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The Prevalence of *Proteus mirabilis* isolated from different clinical specimens in Zakho City, Iraq

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Abstract

Proteus mirabilis is the most common species among the genus *Proteus* that has been associated with various infections in humans. In this study, the prevalence of *P. mirabilis* among outpatients with UTIs, ear, burns, wounds, respiratory tract infections, and vaginitis who attended the major hospitals and clinics in Zakho city has been investigated. Four hundred specimens were collected from both genders at different ages (≤1 year to over 50 years), from July 2021 to January 2022. All specimens were analyzed according to conventional bacteriological methods for detecting the presence of *P. mirabilis*. Also, a molecular method was used using species-specific ureR primer to confirm the presence of *P. mirabilis* among the isolates. From all collected specimens, 23.75% (95/400) were identified as *P. mirabilis* with a higher rate in males than females (52.63% vs 47.37). Among both genders, the age group ≤ 1-10 years showed the highest rate of infection (61.90%). Married patients had a higher rate (27%) than unmarried ones (16.92%). In terms of residency, rural inhabitants had the highest rate (31.25%) among other inhabitants. Furthermore, infections during the months of the year were at their maximum rate during August (52.27%), while reduced to 11.67% in October. ureR primer gives a precise and specific detection of *P. mirabilis.* This study highlights that; *P. mirabilis* is one of the remarkable bacteria that cause various infections in humans through various risk factors like anatomical abnormalities, gender, age, and chronic diseases that facilitate its spreading in the community.

Keywords: *Proteus mirabilis*, *ureR* gene, risk factors, seasonal variation.

Резюме

Proteus mirabilis е най-разпространеният вид от род *Proteus*, който се свързва с различни инфекции при хората. В това проучване е изследвано разпространението на *P. mirabilis* сред амбулаторни пациенти с инфекции на пикочните пътища, ушите, изгаряния, рани, инфекции на дихателните пътища и вагинити, които са посещавали големите болници и клиники в град Захо. От юли 2021 г. до януари 2022 г. са събрани четиристотин проби от двата пола на различна възраст (от ≤1 година до над 50 години). Всички проби са анализирани съгласно конвенционалните бактериологични методи за установяване на наличието на *P. mirabilis*. Използван е и молекулярен метод с помощта на видовоспецифичен ureR праймер за потвърждаване на наличието на *P. mirabilis* сред изолатите. От всички събрани образци 23.75% (95/400) бяха идентифицирани като *P. mirabilis* с по-висок процент при мъжете, отколкото при жените (52.63 % срещу 47.37). И сред двата пола, възрастовата група ≤ 1-10 години показа най-висока честота на инфекция (61.90%). Омъжените пациенти имаха по-висока честота (27%), отколкото неомъжените (16.92%). По отношение на местоживеенето, жителите на селските райони имаха най-висок процент (31,25%) сред останалите жители. Освен това, инфекциите през месеците на годината са с максимална честота през август (52.27%), докато през октомври са намалели до 11.67%. ureR primer дава възможност за точно и специфично откриване на *P. mirabilis*. Това проучване подчертава, че *P. mirabilis* е една от забележителните бактерии, които причиняват различни инфекции при хората чрез различни рискови фактори като анатомични аномалии, пол, възраст и хронични заболявания, които улесняват разпространението ѝ в обществото.

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Introduction

Proteus mirabilis is a common causative agent of many invasive infections in the community. *P. mirabilis* belongs to Enterobacteriaceae (Hamilton *et al.,* 2018). The most prominent feature that distinguishes *Proteus* species from other members of *Enterobacteriaceae* in the clinical labs is the swarming phenomenon on blood agar (Gorički *et al*., 2017). Besides the saprophytic mode of life in the intestines of humans and animals, *Proteus* bacilli under favorable circumstances can cause pathological events such as infections in the respiratory tract, burns, wounds, as well as infections of the gastrointestinal tract, bones, ears, nose, joints and meninges (Armbruster *et al.,* 2018).

