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DIAGNOSIS AND PATHOGENESIS OF THE PROTEUS SPP. INFECTION LIKELIHOOD IN URINE SAMPLES AND DESCRIPTIVE STUDY COLLECTED FROM PATIENTS IN WASIT PROVINCE-IRAQ

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Abstract:

Background: Proteus spp. is one of the most important agents of urinary tract infection (UTI). It is well known in clinical and microbiology survey courses as the species that swarms across agar surfaces, overtaking any other species present in the process. Urease production and robust swarming motility are the two hallmarks of this organism. This species can be identified as a Gram-negative rod that is motile, urease-positive, lactose-negative, indole-negative, and produces hydrogen sulfide.

Aim: As there are limited data about the pathogenicity Proteus isolated from Iraq, we investigated the virulence characteristics and antibiotic resistance in the isolates. Finally, a community-based descriptive study was carried out during the same period of sample collection.

Results: From isolates Proteus P. mirabilis represents (91.7%) of specimens. Which supports the idea that P. mirabilis is more commonly encountered in clinical infections. According to the age and gender, out of 218 Proteus spp., 115 (52.7%) were Females and 84 (38.5%) were above 40 years. Regarding Antibiotic resistance, interesting fiending was that Cotrimoxazole has reached the peak level of antibiotic resistance.

Keywords: P. mirabilis, Antibiotic resistance, Cotrimoxazole, Descriptive study.

Introduction

Proteus spp. is a Gram-negative, rod-shaped bacterium that belongs to the Enterobacteriaceae family. It is known for its ability to cause urinary tract infections (UTIs) in humans, particularly in patients with urinary tract abnormalities or catheterization [1].

The bacterium possesses several virulence factors that contribute to its pathogenicity. One key factor is its ability to swarm, which refers to the movement of bacteria in a coordinated manner

across solid surfaces, including urinary catheters and the urinary tract epithelium. This swarming behavior allows Proteus to colonize and persist in the urinary tract, leading to infection **[2, 3].**

Proteus is also capable of producing urease, an enzyme that hydrolyzes urea into ammonia and carbon dioxide. The increase in ammonia levels raises the pH of the urine, creating an alkaline environment that promotes the formation of urinary stones. These stones can serve as a nidus for bacterial colonization and contribute to recurrent UTIs **[4, 5].**

In terms of diagnosis, Proteus can be identified through urine culture and subsequent identification of characteristic colony morphology and biochemical tests. Specific tests include positive urease and phenylalanine deaminase tests. Additionally, molecular techniques, such as polymerase chain reaction, can be employed for specific detection and confirmation of the bacterium **[6, 7].**

Understanding the pathogenicity and diagnostic methods for Proteus infection is crucial for appropriate management and treatment of UTIs caused by this bacterium. Antibiotic susceptibility testing should also be performed to guide the selection of effective antibiotics for targeted therapy **[8].**

The genus Proteus currently consists of five named species, but P. vulgaris, P. mirabilis and P. penneri are opportunistic human pathogens **[9].**

Extended-spectrum β-lactamases (ESBLs), as one of the major resistance mechanisms among gram-negative bacteria, have been associated with resistance to different classes of antibiotics **[10].** P. mirabilis possesses a host of potential virulence factors that may aid its pathogenesis, including hydrolysis of urea by urease, cell invasiveness and colonization due to swarming, cytotoxicity by hemolysins **[5, 11].**

To understand the pathogenic role of Proteus, we selectively isolated strains of this bacterium from urine samples and investigated their properties at the biochemical and microbiologic levels.

Material and Methods

Community-descriptive study

A descriptive study was conducted between November 2022 and January 2023, targeting UTI patients at care centers and academic hospitals in Al-kut region of Iraq. A total of 287patients participated in the survey. The participants were provided with a self-reported questionnaire in a paper-based format. The questionnaire consisted of inquiries related to their response, infection symptoms, and clinical aspects concerning Proteus spp. Assessment of the study variables among the study participants using a pre-tested self-administered questionnaire.

The collected data were analyzed using SPSS version 25 software (IBM Inc., Chicago, Illinois, USA), aligning with the study's objectives.

Human Samples Collection and Identification

This study was performed in Microbiology Department, Collage of medicine, Wasit university, Iraq. Ethical approval for this research was confirmed by the scientific and ethics committee (add your number). A total of 218 urine samples were collected from patients with UTI symptoms in several hospitals in Wasit, Iraq between November 2022 to January 2023. The

samples were immediately stored at –80^{C} in glycerol (25% v/v). Identification of the samples was done by a series of microbiological and biochemical tests that routinely are used.

