Original Article

Investigation of *blaTEM* among Multidrug-Resistant *Proteus mirabilis* Isolated from Urinary Tracts Infection

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Abstract

Background: Any component of the urinary system can get infected, which is what a urinary tract infection (UTI) is. Most infections affect the lower urinary tract (bladder and the urethra). UTIs are frequent infections that develop when germs, frequently from the skin or rectum, pass through the urethra and infect the urinary system. **Objective:** The current study aims to investigate *blaTEM* gene among multidrug-resistant (MDR) and nonmultidrug-resistant (non-MDR) *Proteus mirabilis* isolated from patients with UTI. **Materials and Methods:** Collected 450 (325 male and 125 female) with UTIs. These patients ranged in age from 17 to 70 years old from three Iraqi hospitals (Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, Merjan Hospital, and Shomali General Hospital) from the period from September to December. Samples were grown on MacConkey agar and blood agar to separate Gram-negative, nonlactose-fermenting colonies exhibiting swarming behavior. Biochemicals were also used to confirm *P. mirabilis*. Molecular detection of *P. mirabilis* achieved by amplification of *atpD* gene. Antibiotic susceptibility tests by disc diffusion method were performed for 15 antibiotics from 6 different classes to assign the isolated to MDR or non-MDR. Finally, the amplification of *blaTEM* gene was achieved using polymerase chain reaction (PCR) technique and specific primer pairs. **Results:** The result of culture, biochemical, Gram stain, and genetic (*atpD* gene) present the percentage of samples positive for *P. mirabilis* was 70 samples (15.5%). The result of antibiotic susceptibility showed that all isolated resistant to Nitrofurantoin (92.8%), Trimethoprim–Sulfamethoxazole (85.7%), Cefotaxime (78.5%), Cefuroxime (68.5%), Ceftazidime (58.5%), Gentamycin (38.5%), Ciprofloxacin (37.1%), and Levofloxacin (28.5%). While the lower resistance against Amikacin (10%), Imipenem (4.2%), Aztreonam (2.8%), and Meropenem (1.4%). The result showed 64 (91.4%) of 70 were positive for the *blaTEM* gene; among them, 60 (93.75%) were MDR and 4 (6.6%) were non-MDR. **Conclusions:** Most *P. mirabilis* isolates were found to MDR with different percentages of resistance to different antibiotics, and *blaTEM*-carrying isolates showed high resistance to Nitrofurantoin, Trimethoprim–Sulfamethoxaz.

Keywords: Antibiotic susceptibility test, *atpD*, *blaTEM*, MDR, *Proteus mirabilis*, UTIs

Introduction

The presence of bacteria in the urinary tract system causes a urinary tract infection (UTI). It is typically caused by digestive tract bacteria that enter the urethral opening and start to reproduce, leading to an infection.[1] UTIs, one of the most common bacterial illnesses, affect approximately 150 million people worldwide.[2,3]

Gram-negative rods known as *Proteus* spp. are members of the family Morganellaceae and the order Enterobacterales. These species constitute a digestive tract's bacterial flora in humans and animals.[4,5] *Proteus mirabilis* is a member of the Enterobacteriaceae family and has the ability to change its shape from a rod to an elongate and swarm motility via the use of flagella.[6]

Extended-spectrum b-lactamase (ESBL)-producing Gram-negative pathogens are a major cause of resistance to expanded-spectrum b-lactam antibiotics. There are many diagnostic tools available to the clinical

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Table 1: The sequencing and PCR condition of the gene used in this study

Genes	Sequences of primers (5′ to 3′)	Product size (bp)	PCR conditions (30 cycles)	References
blaTEM	F:CGTGTCGCCCTTATTCCCTT R:CAGTGCTGCAATGATACCGC	723	Denaturation: 95°C, 30 s Annealing: 60°C, 30 s Extension: 72°C, 30 s	This study
atpD	F:GGTGCGGGTGTTGGTAAAAC R:TGAATCCAGTGGGTCAACCG	570 pb	Denaturation: 95°C, 30 s Annealing: 55°C, 30 s Extension: 72°C, 30 s	This study

