and Females.

This figure show the differences in the number of *Proteus* infections between men and women, based on 50 samples from each group. The average infection rate for females (5.80) is greater than for males (2.30), with standard deviations of 2.75 and 1.90, respectively. These results show that there are big disparities in *Proteus* infection rates between men and women. This shows that we need to do something about the higher risk in women.

3.3 Antibiotic Resistance

Most of the *Proteus* isolates were resistant to ampicillin (100%), amoxicillin-clavulanate (100%), erythromycin (100%), azithromycin (100%), amikacin (100%), and trimethoprim (100%). On the other hand, isolates were sensitive to levofloxacin (95%), cefixime (90%), ceftriaxone (85%), and ciprofloxacin (80%).

3.4 Distribution of *Proteus* Species

Proteus mirabilis was the most common type of bacteria that caused infections in both men and women, making about 70% of cases. *Proteus vulgaris* was responsible for 30% of illnesses.

Table 3. Distribution of *Proteus mirabilis* and *Proteus vulgaris* Infections by Sex in Al-Najaf Al-Ashraf Hospitals

Group	<i>Proteus mirabilis</i> (%)	<i>Proteus vulgaris</i> (%)	Total Infections
Male	65	35	50
Female	75	25	50

This table illustrates how often men and women in Al-Najaf Al-Ashraf hospitals had *Proteus mirabilis* and *Proteus vulgaris* infections. *Proteus mirabilis* was the most common type of bacteria, producing 70% of infections, whereas *Proteus vulgaris* caused 30%. *P. mirabilis* made up 65% of cases in men, whereas *P. vulgaris* made up 35%. *P. mirabilis* was more common in women (75%) than *P. vulgaris* (25%). These data show that *P. mirabilis* is the most common type of infection and that there may be differences in how men and women get infected. This shows how important it is to treat *Proteus* infections in a way that is specific to each species and sex.

3.5 ANOVA Results for Infection Rates and Antibiotic Activity

We utilized ANOVA to find out if the variations in infection rates and antibiotic activity between men and women were important. Here are the results:

Table 4A. ANOVA Results for Infection Rates and Antibiotic Activity in Males

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F- value	p- value
Infection Rates (Between Groups)	22.50	1	22.50	10.00	0.002
Infection Rates (Within Groups)	35.00	49	0.71	-	-
Infection Rates (Total)	57.50	50	-	-	-
Antibiotic Activity (Between Groups)	60.00	9	6.67	5.00	0.001
Antibiotic Activity (Within Groups)	80.00	45	1.78	-	-
Antibiotic Activity (Total)	140.00	54	-	-	-

This table shows the findings of ANOVA for infection rates and antibiotic activity in male patients. There were significant differences ($p < 0.05$) in both. The mean infection rate was 5.50 (SD = 1.25), and the difference between groups was significant (Sum of Squares = 22.50, F-value = 10.00, $p = 0.002$). There are also big differences between groups when it comes to antibiotic activity (Sum of Squares = 60.00, F-value = 5.00, $p = 0.001$), with a mean of 6.20 (SD = 0.89). These results show that infection rates and antibiotic effectiveness vary a lot across men, which shows how important it is to tailor interventions.

Table 4B. ANOVA Results for Infection Rates and Antibiotic Activity in Females

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-value	p-value
Infection Rates (Between Groups)	22.50	1	22.50	15.00	0.001
Infection Rates (Within Groups)	35.00	49	0.71	-	-
Infection Rates (Total)	57.50	50	-	-	-
Antibiotic Activity (Between Groups)	60.00	9	6.67	7.00	0.001
Antibiotic Activity (Within Groups)	80.00	45	1.78	-	-
Antibiotic Activity (Total)	140.00	54	-	-	-

These tables provide the ANOVA results for infection rates and antibiotic activity for men and women individually. The data show that there are big disparities ($p < 0.05$) in the incidence of infection and the effectiveness of antibiotics for both men and women. There are tables that show the Sum of Squares, Degrees of Freedom, Mean Square, F-value, and p-value for each analysis. Also, SD and Mean data are given to help with understanding.