Urine cultures

Proteus samples involve the process of isolating and identifying the bacterium. A small portion of the urine sample is streaked onto a culture plate containing a specific growth medium, blood agar or MacConkey agar. The streaking technique helps to obtain isolated colonies of bacteria. The culture plate is then incubated at the appropriate temperature (37°C) for a specific duration, 18 to 24 hrs. This allows the bacteria to grow and form visible colonies.

Colony identification

After incubation, the colonies on the culture plate are examined for their appearance, morphology, and characteristics. Proteus colonies typically exhibit swarming motility, which is a characteristic feature of this bacterium. The colonies may appear large, grayish, and exhibit a "swarming" pattern, spreading across the agar surface.

Biochemical tests

To confirm the identification of Proteus mirabilis, various biochemical tests are performed using API 20E kit (BioMerieux, Inc.). Thes tests include urease production, indole production, citrate utilization.

Antibiotic susceptibility testing

This is done by testing the susceptibility of the bacteria to different antibiotics using standard Kirby-Bauer disk diffusion method against all P. mirabilis isolates. The antibiotics used were shown Table 1. Resistance to three or more classes of antimicrobial agents was defined as MDR.

Antibiotic	Abbreviation	Concentration
piperacillin	PRL	(100 µg)
amoxicillin/clavulanic acid	AMC	(20/10 µg)
aztreonam	ATM	(30 µg)
imipenem	IPM	(10 µg)
cefoxitin	FOX	(30 µg)
ceftazidime	CAZ	(30 µg)
cefotaxime	CTX	(30 µg)
ciprofloxacin	CIP	(5 µg)
cotrimoxazole	TS	(25 µg)
gentamicin	GM	(10 µg)
amikacin	AK	(30 µg)

ESBL Confirmation Test

The ESBL enzymes release was examined using a double-disk synergy test (DDST). The surface of Mueller-Hinton agar (MHA) plates was dried, and overnight cultures of the tested isolates were diluted to a 0.5 McFarland standard. Then, ceftazidime (30 µg) and cefotaxime (30 µg) disks were placed on the agar surface, positioned 15 mm away from the centrally placed amoxicillin-clavulanic acid (20/10 µg) disk. The plates were incubated at 37 °C for 24 hours.

Results

Participants' characteristics

A total of 218 patients with UTIs were enrolled in the study. Of them, 115 (52.7%) were Females and 84 (38.5%) were above 40 years (Fig. 1).

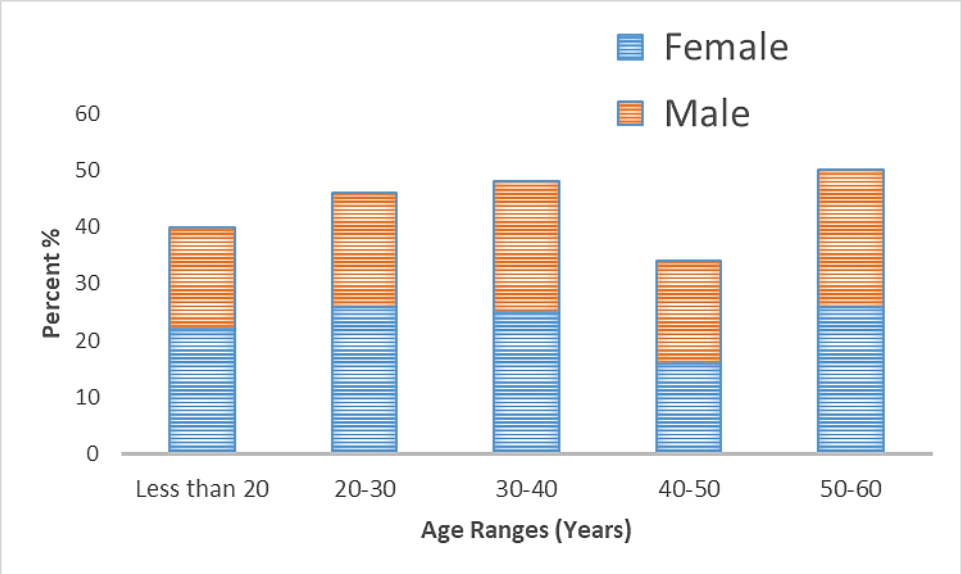

Figure 1 Distribution of isolates according to the age and gender of UTI patients.