PCR: polymerase chain reaction

microbiology laboratory and include both phenotypic and genotypic tests to detect b-lactamases. ESBL-producing Gram-negative pathogens will continue to be a major contributor to antimicrobial resistance worldwide. It is essential that we remain vigilant about identifying them in both inpatient isolates and through surveillance studies. TEM-type ESBLS are variants of the original plasmid-mediated b-lactamase, TEM-1, which was described in the early 1960s.[7]

Resistance is one of the most important challenges for global public health, as the likelihood of bacteria curing diseases brought on by multidrug-resistant (MDR) bacteria has drastically decreased. The number of clinically effective antibiotics available for the treatment of infections caused by MDR bacteria has been reducing at an alarming rate in recent decades as a result of increasing resistance. By antibiotic agent and duration, the risks of adverse medication events and possible microbiome-related events vary greatly.[6] The present study aims to isolate and identify *Proteus* spp. from urine samples collected from urinary tract infection patients in the different hospitals in Babylon province and reveal the antibiotic resistance among these isolated.

Materials and Methods

Specimens collection

Collected 450 (325 male and 125 female) with UTIs. These patients ranged in age from 17 to 70 years old from three Iraqi hospitals (Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, Merjan Hospital, and Shomali General Hospital) from the period from September to December.

Isolation and identification of *Proteus mirabilis*

According to culture and biochemical testing, nonlactose fermenting colonies on MacConkey agar with swarming on blood agar plates, a characteristic fishy odor, microscopic examination revealing Gram-negative pleomorphic bacteria with active motility, and the findings of the biochemical tests, oxidase, and indol, the isolates were identified as *P. mirabilis*. However, we tested urease, methyl red, citrate, Vogas–Proskeur, catalase, and glucose fermentation (together with gas and acid).[8] Also, the genetic identification of *P.mirabilis* by using *atpD* gene, the nucleotide sequences of the gene, and the Polymerase chain reaction are described in Table 1.

Antibiotic susceptibility test

On Mueller–Hinton agar, the Kirby–Bauer disc diffusion method was used, the antibiotic sensitivity test of the isolates against 13 antibiotics was determined. The test involves placing tiny (6 mm) filter paper disks on an agar plate containing bacteria that have been swabbed, each of which has been impregnated with a standard dose of antibiotic. Following an overnight 37°C incubation of the plates, the diameter of the inhibition zone is used to determine the susceptibility.[9]

Detection of *bla TEM* gene in *P. mirabilis* isolates

According to the manufacturer's instructions, DNA extraction from all isolates was performed using a high pure polymerase chain reaction (PCR) template preparation kit (FavorPrep™ Cultured Cell Genomic DNA Extraction Mini Kit). The extracted DNAs were stored at -20°C for subsequent steps. The ESBL-encoding loci, *blaTEM* were amplified by conventional PCR using the primers and conditions described in Table 1 designed in this study.

Ethical approval

The investigation was carried out in conformity with the Declaration of Helsinki's ethical precepts. Before a sample was taken, it was done with the patient's verbal and analytical consent. The study protocol and the subject information, and consent form were reviewed and approved by a local ethics committee with Project No.: M220901 was approved on September 28, 2022.

Results

In the present study, a total of 450 samples were collected from patients suffering from UTI were distributed in seven groups according to the age <20 years (76) patients, 20–30 years (206) patients, 31–40 years (89) patients, 41–50 years (37) patients, 51–60 years (29) patients, 61–70 years (12) patients, and >70 years (1), also the samples include 325 female and 125 male.

The result of culture, biochemical test, Gram stain, and genetic (*atpD* gene) present that among 450 tested samples, 70 (15.5 %) were positive for *P. mirabilis*. Rest of the isolates were belonging to Gram-positive and Gram-negative bacteria. The *P. mirabilis* isolates are distributed for 24(34.2%) from male and 46(65.7%) from female [Table 2] [Figure 1].

The result of antibiotic susceptibility showed higher resistance against Nitrofurantoin (92.8%), Trimethoprim–Sulfamethoxazole (85.7%), Cefotaxime (78.5%), Cefuroxime (68.5%), Ceftazidime (58.5%), Gentamycin (38.5%), Ciprofloxacin (37.1%), Levofloxacin (28.5%). While the lower resistance against Amikacin (10%), Imipenem (4.2%), Aztreonam (2.8%), and Meropenem (1.4%) [Figure 2].