P. mirabilis has developed various virulence factors to multiply, damage, and survive in the host. Some virulence factors are associated with disease-producing potentials including; urease production, flagella, fimbriae, protease enzyme production, and swarming regulator genes that damage the host tissue (Abd Al-Mayahi, 2017). Additionally, several risk factors such as age, hydronephrosis, gender, hyperthermia, and diabetes mellitus increase the rate of infection with *P. mirabilis* but most frequently the rate of infections is increased in persons with physiological and anatomical defects, especially in UTI infections. Infections occur predominantly in the urinary system, where *P. mirabilis* facilitates stone formation and kidney failure (Armbruster *et al.,* 2018). The illustrated rates of *Proteus* spp. infections have increased worldwide, especially in developing countries. *P. mirabilis* is the third most common cause of UTI infections, after *Escherichia coli* and *Klebsiella pneumoniae* (Kwiecinska-Pirog *et al.,* 2016). Additionally, it has been estimated that *P. mirabilis* accounts for 90% of all *Proteus* infections in healthcare facilities (Zafar *et al.,* 2019).

Several studies have recorded alarming results in terms of antibiotic resistance and distribution of *P. mirabilis* for example, Alabi *et al*. (2017) demonstrated that 55% of *P. mirabilis* stains were multi-drug resistant. Another study in Kurdistan by Naqid *et al*. (2020) recorded alarming findings about multidrug-resistant *P. mirabilis*. Furthermore, in Zakho City Al-Berfkani *et al.* (2016) have estimated that *P. mirabilis* was responsible for 8.3% of UTI infections among both genders. While higher rate was recorded in Erbil City by Hamid *et al.* (2020) who revealed that 21.42% of *P. mirabilis* were responsible for diabetic foot infections. Additionally, in Sulaimani City, Qadir *et al.* (2020) recorded that the distribution of *P. mirabilis* in diabetic foot ulcer patients was 17%. Due to the limited availability of studies in this aspect in Zakho City, the current study was adapted to investigate the prevalence of *P. mirabilis* among different clinical specimens in major hospitals and clinics of Zakho City by using biochemical tests and molecular detection of species-specific genes among the isolated *P. mirabilis*. Furthermore, to investigate the relationship between *P. mirabilis* and some risk factors along with seasonal variation.

Materials and Methods

Sample collection

Four hundred clinical specimens of both genders and different ages (≤1 to above 50 years) were aseptically collected from symptomatic outpatients after receiving their verbal consent. Samples were from different regions of Zakho City and some nearby villages who visited Zakho General Hospital, Emergency Hospital, and some other private clinics during the period from July 2021, to January 2022. The examined specimens included urine (150), burn swabs (23), wound swabs (30), ear swabs (90), vaginal swabs (45), and sputum (62). The collected specimens were directly streaked on labeled blood agar and MacConkey agar plates then were incubated aerobically at 37°C for 24 hrs. for further identification purposes.

Microbiological investigation

The identification of the isolates was based on morphological and cultural characteristics of blood agar and MacConkey agar (Ahmed, 2015). The isolated colonies then were tested by gram staining according to the protocol of the commercial kit (Himedia-India). For more confirmation, certain biochemical tests were done such as urease test, indole test, citrate utilization test, methyl red test, and triple sugar iron agar (TSI) (Jain *et al.,* 2020). This study was performed at the Microbiology and Molecular Laboratory of the Biology Department at Zakho University.

Genomic DNA extraction and molecular diagnosis

A genomic extraction kit (GeNet Bio, Taiwan) was used to extract DNA from *P. mirabilis* strains following the manufacturer's instructions. The concentration and purity of the extracted DNA for 52 multidrug resistance *P. mirabilis* samples were determined by using a Nanodrop spectrophotometer 2000 (Thermo Scientific, USA). For molecular identification of *P. mirabilis* the *ureR* primer was used as a species-specific to amplify the target *ureR* gene as shown in Table 1.

Table 1. Species-specific primer for detection of *P. mirabilis*

Primer	DNA Sequences (5'-3')	Size (bp)	References
ureR	F-GGTGAGATTTGTATTAATGG R-ATAATCTGGAAGATGACGAG	225bp	(Zhang *et al.,* 2013)

The PCR amplification mixture for each sample is shown in Table 2. The PCR conditions were: the initial denaturation at 94°C for 4 minutes, 40 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 1 minute, extension at 72°C for 20 seconds, and a final extension at 72°C for 4 min. The amplicons of PCR products were resolved on 1.2% agarose gel containing RedSafe dye with green fluorescence (GDSBio-China). DNA ladder with a molecular weight of 100 –1000 bps (GDS-Bio-China) was also included.