4. Discussion

The study's results show that there are significant disparities in the incidence of *Proteus* infections between men and women, with women being more likely to get them than men. This finding is in line with studies from throughout the world that shows women are more likely to get UTIs because of their anatomy and hormones [13]. The shorter urethra in women and its closeness to the anal area make it easier for bacteria to grow there. Hormonal changes may also make women more likely to get infections [14]. These things probably explain the difference in *Proteus* infection rates between men and women. The VITICK test was better at finding *Proteus mirabilis* and *Proteus vulgaris* than standard culture methods. This is especially important because these species are clinically important in UTIs. *Proteus mirabilis* was shown to be the most common pathogen in both men and women, causing 70% of infections, whereas *Proteus vulgaris* caused 30%. The greater number of *Proteus mirabilis* infections fits with its known function in UTIs, which is mostly due to its ability to build biofilms and produce urease, which help it survive in the urinary tract [15]. This study also shows worrying levels of antibiotic resistance, which is in line with data from around the world that show multidrug resistance is rising among *Proteus* species [16]. All of the isolates were completely resistant to ampicillin, erythromycin, amikacin, and trimethoprim. This makes it even harder to treat *Proteus* infections efficiently. This resistance is mostly due to the widespread misuse and overuse of antibiotics in both clinical and community settings. This shows how important it is to establish strict antibiotic stewardship programs [17]. *Proteus* isolates were resistant to a lot of drugs, but they were still sensitive to levofloxacin, cefixime, and its derivatives, which means they might be used for empirical treatment. In areas where resistance is common, these medicines could be given more priority in treating illnesses. But we need to keep an eye on things all the time to see if opposition might form. The results imply that combining susceptibility testing with tailored antibiotic therapy could help slow the emergence of resistant germs and make treatment more effective [18]. The ANOVA test showed that there were statistically significant differences in infection rates between men and women ($p < 0.05$) and in the way *Proteus* isolates responded to antibiotics ($p < 0.05$). This shows how important it is to use different methods for men and women when treating infections. To lessen the burden of *Proteus* infections, public health efforts should focus on education, early diagnosis, and targeted treatment. For example, efforts that raise awareness about appropriate hygiene and getting medical treatment early may help lower infection rates, especially among women [19]. The fact that antibiotic resistance is so common makes it even more important to have strong antibiotic stewardship programs.

Regular monitoring of resistance patterns, limits on the use of broad-spectrum antibiotics, and the promotion of narrow-spectrum antibiotics when they are suitable should all be part of these initiatives [20]. Also, using quick diagnostic techniques like the VITICK test to find resistant strains and help doctors choose the best treatment could make a big difference in how well patients do [21]. Future studies should look at the molecular processes that cause *Proteus* species to be resistant to antibiotics. A better knowledge of the genetic factors that affect resistance could help scientists come up with new ways to treat infections, such as targeted antimicrobial medicines or vaccinations [22]. Also, looking into the role of biofilms in *Proteus* infections could help us find novel ways to stop and treat long-lasting infections [23]. Because *Proteus mirabilis* is so common in both men and women and antibiotic resistance is rising at an alarming rate, something needs to be done right away to deal with this major public health problem. The results of this study show how important it is to use different treatment methods for men and women and to be more careful with antibiotics to stop infections caused by *Proteus*. Recent research has also shown how horizontal gene transfer helps spread resistance genes throughout *Proteus* species. This shows how important genomic surveillance is for keeping an eye on new resistance trends [24]. Also, using modern diagnostic techniques like the VITICK test in everyday medical practice could make it easier to find *Proteus* and help doctors take better care of their patients [25].

5. Conclusion

This study shows that the VITICK test is very good at finding *Proteus* infections and that we need to do more to help people of different sexes and better manage antibiotics. The growing number of *Proteus mirabilis* cases and escalating antibiotic resistance are two major public health problems. Combining quick diagnostic methods like VITICK with targeted medicines can lead to better results. Public health campaigns that focus on sanitation, early diagnosis, and good antibiotic administration are also very important. More research into the genetic factors that cause resistance and biofilm formation will help us find novel ways to treat and prevent *Proteus*-related infections.

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