Nitrofurantoin (F), Trimethoprim–Sulfamethoxazole (SXT), Cefotaxime (CTX), Cefuroxime (CXM), Ceftazidime (CAZ), Gentamycin (CN), Ciprofloxacin (CIP), Levofloxacin (LEV), Amikacin (AK), Imipenem (IPM), Aztreonam (ATM), and Meropenem (MEM).

Table 2: Distribution of *Proteus mirabilis* age groups and gender or patients in the current study

Age	Gender		
	Male No. (%)	Female No. (%)	Total No. (%)
<20	3 (4.2)	8 (11.4)	11 (15.7)
20–30	8 (11.4)	19 (27.1)	27 (38.5)
31–40	9 (12.8)	9 (12.8)	18 (25.7)
41–50	3 (4.2)	3 (4.2)	6 (8.5)
51–60	1 (1.4)	2 (2.8)	3 (4.2)
61–70	0 (0)	5 (7.1)	5 (7.1)
>70	0(0)	0(0)	0 (0)
Total	24 (34.2)	46 (65.7)	70 (100)

The prevalence of *blaTEM*-producing *Proteus* spp. is increasing worldwide. In the present study, among 70 *P. mirabilis* isolates, 64 (91.4 %) have *blaTEM* gene [Figure 3]. The occurrence of blaTEM among MDR isolates was 60 (93.75%) and 4 (6.6%) were non-MDR.

Discussions

The result of culture, biochemical, Gram stain, and genetic (*atpD* gene) present the percentage of samples positive for *P. mirabilis* was 70 samples (15.5%) were found positive for *P. mirabilis*. Rest of the isolates were belonging to Gram-positive and Gram-negative bacteria. Because of the presence of bile salts, *Proteus* isolates produced pale-colored colonies on MacConkey agar medium. *Proteus* appeared as short pink Gram-negative rods under the microscope.[8]

This result differed from that of other studies, which reported that a total of 38 patients with UTIs at Al-Diwaniyah Teaching Hospital had a total of 15 isolates (or 39.4%) of *P. mirabilis*.[10] There were 250 samples collected from patients with UTIs, and 60 of those samples (or 24%) had *P. mirabilis* that was positively proliferating,[8] and a total of 132 urine samples were collected from different hospitals in Baghdad city *P. mirabilis* in a percentage of 53.[11] The percentage of *P. mirabilis* isolates was 8 (13.8%) more than the result of this study Ullah *et al*.[12]

Based on the finding that the atpD gene was more conservative than the *16 S rRNA* gene in bacterial species, the atpD gene was chosen as the PCR target for *Proteus*