Table 2. Contents of PCR mixture

Component	Volume
Master Mix (2x)	10 µl
Forward primer (10pm/µl)	1 µl
Reverse primer (10pm/µl)	1 µl
Extracted DNA (30-60 ng/µl)	2 µl
Nuclease free dH2O	6 µl
Final volume	20 µl

Statistical analysis

Descriptive statistics including frequencies, graphs, and percentages for determining a logical representation of the analyzed data was done by statistical package for social science version 17.0 (SPSS). The relationship between discrete variables was determined using the Chi-square test, $p<0.05$, was considered significant.

Results

Cultural characteristics of *P. mirabilis* were as follows: swarming phenomenon along with fishy odor on blood agar, and non-lactose fermenting ability on MacConkey agar. For further confirmation standard biochemical techniques were also done to the suspected isolates. *P. mirabilis* isolates were positive for urease, citrate utilization test, and Catalase produced black precipitate (H_2S) at the bottom of TSI agar medium but were negative for to indole test (Procop *et al.,* 2020).

The cultural and biochemical examination revealed that 23.75% (95/400) of the isolates were *P. mirabilis*. Regarding gender, in general, a higher rate of *P. mirabilis* was found in males than females (53.68% vs 46.31%), except for urine samples that were higher in females than males, as shown in Table 3. On the other hand, the highest rate of infections among males was observed in sputum samples with 76.92%, while in females, the highest rate was in women who had vaginitis, followed by urine samples. The difference between both genders among all sources was highly significant ($p< 0.0001$).

It is obvious from Table 4 that the rate of infection in males among all age groups was higher than that of females except for the ≤1-10 age group which had a higher rate in females with 73.07%. On the other hand, the highest rate in males was within 41-50 years which was 84.61%. Among both genders, the highest rate of *P. mirabilis* was seen in females aged between 1-10 years, which accounted for 61.90% (26/42), with highly significant ($P<0.0001$) differences among males and females in different age groups.

In terms of marital status, the distribution of the isolated *P. mirabilis* was at the highest rate among married patients which was 27.07 %, while

Table 3. Distribution of *P. mirabilis* from different clinical specimens among both genders in Zakho City

source	No. of examined samples	No. of *P. mirabilis* isolates from each source	Gender	
			Males (%)	Females (%)
urine	150	42	19(45.24)	23(54.76)
ear	90	20	15(68.18)	7(31.81)
wound	30	7	5(80.00)	1(20.00)
burn	23	4	3(75.00)	1(25.00)
sputum	62	13	10(76.92)	3(23.08)
vagina	45	9	-	9(100)
total	400	95	51(53.68)	44(46.31)

****P<0.0001

Table 4. Distribution of *P. mirabilis* among different age groups and both genders

Age groups	No. of examined samples	*No. P. mirabilis* isolates (%)	Gender	
			Males (%)	Females (%)
≤ 1-10	42	26(61.90)	7(26.92)	19(73.07)
11-20	89	8(8.99)	5(62.50)	3(37.50)
21- 30	96	11(11.46)	6(54.54)	5(45.45)
31- 40	70	17(24.29)	10(58.82)	7(41.17)
41- 50	45	13(28.89)	11(84.31)	2(15.38)
51≤	58	20(34.48)	12(60.00)	8(40.00)
Total	400	95(23.75)	51(53.68)	44(46.31)

****P <0.0001

Table 5. Occurrence of *P. mirabilis* in both genders

Marital Status	No. of examined sample	No. of Isolated *P. mirabilis* (%)	Gender	
			Males (%)	Females (%)
Married	270	73 (27.04)	35 (47.95)	38 (52.05)
Single	130	22 (16.92)	16 (72.72)	6 (27.27)
Total	400	95 (23.75)	51 (53.68)	45 (46.31)

**P<0.0042

unmarried ones had a rate of 16.92% as shown in Table 5. Statistically, this difference was highly significant *P. mirabilis* infections were more distributed among married females than those of males (53.68% vs. 46.31%), respectively. While single males had a higher rate than single females, as shown in Figure 1.

Under residency, the patients who lived in rural areas were more vulnerable to getting infected with *P. mirabilis* than patients living in urban areas and camps with the highest rate of 31.52% as shown in Table 6, with a highly significant (P <0.0001) differences between residency and genders.

Throughout seven months the rate of *P. mirabilis* varied among patients, as represented in Figure 2. Infections among both genders were at their maximum rate in August with 52.27%, while dramatically reduced in October to reach 11.63%.