Biochemical and microbiological results

According to the biochemical tests, two species were identified in our samples; they were P. mirabilis and P. vulgaris (Table2, fig.2,3). Two hundred specimens (91.7%) of all specimens were identified as P. mirabilis, and 18 specimens (8.3%) were identified as P. vulgaris. Results can be explained as P. mirabilis is more commonly encountered in clinical infections compared to P. vulgaris because it is typically found as a normal part of the microbial community in humans and other mammals. As a result, contamination of water or food with fecal matter can occur, leading to the spread of P. mirabilis.

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Table 2- Biochemical and microbiologic properties of P. mirabilis and P. vulgaris

Biochemical Tests		
Test	**P. mirabilis**	**P. vulgaris**
Urease production	**+ (within 4 hr)**	**+ (within 4 hr)**
Indole production	-	+
Sucrose fermentation	-	+
H_2S production	+	+
Phenylalanine deaminase	+	+
Microbiological Tests		
Lactose fermentation	-	-
Swarming on Blood agar	+	+
Hemolytic activity	-	-

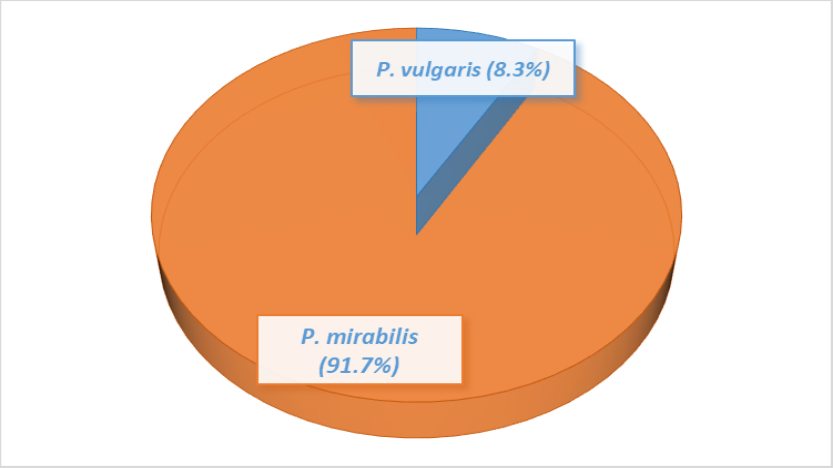

Figure 2. Distribution Percentage of P. mirabilis and P. vulgaris.

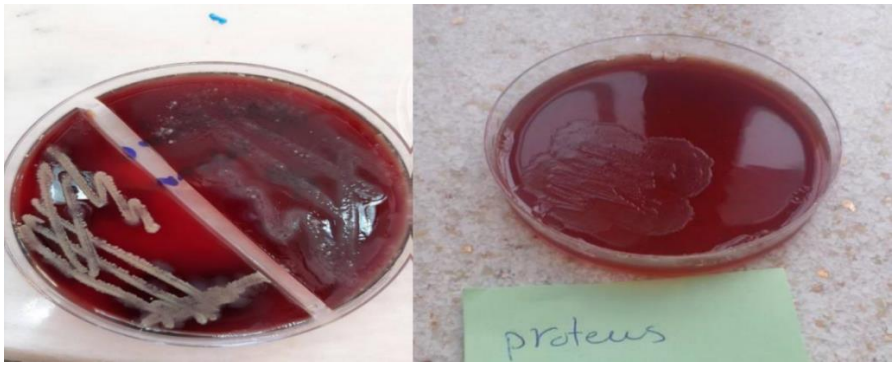

Fig 3. Proteus diagnostic media.

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Antibiotic resistance profiles of P. mirabilis

The susceptibility of isolated P. mirabilis to different antibiotics performed by Kirby-Bauer method. Our results showed that P. mirabilis isolates had varying resistance degrees towards different antibiotics categories (Fig.4). The bacterial isolates showed a high resistance to cotrimoxazole (TS) with 58.6%, then amoxicillin/ clavulanic acid (AMC) with 45%, 36% to GM, 25.5% to CIP ,32.6% to FOX, 32.7% to CAZ. Resistance to other antibiotics was lower than 20%.