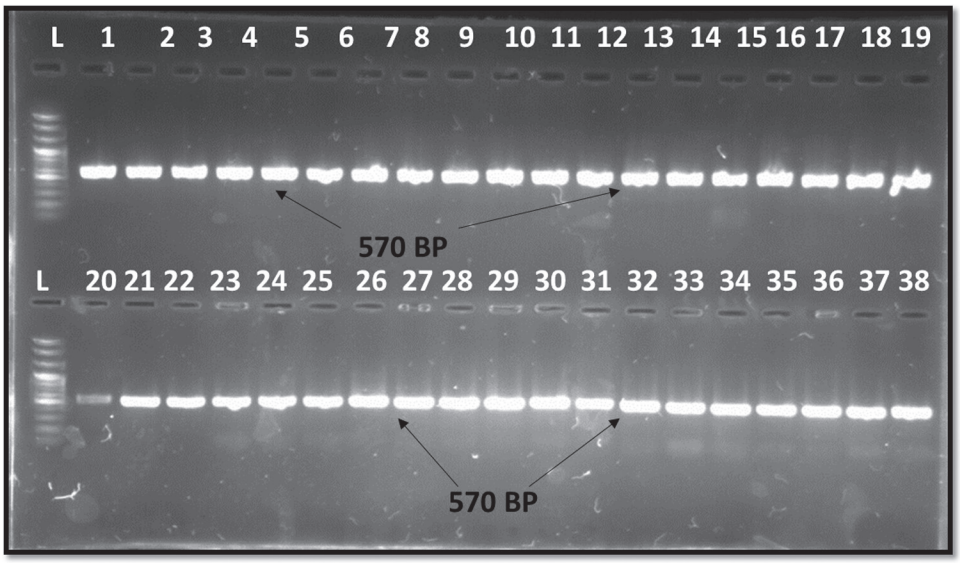
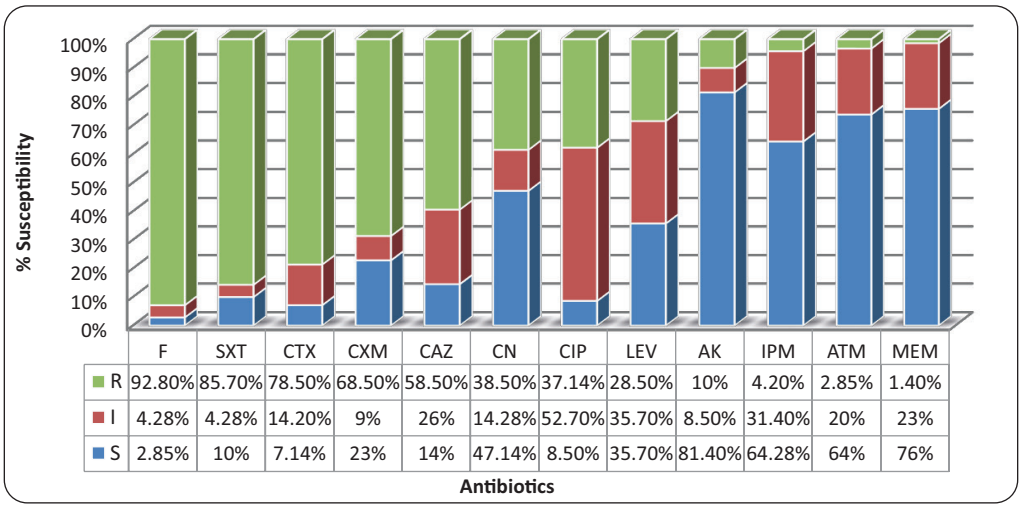


Figure 1: 1.5% agarose gel electrophoresis at 70 V for 60 min after staining with redSafe for *atpD* PCR products. Lane (L) molecular size marker for DNA molecules (1500-bp ladder). Show positive results; the size of the product is 570 bp



	F	SXT	CTX	CXM	CAZ	CN	CIP	LEV	AK	IPM	ATM	MEM
R	92.80%	85.70%	78.50%	68.50%	58.50%	38.50%	37.14%	28.50%	10%	4.20%	2.85%	1.40%
I	4.28%	4.28%	14.20%	9%	26%	14.28%	52.70%	35.70%	8.50%	31.40%	20%	23%
S	2.85%	10%	7.14%	23%	14%	47.14%	8.50%	35.70%	81.40%	64.28%	64%	76%