In terms of molecular characterization of *P. mirabilis* all 52 multidrug-resistance selected isolates revealed a successful amplification for the specific-species locus by producing a single band of a target-specific gene *ureR* with a molecular weight of 225bp, as shown in Fig. 2.

Discussion

Proteus mirabilis is an opportunistic bacterium that causes many infections in the community and hospital environments (Bahashwan and El Shafey, 2013). The results of the current study indicate that 23.75% of *P. mirabilis* were responsible for various infections in suspected patients. Nearly findings were reported in Erbil city by Kamil and Jarjes (2019) who illustrated that *Proteus* spp. had

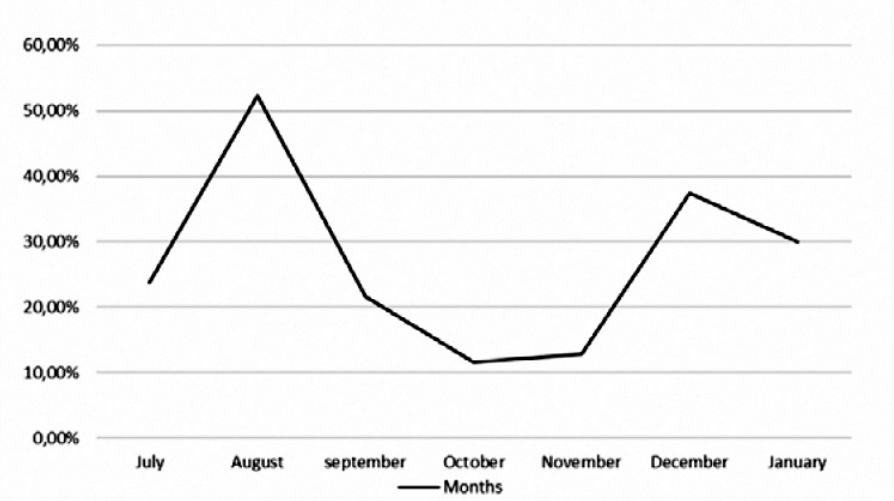

Fig. 1. Month-wise occurrence of *P. mirabilis*

Table 6. Distribution of *P. mirabilis* according to residency

Residency	No. of examined sample	No. of isolated *P. mirabilis*	Gender	
			Males (%)	Females (%)
Urban	210	47 (22.38)	30 (63.82)	17 (36.17)
Rural	92	29 (31.52)	17(58.62)	12 (41.38)
Camp	98	19 (19.39)	4(21.05)	15 (78.95)
Total	400	95 (23.75)	51(53.68)	46 (46.31)

****P <0.0001

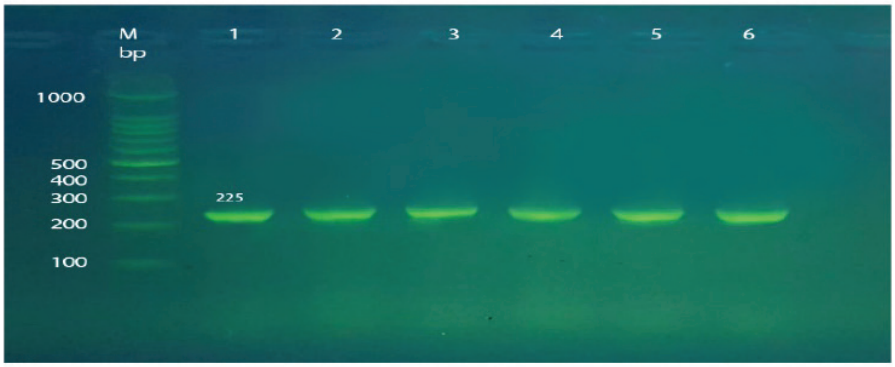

Fig. 2. The amplification of PCR for *P. mirabilis* specific gene *ureR* for the chosen multidrug resistance samples were done using ureR primer with molecular size of 225 bps. The separation was performed on 1.2% agarose gel electrophoresis for 1 hr. Lane M=DNA marker is (100-1000bp).

a rate of 26% among 200 screened samples. While lower rates were observed by Ahmed (2015) in Baghdad City and Jabur *et al.* (2013) in Babylon City who reported that *P. mirabilis* among different clinical specimens have rates of 13.16 % and 7%, respectively. The variation in the isolate rates may be due to the variations in sample size and diagnostic criteria, the source of samples, and the number of hospitals assessed.