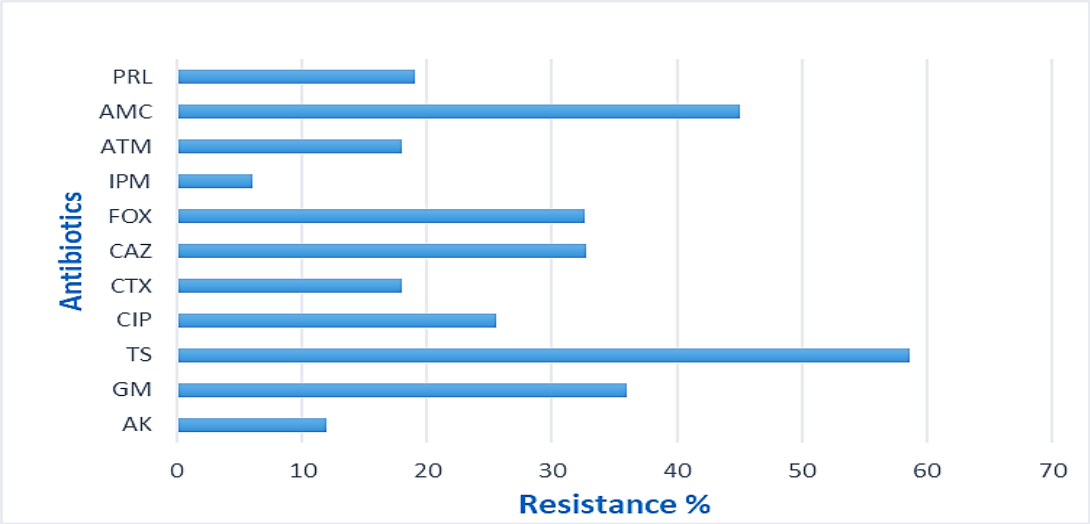

Figure 4. P. mirabilis Susceptibility to different antibiotics.

ESBLs production

Among the 200 P. mirabilis isolates, 117 (58.5%) were ESBL producers, as determined by DDST test. All ESBL producing isolates had high rates of resistance toward TS antibiotic, while being susceptible to imipenem (IPM). Significantly, PRL resistance rates recorded (31.3%), CTX (36.7%) and CIP (33.4%) were indicated (Fig. 5).

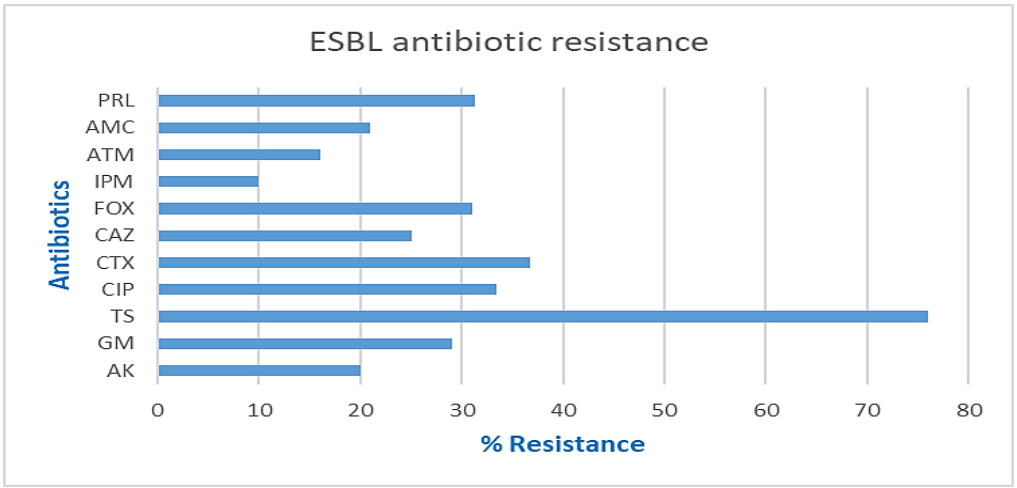

Figure 5. EBSL P. mirabilis Susceptibility to different antibiotics.

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Descriptive study questionnaire.

The model was designed to determine response, infection symptoms, and clinical aspects among patients. Of 287 total respondents, 227(79%) considered previous Proteus spp. infection. Regarding the symptoms, 68% indicated Hematuria and16.7% listed abdominal cramps (Table 3). In addition, evaluating medical history, the majority of responds don't concern with it. This may contribute for poor medical practice as 98.6% never doing laboratory tests. Also, from table 4 the majority of the participant were from women's (74%), whereas they receive poor level of education (67%). These all contribute to a high percentage of infection found.