Figure 2: Antibiotic Susceptibility of *Proteus mirabilis n* = (70) isolates

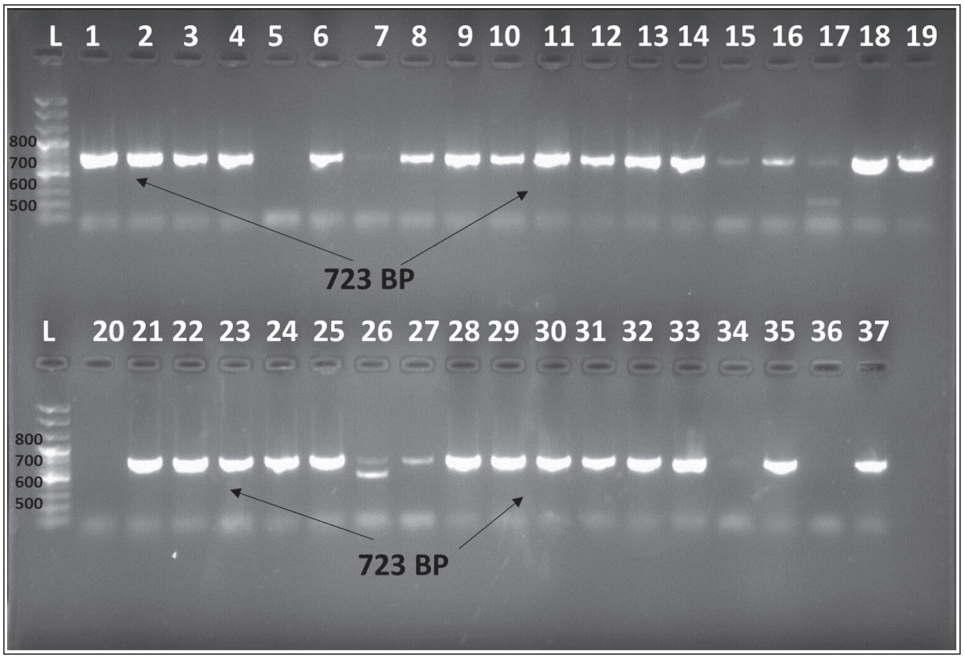


Figure 3: 1.5 % agarose gel electrophoresis at 70 V for 60 min after staining with GoldView for *blaTEM* PCR products. Lane (L) molecular size marker for DNA molecules (1500-bp ladder). Show positive results; the size of the product is 723 bp

species detection.[13] who isolated 30 *P. mirabilis* strains from 541 samples (5.55%) using *atpD* PCR detection.[14]

The variation in result may be related to the geographic distribution, sample type, number of tested samples, uptake of the antibiotic, environmental and socioeconomic factors, accuracy with a gynecologist takes a sample from a patient, the method of diagnosis, the number of tested samples, the type of samples, and cultural factors.[15] The result of antibiotic susceptibility in the current study showed high resistance against Nitrofurantoin (92.8%), Trimethoprim–Sulfamethoxazole (85.7%), Cefotaxime (78.5%), Cefuroxime (68.5%), Ceftazidime (58.5%), Gentamycin (38.5%), Ciprofloxacin (37.1%), Levofloxacin (28.5%). While the higher sensitive against Amikacin (10%), Imipenem (4.2%), Aztreonam (2.8%), and Meropenem (1.4%). This result is similar to the result obtained by Mirzaei *et al.*[14] who found the bacterial isolates showed high resistance to amoxicillin at 44.5% and were sensitive to imipenem and meropenem in percentages 11.8% and 4.5%, respectively. In another study, out of 150 isolates, 46 were confirmed as *P. mirabilis*, while resistance towards levofloxacin and imipenem was noticed as 60% and 58%, respectively.[16]

The prevalence of *blaTEM*-producing *Proteus* spp. is increasing worldwide. In the present study, the prevalence of *blaTEM* gene present 64 (91.4%) from 70 *P. mirabilis* isolated present positive for this gene. Other studies found the occurrence of *blaTEM* among MDR isolates

60 (93.75%) and 4 (6.6%) were non-MDR, while other researchers found 30 *Proteus* spp. isolates, 7 (23.3%) were positive for *blaTEM*.[17] These differences in the distribution of blaTEM may be due to geographical distribution, type of organisms, and source of infections. In Iraq, the prevalence of *blaTEM* was 60% among 30 isolated *Proteus* spp.,[18] while in China, it was around 52% in the same bacteria.[19]

In Argentina, investigating the resistance to β-lactam/β-lactamase inhibitors in enterobacteria revealed that all *Proteus* spp. isolates harbored the *blaTEM* gene.[20] In India, the *blaTEM* rate among *P. mirabilis* isolates was 81.9%,[21] while in Egypt, it was 35%.[22]

In Tehran, Iran, Malekjamshidi *et al*.[23] estimated *blaTEM* prevalence at 83% among ESBL-positive *P. mirabilis* specimens. The *Proteus* ssp. isolates showed resistance to gentamycin.[24] Other studies indicated variable rates ranging from 8.3% to 91%.[25–30]

The evolution of antibiotic resistance in the treatment of UTIs is a severe public health issue, particularly in developing nations with high levels of illiteracy, poverty, and poor hygiene habits.[31]

Conclusions

The majority of *P. mirabilis* isolates were discovered to be MDR with varying degrees of resistance to various antibiotics, while isolates expressing the blaTEM gene exhibited high resistance to Nitrofurantoin and Trimethoprim–Sulfamethoxazole.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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