In the present study, *P. mirabilis* was more predominant among male patients than females, but this picture was different in urine samples in which females recorded a higher rate of UTI infections than males (54.76% vs., 45.24%). Similarly, in Duhok City, Khalid and Yassin (2017) illustrated that the majority of *P. mirabilis* 54% were detected in urine samples isolates rather than wounds and middle ear samples. On the other hand, among males, sputum samples had the highest rate accounting for 78.92%, however, it is worth mentioning that among females who suffered from vaginitis 100% of samples were positive for *P. mirabilis* since the small number of samples were taken, it does not represent the whole society. The recorded rate was somewhat agreed with Bahashwany and El Shafey (2013) who illustrated that the highest rate of 80% of *Proteus* spp. was found in sputum samples of males. The possibility of the elevated prevalence of *P. mirabilis* among male patients is predominance due to the fact that they are more exposed to the workforce hence they have a greater chance of gaining infectious diseases, accidents, and operations (Zafar *et al.,* 2019).

P. mirabilis was recovered from all age groups but predominantly was found among children aged between ≤ 1-10 years. A similar investigation was reported by Feglo *et al.* (2010) who illustrated the highest rate of *P. mirabilis* among the 1-9 years age group. According to Madana *et al.* (2011), the higher occurrence of bacterial infection among these age groups may be due to that children usually suffer from chronic diseases and weak immunity. However, some studies stated that urinary tract infections are high among children wearing diapers due to bacterial accumulation especially when unchanged for a longer period (Tawfeeq, 2014).

In general, the distribution of *P. mirabilis* was higher in married patients than in single which were 27.04% and 16.92%, respectively. But in particular, women had a higher rate of infection with *P. mirabilis* than married men. This agrees with Parajuli *et al.* (2021). Such findings might be induced by hormonal and physiological changes during pregnancy which enhance the chance of infection (Al-Berfkani *et al.,* 2016). However, in UTI infection the shortage of urethra in female genitals assists in the transfer of pathogens from the anus to the urethra (Mirzaei *et al.,* 2019).

In terms of residency, an elevated rate of infection in rural areas was recorded. This observation concords with the findings of Edrees *et al.* (2021) who noticed an elevated rate of 40.62% among wound infections in rural areas. Furthermore, Kishore *et al.* (2015) found that more UTI infections were recorded in rural areas than in urban areas with a rate of 35.4% vs., 28.7%, respectively. On the other hand, these results disagree with a study carried out by Almukhtar (2018) in Kirkuk City who illustrated that the rate of UTIs in women in urban areas was higher than in those living in rural areas (56.4% vs. 43.6%). The high occurrence of *P. mirabilis* in rural areas could be attributed to low sanitation and limited concern regarding personal hygiene. Similarly, a lack of awareness about infections are significant cause of the elevated rate of infection in rural areas (Al-Duliami *et al.*, 2010).

A higher rate of *P. mirabilis* infections with 52.27% was recorded in August. As per our knowledge few studies according to this aspect have been done for instance, Al-Berfkani *et al.* (2016) in Zakho City have conducted a study that was limited to UTI infections and found high infections of *P. mirabilis* in hot months. Similarly, Bahashwan and Shafey (2013) illustrated that 40% of *P. mirabilis* infections among different clinical samples occur in summer, while in cold weather half of the aforementioned rate was recorded. This might be due to the fact that bacterial infections especially among UTIs are induced by dehydration that allows urine to be more concentrated (Tawfeeq, 2014). Nevertheless, cold seasons are more linked with respiratory system infections (Pandey *et al.,* 2011). The recorded variation in the rate of *P. mirabilis* over different months could be induced by the fluctuation of temperatures throughout the year.

All of the 52 multidrug-resistant selected samples for molecular detection gave positive bands of the expected size of 225 bp. According to Zhang *et al*. (2013), the *ureR* gene which codes for urease enzyme is considered a hallmark for molecular identification of *P. mirabilis* and is an important virulence factor among *Proteus* spp. A similar *ureR* primer of 225bp in Erbil City was used by Adnan *et al.* (2014), and Kamil and Jarjes (2021). Moreover, this method establishes a quick and effective identification of *P. mirabilis*; thereby providing a new avenue in molecular applications.

Conclusion

P. mirabilis is a significant causative agent that induces various infections in the community. Additionally, it can infect both genders at all ages, but is more commonly found in children. Several risk factors enhance the spread of *P. mirabilis* in the community. Furthermore, the *ureR* primer gives a precise detection of *P. mirabilis.*

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