Table 3- The percentage of response, infection symptoms, and clinical aspects (n=287).

Variables		Statement	Frequency	%
1.	Previous Proteus spp Infection (n=287)	Yes	**227**	**79**
		No	**60**	**20.9**
2.	known someone who had a Proteus spp infection (n=287)	Yes	**193**	**67.2**
		No	**94**	**32.8**
3.	Public education campaigns can help raise awareness about Proteus spp (n=287)	Yes	**287**	**100**
		No	**0**	**0**
4.	Precenting Symptoms (n=287)	High fever	**8**	**2.78**
		Hematuria	**197**	**68.64**
		Nausea and vomiting	**34**	**11.8**
		Abdominal cramps	**48**	**16.7**
5.	Medical History (n=287)	other bacterial infection	**18**	**6.3**
		Allergic reaction	**2**	**0.7**
		Wound infection	**14**	**4.9**
		Don't know	**253**	**88.1**
6.	Laboratory tests (n=287)	Routine doing it	**4**	**1.4**
		Never doing it	**283**	**98.6**
7.	Received treatment for a Proteus spp infection (n=287)	Yes	**201**	**70**
		No	**86**	**29.9**
8.	Received any other antibiotics (n=287)	Current or recent use	**198**	**68.9**
		Never use	**89**	**31**

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Table 4: Domain's satisfaction according to gender and education level.

Domains	Gender		Education level	
	M Responses (%)	F Responses (%)	Higher education Responses (%)	Read and write. Responses (%)
	75 (26.13)	212 (73.86)	94 (32.7)	193 (67.24)

Discussion

Proteus is a genus of bacteria that naturally resides in the gastrointestinal tract of humans. It is known to be a primary source of infections acquired outside healthcare settings and is also responsible for a significant number of infections acquired within hospitals. In terms of frequency, Proteus ranks third among the causative agents of hospital-associated infections **[12, 13].**

In the present study, 218 Proteus spp. isolates were collected from urine clinical samples. The highest percentage of isolates was from P. mirabilis (91.7%), followed P. vulgaris (8.3%). The production of urease and subsequent accumulation of ammonia by P. mirabilis create an environment that favors its survival and colonization within the urinary tract. This ability increases the risk of developing pyelonephritis and upper urinary tract infections (UTIs) **[14].**

The founded high percentage of locally isolated Proteus bacteria may be attributed to the genus' extensive capacity to invade tissues and surfaces, facilitated by its virulence factors. Additionally, incorrect usage of antibiotic drugs contributes to the rise in Proteus infections as found in our descriptive study result 69% receive antibiotics. Moreover, the presence of contaminated urinary catheters or other indwelling medical devices in unclean hospital environments can further contribute to the increased prevalence of Proteus infections **[2, 15].**

The simultaneous presence of resistance to multiple antibiotics indicates an ongoing and continuous transfer of resistance characteristics among bacterial pathogens. This emphasizes the inclination for multidrug-resistant (MDR) strains to emerge within Proteus spp. **[16].** Our study found high resistance for cotrimoxazole antibiotic (58.6%) and amoxicillin/clavulanic acid (45%) which agree with results reported by (Shaaban et al, 2022) **[17].**

Obviously, the results indicated high P. mirabilis sensitivity to imipenem. A result reported by other studies **[16, 18, 19].**

Furthermore, our study indicated that the rate of P. mirabilis able to produce high levels of ESBL enzymes increase. These may be the reason of a significant levels of antimicrobial resistance, our study reported the highest resistance to cotrimoxazole followed by amoxicillin. Same results mentioned by other studies **[20, 21].**

Concerning the descriptive questionnaire study, we obtain to record the relation between practical production and society (Table. 3), this study showed that wrong use of antibiotics a long with poor education level aid in a strong way in infection increase. Therefore, increasing community awareness about parasitic infection and antibiotic usage control should be created to help reduce complications. In addition, further research is needed with the molecular side.

Conclusion

The overall data results obtained in this study revealed a high prevalence of ESBL production among P. mirabilis clinical urinary isolates in Iraq. While a significant proportion of isolates showed resistance to the tested antimicrobials, imipenem remains one of the few remaining options for effectively controlling and managing the pathogenicity of Proteus. Furthermore, it is imperative to emphasize the importance of implementing proper sanitary procedures to prevent the future escalation of antibiotic resistance among the isolated strains.

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